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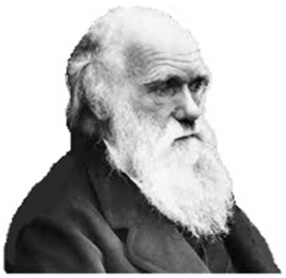

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Study on the survival in liquid nitrogen (LN) of the three rice seed cultivars collected from Nagaland

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ABSTRACT

The effect of cryopreservation on seed germination and seed vigour, and seeds with different moisture regimes have been studied to find out if they are a good candidate for LN by storing seeds in liquid nitrogen (–196 °C) for 24 and 72 hours from three rice seed cultivars, namely Lisem, Manen, and Mapok. For all the three cultivars studied, cryopreservation did not decrease germination percentages or seed vigour and it also appears that seed moisture content ranging from 10% to 18% is not a determining factor for the survival of seeds up to 72 hour storage of the seed in LN.

***Keywords*:** Germination, Seed Vigour, Moisture content, Seed viability, Liquid Nitrogen, Preserved, cryostorage

1. INTRODUCTION

Seeds are units of propagation for many species both, wild and domesticated, hence, seed viability is of utmost importance for the continuation of the race of a particular plant species. When seeds are viable, they are capable of germinating to produce new seedlings provided dormancy is over. Seed viability is an important attribute of seeds. Seed longevity is different from viability as it refers to the "*life span of seed*". However, both viability and longevity of seeds have the common factors affecting them.

Viability can be measured directly by tetrazolium test or by germination test; germination test is one of the standard means by which seed viability can be tested, so is the longevity of seeds. In short, both viability and longevity of seeds can only be determined by germination test and other tests. Seed vigour, although it cannot be determined directly, is an important

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component of seeds. Seeds have the maximum germinability and vigour at physiological maturity, after which when the seeds are harvested they tend to lose their vigour which precedes the germinability during the course of storage and more so when the storage conditions are unfavourable, that is, of high seed moisture content and high storage temperature. Seed vigour and deterioration are the two aspects in seed ageing. Seeds, like other biological organisms, during storage tend to age and ageing is due to deteriorative changes (physiological and biochemical) occurring in seeds soon after physiological maturity to the next planting. Seed vigour decreases as deterioration continues till and until seeds are dead. Vigorous seeds are more likely to loose their viability at a much slower rate as compared to the less vigorous ones. Seed vigour is also an abstract component of seeds, which cannot be measured directly but it can be determined indirectly by measuring the respiration rate, ATP production, seedling length and dry weight, reduction of tetrazolium salt, conductivity of seed leachate and so on.

Harrington (1972) after having worked with many seeds found that viability loss is preceded by loss in vigour. Seed vigour, like viability can be maintained by proper storage conditions of low seed moisture content and storage temperature, particularly in orthodox species. Humans have been relentlessly and ruthlessly exploiting the natural habitat for plants, especially the wild species, in the context of improving the life of human beings through socio-economic activities.

New varieties of agricultural species have been introduced through agriculture, especially in rice, local cultivars are being pushed to extinction by replacing them with evolved varieties; local cultivars start disappearing much before the good qualities could be identified. Hence, attempts have been made that at least local cultivars may be preserved for future use, which may be achieved only by storing them in recognized seed banks. Such attempts on tef and niger by Zewdi and Ellis (1991) and also on wild species (Pence, 1991) by identifying them as potential candidates for cryostorage (LN storage).

2. MATERIALS AND METHODS

2. 1. Seed Source and Cultivars

Seeds of three different cultivars of *Oryza sativa* were collected from different localities in Nagaland and they belong to the harvest of the month of August and September, 2016. *Mapok*, *Lisem* and *Manen* cultivars were collected from different farmers of different localities in Nagaland.

2. 2. Methodology

2. 2. 1. Seed Moisture content

The seed moisture content of the seeds shall be determined by the standard method prescribed by the International Seed Testing Association, (2010). 4-5 g seeds will be weighed in the moisture bottles (**Tables 1-3**). The bottles containing seeds shall then be transferred to the oven maintained at 103 ±2 ºC for 17 hours. The weight of the dried samples and bottles were again taken and the moisture content shall be calculated according to the formula below:

$$\text{Seed Moisture Content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100\%$$

where: W_1 = weight of empty bottle
W_2 = weight of fresh seeds and moisture bottle
W_3 = weight of dried seeds and moisture bottle.

The results will be expressed in percentage (fresh weight basis).

2. 2. 2. Germination Test

In order to know the germinability percentage of the seeds**,** seeds shall be sterilized in 0.1% $HgCl_2$ solution followed by repeated washings (about three times) in distilled water. Routinely, 50 seeds in three replicates will be rolled into a moistened towel paper and incubated at 25 ºC; on the 14th day germination shall be evaluated by classifying seedlings into Normal, Abnormal, Dead seed and Hard Seeds (if any). Each category shall be expressed in percentage.

2. 2. 3. Seedling length

The length of 10 seedlings in three replicates was recorded and results were expressed as the mean (in cm/seedling) of means of three replicates of 10.

2. 2. 4. Seedling dry weight

The seedling dry weight was determined by drying separately 10 seedlings in three replicates and the mean of three replicates of 10 was expressed in g/seedling, after drying at 90 ºC for 24 hours.

2. 2. 5. Conditioning of seeds and exposure to LN

Seeds of different cultivars were conditioned to targeted moisture content of the range of 10 to 12% by exposing them to the sun with the day temperature of 22-25 ºC for a few hours followed by exposure to LN for about 24 and 72 hour duration to determine if they are the candidates for cryopreservation. At the end of 24 and 72 hour exposure to LN seeds were thawed at room temperature for three hours followed by germination test; on the fourteenth day geminatibility, seedling length and dry weight were assessed as described above.

3. RESULT

Table 1. Showing the original seed moisture content of the three rice cultivars

Cultivar	Moisture content (%)
Lisem	17.74 (±0.05)
Mapok	17.37 (±0.11)
Manen	16.35 (±0.13)

± = Standard Error

Table 2. Showing the germination percentage of the seeds from freshly arrived seeds

Cultivar	Normal seedlings (%)	Hard seeds (%)	Dead seeds (%)
Lisem	84.67 (±2.94)	15.33 (±2.94)	0.00 (±)
Mapok	96 (±4.89)	2.67 (±3.26)	1.33 (±1.63)
Manen	81.33 (±0.81)	18.67 (±0.81)	0.00 (±)

Table 3. Showing the seedling length and dry weight from freshly arrived seeds after 14 days of germination test

Cultivar	Seedling length (cm)	Seedling dry weight (gm)
Lisem	18.25 (±1.57)	0.124 (±0.002)
Mapok	23.26 (±0.09)	0.160 (±0.018)
Manen	14.43 (±0.26)	0.095 (±0.001)

± = Standard Error

Table 4. Showing germination percentage of normal seedlings, hard seeds and dead seeds from seeds exposed to liquid nitrogen (LN) for 24 and 72 hours, respectively

Cultivar	Moisture Content (%)	24 hours in LN			72 hours in LN		
		Normal Seedlings (%)	Hard seeds (%)	Dead seeds (%)	Normal Seedlings (%)	Hard seeds (%)	Dead seeds (%)
Lisem	10.87 (±0.15)	75.33 (±3.26)	24.00 (±3.74)	0.7 (±0.81)	82.66 (±3.55)	17.33 (±3.55)	0.00
	17.74 (±0.05)	83.33 (±0.81)	16.67 (±0.81)	0.00	82.66 (±4.32)	17.33 (±4.32)	0.00
Mapok	10.82 (±0.09)	86.00 (±3.74)	14.00 (±3.74)	0.00	90.00 (±1.41)	10.00 (±1.41)	0.00
	17.37 (±0.11)	86.66 (±4.96)	13.33 (±4.96)	0.00	88.66 (±4.08)	11.33 (±4.08)	0.00

Manen	10.07 (±0.03)	69.33 (±5.71)	30.67 (±5.71)	0.00	78.66 (±1.63)	21.33 (±1.63)	0.00
	16.35 (±0.13)	76.67 (±0.81)	23.33 (±0.81)	0.00	82.66 (±0.81)	17.33 (±0.81)	0.00

± = Standard Error

Table 5. Showing the seedling length and dry weight from LN exposed seeds after 14 days of germination test

Cultivars	Moisture Content (%)	24 hour in LN		72 hour in LN	
		Seedling length (cm)	Seedling dry weight (g)	Seedling length (cm)	Seedling dry weight (g)
Lisem	10.87 (±0.15)	25.19 (±1.26)	0.110 (±0.006)	26.31 (±2.12)	0.108 (±0.000)
	17.74 (±0.05)	26.21 (±1.20)	0.122 (±0.002)	28.61 (±0.36)	0.094 (±0.003)
Mapok	10.82 (±0.09)	31.08 (±0.16)	0.141 (±0.002)	28.83 (±1.11)	0.154 (±0.004)
	17.37 (±0.11)	34.16 (±1.97)	0.172 (±0.019)	36.30 (±1.23)	0.158 (±0.009)
Manen	10.07 (±0.03)	25.55 (±1.51)	0.087 (±0.002)	25.83 (±0.51)	0.090 (±0.002)
	16.35 (±0.13)	27.74 (±1.14)	0.096 (±0.001)	28.24 (±0.94)	0.127 (±0.005)

± = Standard Error

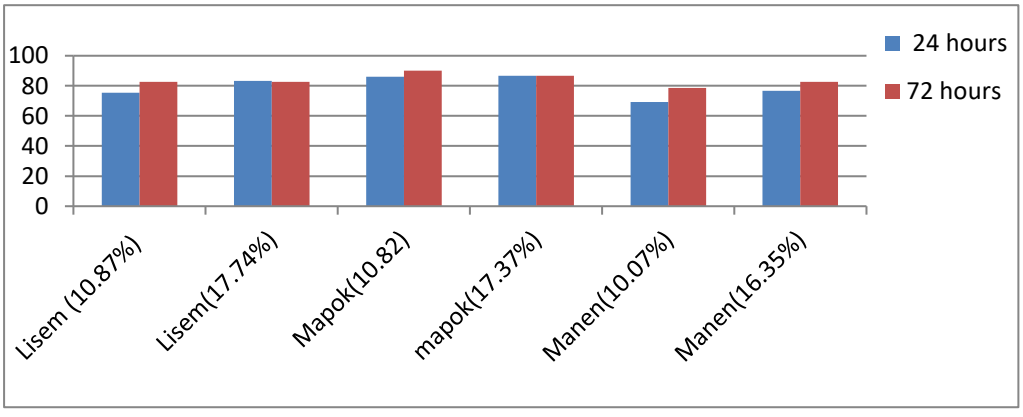

Figure 1. Percentage of seedling length (in cm) of each cultivar with two different moisture content exposed to liquid nitrogen for 24 and 72 hours

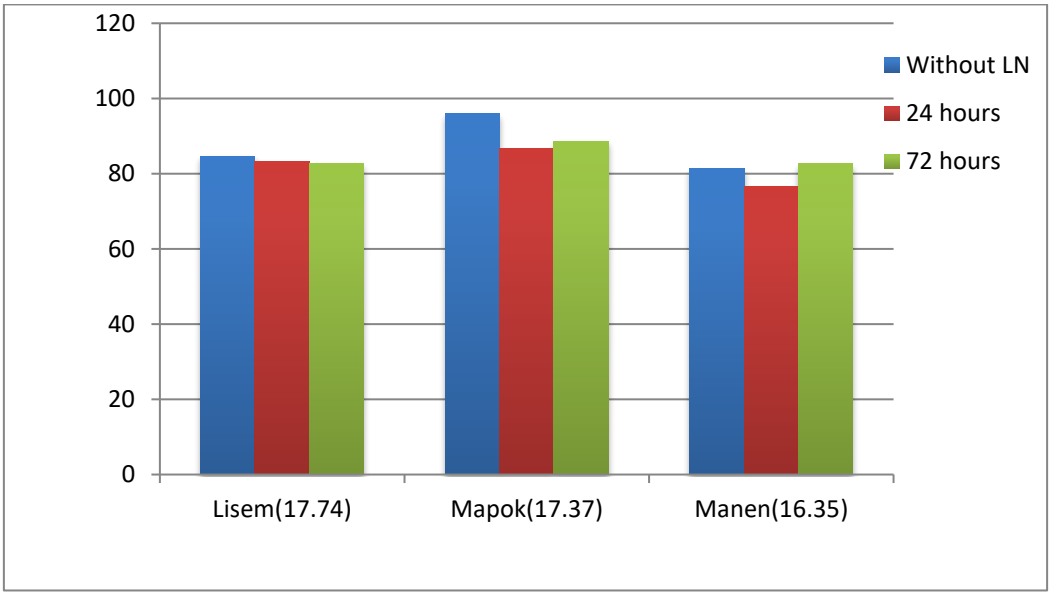

Figure 2. Differences in percentage of normal seedling without and with exposure to LN of moisture content – Lisem (17.74), Manen (17.37) and Mapok (16.35)

4. DISCUSSION

In the present investigation, it has been observed that the original seed moisture content of all the three rice cultivars ranges from 16.35% to 17.74% and that the conditioned seed moisture content ranges from 10.07 to 10.87% on wet weight basis (**Figure 1**). The seeds of cultivars with different moisture regimes were subjected to 24 and 72 hours of exposure to liquid nitrogen (LN).

It has been observed that after exposure to LN for the above mentioned durations, there is not much difference in the percentage of normal seedlings in all the three cultivars (**Table 4**), despite the fact that lower values have been observed in all cultivars for any moisture regime, which may be attributed to experimental error. Seeds exposed to LN for 72 hours show higher values indicating that the germinability of seeds after LN exposure is not affected at all although cannot be confirmed with confidence as there is no evidence to support our assumption. It may be argued that for the seed moisture regimes under our investigation, seeds of all the three cultivars may be the potential candidates for LN storage (**Figure 2**).

So far as hardseededness is concerned the percentage of lower values have been observed in all cultivars stored for 72 hours in LN as compared to those stored for 24 hour; higher values at 24 hour of storage (**Table 4**) may again be attributed to experimental error, which may be eliminated by better handling during the investigation, but apparently with a little deviation from values from freshly arrived untreated seeds of all the cultivars (**Table 2**).

In short, according to the objectives to ascertain seed survival in LN, it may be argued that seeds are candidates for LN storage. In the present investigation, all data collected from seeds which experience LN storage for 72 hours are more reliable for survival at sub-zero temperature. Like normal seedling percentage parameter, it appears that seedling length and dry weight values are a little higher from seeds stored for 72 hours in LN in all cultivars for any

seed moisture content as compared to seeds stored for 24 hours in liquid nitrogen (**Table 5**) and also higher values in the seedling length from the seeds stored in liquid nitrogen as compared to freshly arrived untreated seeds (**Table 3**). As discussed above lower values on the above parameters may be attributed to experimental error. Hence, it may be concluded that seed vigour is not affected by storage at sub-zero temperature, because if seed vigour is affected that would have been reflected in reduced seedling length and dry weight.

As evident from the results obtained, it may be argued that the three rice cultivars in our investigation may be recommended as candidates for sub-zero temperature as it appears that seed moisture content is not a determining factor for the survival of seeds upto 72 hour storage of seed in LN. For the survival of seeds at sub-zero temperature it has been noted by some workers that seed moisture content is a determining factor (Roos and Stanwood, 1981; Becwar *et al*., 1983; Stanwood, 1987; Roberts and Ellis, 1989; Vertucci, 1989) as well as seed biochemical composition (Touchell and Dixon, 1994; Ashutosh, 2015; Bashyal, 2013, 2016; Wulff, 2010; Bhanu, 2019; Vinay, 2019; Bewket, 2018; Zewdineh, 2019).

5. CONCLUSION

Hence, based on the results obtained in the present investigation, it may be concluded that: Seeds of the three rice cultivars *Lisem*, *Mapok* and *Manen* survive LN storage at moisture content of about 10 to 18% exposed to LN. Seeds of three rice cultivars, namely, *Lisem*, *Mapok* and *Manen* may be recommended for LN storage with the seed moisture content of about 10% (even lower) to 18%. Hence, the three cultivars are candidates for LN storage. Apparently there is no loss of seed vigour for any moisture regime and different duration of storage in LN. This further strengthens our view that the seeds of all cultivars are candidates for LN storage.

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