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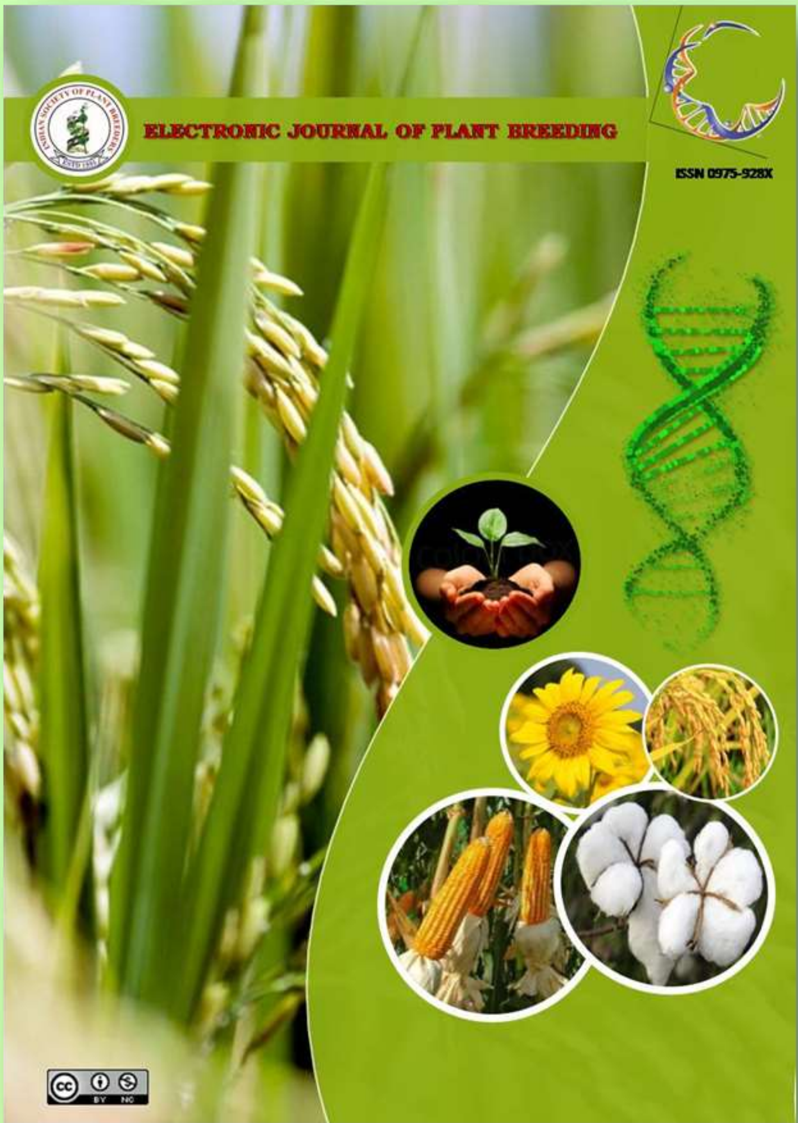

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Research Article

Effect of cryo-storage on the germinability of bitter gourd (*Momordica charantia L.*) seeds

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Abstract
For long term storage of seed, cryo-storage at -196°C is an effective approach among the ex-situ conservation methods. Even though the bitter gourd (*Momordica charantia L.*) seed has been classified as orthodox, various previous studies reported its sensitivity to sub-zero temperature. Hence, the present study was carried out to investigate the seed germination behavior of bitter gourd seeds after cryo-treatment with a view to test the cryo-fitness of the seeds. Results of seed viability experiments conducted in four germplasm lines such as *Arka Harit*, Co 1, *Preethi* and *Bitter gourd long* indicated significant differences in germination percentage in all the bitter gourd lines except *Arka Harit.*

Introduction

Bitter gourd (*Momordica charantia L.*) is a vegetable cultivated predominantly in tropical and subtropical Asia for its immature fruits and tender shoot tips (Vogel, 1996) and is also used in traditional African and Chinese medicine (Grover and Yadav, 2004), as well as in modern medicine where the focus is on its presumed beneficial effect in the treatment of diabetes (Ahmed *et al*., 2004). It is traditionally known for its medicinal properties such as anti-diabetic, anti-tumorous, anti-cancer, anti-inflammatory, antiviral and cholesterol lowering effects, wound healing activity. The protein in bitter gourd, α-momorcharin and β-momorcharin were shown to have potential against HIV (Behera *et al*., 2010) infections.

In the past, bitter gourd breeding mainly relied on the selection of suitable landraces. During the last decade, concerted hybridization based breeding programmes have been undertaken due to increasing attractiveness of the species for its nutrition and drugs (Behera *et al.*,2010). Hence, conservation of available bitter gourd germplasm is mandatory for future use in breeding programmes for its further genetic improvement. Ex-situ conservation in gene banks is the widely prevalent approach of conserving plant genetic resources. These gene banks offer the convenience of conserving germplasm seeds in specially designed cold modules. However for long term storage, cryopreservation at -196 °C in liquid nitrogen (LN) is an effective approach for conserving the germplasm accessions (Rao, 2004; Reed, 2008). Since, bitter gourd seeds are classified as orthodox in several reports (Doijide, 2001; Royal Botanic Garden Kew,2019), they are expected to survive long-term dry storage at subzero temperatures (Hong and Ellis,1996). However, there are some reports indicating deterioration in germination of bitter gourd seed samples if stored at sub-zero temperatures for more than six months (Zhang *et al*., 1990) while some literature report no deterioration in germination percentage if stored at 5°C (Ebert *et al*., 2015). Hence, for long term conservation critical assessment of cryo-fitness of the bitter gourd seeds would be essential. Therefore, optimization of conditions for cryo-conservation of bitter gourd seeds will enable us to preserve the enormous genetic diversity of this native medicinally important crop indefinitely for posterity. Hence, this study was taken up to investigate the germinating ability of bitter gourd seeds after cryo-treatment for six months.

Materials and Methods

The material for this study included four bitter gourd germplasm accessions collected from various institutes *viz*., variety Co 1 from Department of Horticulture, Tamil Nadu Agricultural University Coimbatore; cultivar *Arka Harit* from Indian Institute of Horticulture Research, Bengaluru; cultivar *Preethi* from Kerala Agricultural

University, Thrissur and one landrace *Bitter gourd-long* collected from a farmer field at Coimbatore.

The seeds of all the four germplasm were desiccated to a moisture content of 4-5%. The desiccation was performed by placing the seeds over a pre-heated silica gel in an air tight plastic box for a period of 16 hours. The moisture content was measured using the digital moisture meter (Weiber).

After desiccation, the seeds of each genotype were vacuum packed in a tri-laminated aluminium pouches. The seeds were packed in three replicates containing 100 seeds per replication. The packed seeds were incubated at -140^{o}C for a period of 30, 60, 90, 120, 150 and 180 days durations in the vapour phase of liquid nitrogen. The cryo vessel was topped up with liquid nitrogen in two days intervals up to a level of 7cm height in order to maintain the temperature at -140^{o}C. After cryo-storage for six different durations as mentioned previously, the seeds were gradually thawed by immediately placing the seeds at – 20°C for one day followed by exposure to 5°C for one week.

The germination test was carried out by sowing the seeds in sand media as per the recommendations in ISTA rules (2019). The germination test was performed at room temperature. The final count was taken at 14 days after sowing and germination percentage was calculated using the formula,

$$Germination\ percentage = \frac{S}{T} \times 100$$

Where,

S – Number of germinated seeds,

T – Total number of seeds sown.

Data on germination percentage was analyzed by ANOVA and significant difference among control (freshly collected seeds) and cryo-stored seeds were compared by t-tests using the statistical package of SPSS Inc. version 16.0

Results and Discussion

The effect of cryo-storage of seeds of four bitter gourd varieties on seed viability is presented in Figures 1- 4.The result showed that among the four bitter gourd germplasm studied, only *Arka Harit* exhibited very minimal reduction of 4.65% in germination as compared to fresh seeds (Fig.1). In the cryo-stored seeds of *Arka Harit*, the germination percentage ranged between 82% and 86% during entire six months period of cryo-storage. A drastic reduction in the germination percentage from 80% to 0% was observed in Co 1 variety. It was noticed that the change in seed viability was maintained at a same level in both control and cryo-stored seeds of Co 1 during the first four months. However, a sudden decline in germination was observed after five months in the cryo-stored seeds (Fig 2). With respect to the cultivar *Preethi*, the germination behavior was almost the same as that of the cryo-treated seeds of Co 1 *i.e.*, no significant effect on seed viability was found between control and cryo-stored seeds of *Preethi* during first four months. However, the germination percentage decreased drastically to 43.18% at fifth month and the germination percentage was reduced to zero after 180 days of cryo-storage (Fig 3). The results indicated that the mean germination percentage was almost same for both control and cryo-stored seeds of *Bitter gourd long*. However, absolutely no germination of cryo- treated seeds was noticed after 180 days of cryo-storage (Fig 4).

In this study Bitter gourd genotypes *Arka Harit* , CO 1 , *Preethi* and *Bitter gourd long* seed viability reduction of 4.65,100%,100% and 100% were observed respectively in treatment over untreated control. Zhang *et al.,* (1990) observed no (0%) germination in bitter gourd seeds when stored in liquid nitrogen for the period of six months days corroborates with the result of the present experiment. Additionally, the genotype dependent variation in cryo fitness was also observed. Vidhya (2015) has observed drastic reduction in seed viability of bitter gourd seed after cryo-storage for one month. But in this study, the reduction of seed viability occurred only after five months of cryo-storage. Ice nucleation cannot be reason for reduction as the moisture content was already reduced to 4-5%. So the breakdown of macromolecules like carbohydrates, proteins, lipids or nucleic acids could be the reason for reduction in seed viability. Further biochemical and enzymatic studies are to be carried out to confirm the reason for breakdown of seed viability.

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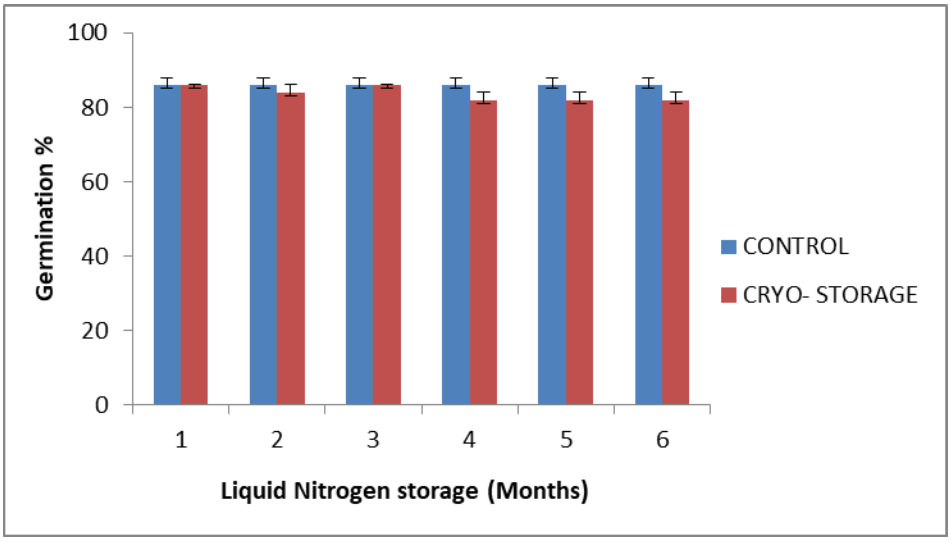

Fig. 1. Seed viability response of cultivar *Arka Harit* genotype after 6 months of cryo– storage.
Bars represent the standard error of mean

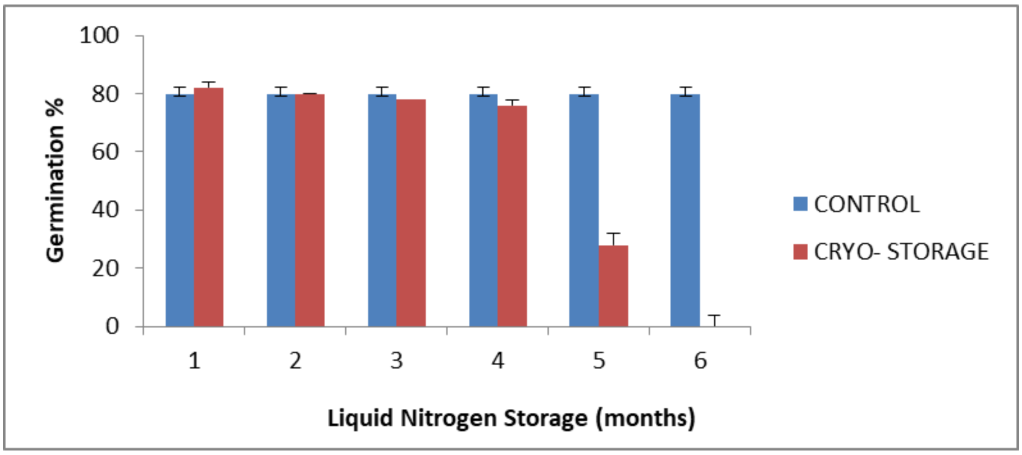

Fig. 2. Seed viability response of variety Co 1 genotype during 6 months period of cryo –storage.
Bars represent the standard error of mean

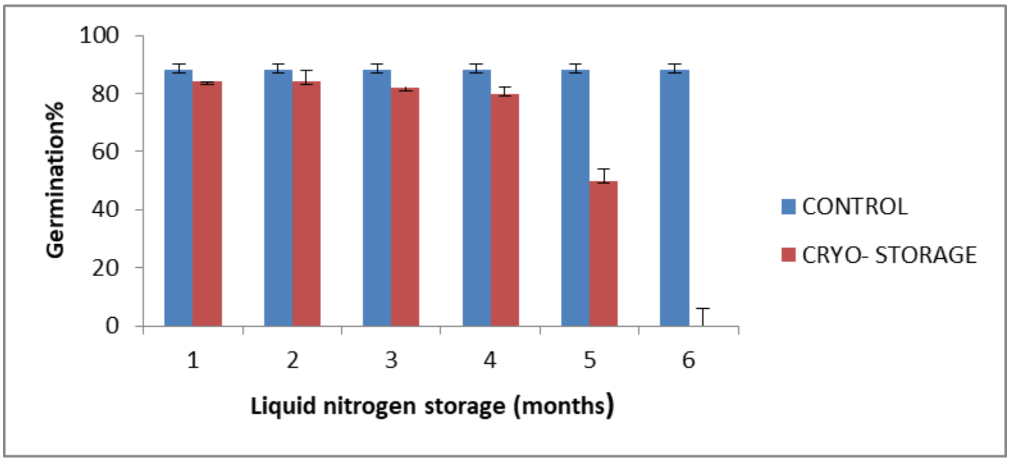

Fig. 3. Seed viability response of cultivar *Preethi* during 6 months period of cryo – storage.
Bars represent the standard error of mean

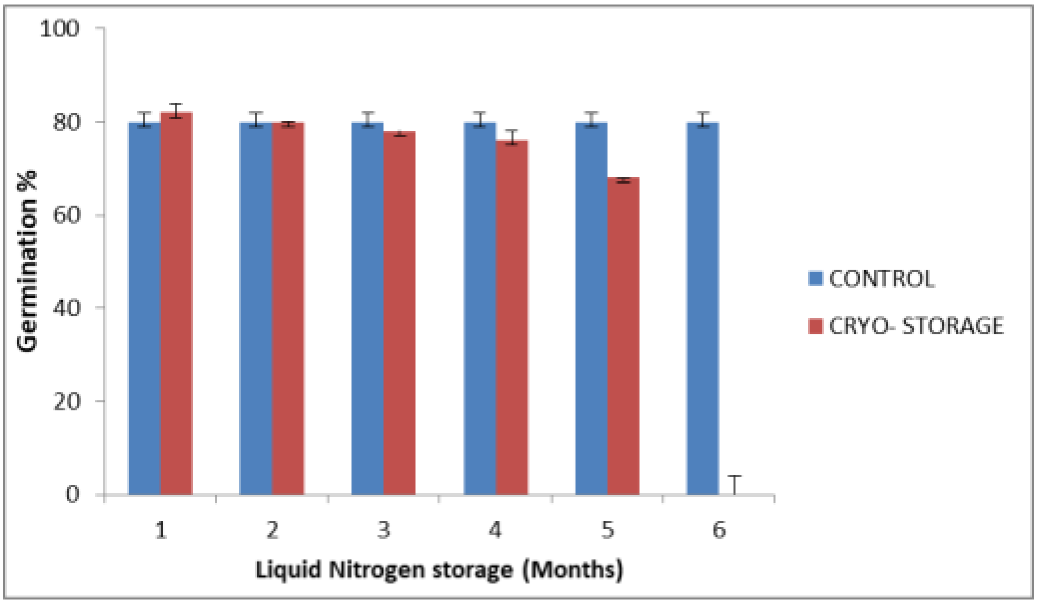

Fig. 4. Seed viability response of bitter gourd long genotype during 6 months period of cryo – storage.
Bars represent the standard error of mean

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