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# CRYOPRESERVATION OF DESICCATED SEEDS OF NEEM (*AZADIRACHTA INDICA* A. JUSS.) FOR GERMPLASM CONSERVATION

**Rekha Chaudhury and K.P.S. Chandel**

National Plant Tissue Culture Repository,
NBPGR, Pusa Campus, New Delhi-110 012

*Among tree species, immensely valued for timber and medicine, neem* (Azadirachta indica) *is extremely important. Neem seeds, inspite of possessing some typical features of recalcitrant seeds like short life, high moisture content (about 46%) at the time of shedding and possessing endocarp which played a significant role in germination and desiccation responses, showed orthodox nature in terms of desiccation and freezing sensitivity. Seeds showed desiccation tolerance upto 4 per cent and freezing tolerance at 196°C for seeds desiccated between 18.5 per cent and 4 per cent moisture level. Cryopreservation, the technique of storage of germplasm at the ultra low temperatures of liquid nitrogen (196°C) can thus be utilized for long term conservation of neem germplasm.*

Neem (*Azadirachta indica* A. Juss.) is a valuable arboreal species found extensively in tropical regions of India. FAO panel of experts on Forest Gene Resources in their recommendations regarding collection, evaluation, and conservation of forest species, have selected neem for urgent action in India. So far, very limited information is available on germination, viability and storage of neem seeds. Neem seeds are reported to be short-lived (Ezumah, 1986; Troup, 1986; Maithani *et al.*, 1989) mainly due to their high moisture levels which causes deterioration in them (Troup, 1986). Attempts at storing seeds under different temperature and storage conditions have been successful only for 16 weeks at room temperature (Ezumah, 1986) and 6 months at 15°C (Maithani *et al.*, 1989). Storage temperatures of 5-11°C were found unfavourable (Ezumah, 1986; Maithani *et al.*, 1989). Controversial re-

ports exist regarding the classification of neem seeds either as orthodox or recalcitrant. Roberts (1983) had earlier designated neem as recalcitrant. In a later report (Holden & Williams, 1984), it was found that viability of seeds is drastically reduced on desiccating the intact neem seeds and endocarp affected the germination responses. Neem seeds have been, however, categorised as orthodox, based on electrical conductivity measurements of fresh seeds (Khare *et al.*, 1989). Present studies were, therefore, undertaken to characterise neem seeds as orthodox or recalcitrant based on the germination, desiccation and freezing sensitivity studies.

## MATERIALS AND METHOD

Seeds were extracted from the ripe yellow fruits following the method described earlier (Radhamani *et al.*, 1990). Moisture content of the seeds was determined (after removing the endocarp) by low constant temperature oven method (Anon., 1985) 103 (103 $\pm$ 2°C for 17 h) and expressed as percentage of fresh weight. Germination was carried out by the method reported earlier (Radhamani *et al.*, 1990). Seedling vigour was determined by average root lengths of seelings. Seeds were desiccated to different levels by two methods, (1) over silica gel in a desiccator at room temperature and (2) in a seed drier (Model MVE 50 SP., Bry Air India Ltd.) supplying forced air at 20-25 per cent relative humidity and 20°C. Desiccated seeds were immediately plunged in liquid nitrogen after packing in polypropylene cryovials. After 24 hours, seeds were retrieved and thawed by immersion in a water bath maintained at 37-38°C.

## RESULTS AND DISCUSSION

Average moisture content of neem seeds was 46$\pm$3.7 per cent. This value is much higher than that reported earlier (Maithani *et al.*, 1989; Khare *et al.*, 1989). Moisture content of endocarp alone was 12.01 per cent. In view of inhibitory role of endocarp (Radhamani *et al.*, 1990), freshly harvested seeds were always germinated after removing their endocarp and the germinability ranged from 90 to 100 per cent. Fresh seeds could be desiccated much faster in seed drier than in desiccators over silica gel (Table 1) and within each of the methods of drying, excised seeds (without their endocarp) lost moisture much faster than the intact seeds especially when desiccation was prolonged. Excised seeds lost considerable moisture (about 43% of fresh weight) after 24 hours of desiccation in a seed drier, whereas, those maintained in silica gel took 144 hours to lose same amount of moisture. Intact seeds could also be desiccated to this low level but only after 161 hours of desiccation over silica gel.

**Table 1. Desiccation of intact and excised seeds of neem for varying periods using seed drier and silica gel**

| Hours of desiccation | Desiccation in seed drier (% moisture lost in seeds)* | | Desiccation over silica gel (% moisture lost in seeds)* | |
|---|---|---|---|---|
| | intact | excised | intact | excised |
| 1 | 1.6 ± 0.04 | 6.9 ± 0.04 | 1.8 ± 0.06 | 2.3 ± 0.10 |
| 4 | 8.5 ± 0.05 | 9.9 ± 0.04 | 2.9 ± 0.10 | 3.0 ± 0.15 |
| 7 | 11.5 ± 0.04 | 16.3 ± 0.09 | 4.3 ± 0.51 | 10.2 ± 0.88 |
| 15 | 17.1 ± 0.10 | 36.4 ± 1.1 | 9.5 ± 1.1 | 25.1 ± 2.3 |
| 24 | 19.3 ± 0.41 | 43.3 ± 1.3 | 12.1 ± 2.1 | 30.2 ± 2.2 |
| 43 | — | — | 15.0 ± 2.2 | 32.5 ± 3.1 |
| 72 | — | — | 28.0 ± 1.8 | 35.2 ± 2.1 |
| 144 | — | — | 35.9 ± 1.1 | 42.0 ± 1.8 |
| 161 | — | — | 41.9 ± 2.1 | 42.0 ± 1.9 |

* Average values of three replicates (% of fresh wt.).

Excised seeds desiccated upto about 21 per cent moisture content maintained their original high viability (Fig. 1). Seeds desiccated further to 13.5 per cent showed the first sign of desiccation injury. Three hours of rehydration of these seeds over 100 per cent relative humidity at room temperature could, however, recover its original germinability. Seeds desiccated to 4 per cent moisture level showed a fall in germination values to 65 per cent, which could be recovered to a maximum of 70 per cent by rehydration for three hours. In all the subsequent experiments, prehumidification of seeds for 3 hours was routinely done before sowing for germination.

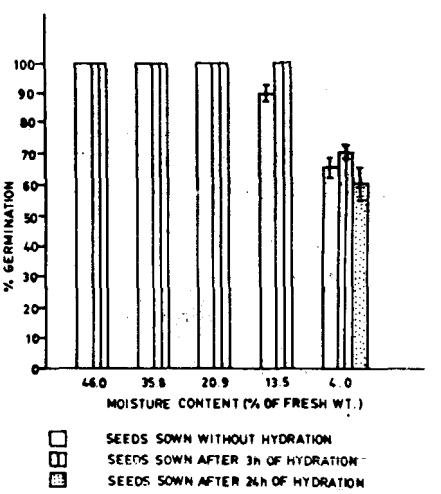


Fig. 1. Extent of desiccation tolerance of excised neem seeds and recovery of desiccated seeds following rehydration at 100 % RH at room temperature for various periods.

Intact seeds desiccated to different moisture levels on sowing for germination developed cracks in their endocarp, indicating initiation of germination by 6 days of sowing in 10 to 30 per cent on the seeds (Table 2). However, these seeds showed further growth only in seeds with

**Table 2. Effect of presence or absence of endocarp on germination of neem seeds desiccated to different levels in intact state**

| Moisture content of seeds (% of fresh wt.) | Endocarp retained/removed | Observations recorded after 6 days of sowing for | |
|---|---|---|---|
| | | per cent Germination | Average root length (cm.) |
| 46.9 | Retained | 5* (30) | 1.2 |
| | Removed | 95 | 3.65 |
| 32.8 | Retained | 0* (20) | - |
| | Removed | 95 | 3.5 |
| 28.3 | Retained | 0 (10) | - |
| | Removed | 90 | 4.6 |
| 22.4 | Retained | 0 (10) | - |
| | Removed | 70 | 2.1 |
| 12.7 | Retained | 0 (0) | - |
| | Removed | 50** | 2.05 |

*Final germination percentage increased to 25.
**Final germination percentage increased to 65.
Values in parenthesis are % of intact seeds that showed only cracks in endocarp

higher than 32 per cent moisture level. A part of the seeds sample at each of the desiccation levels sown after removal of the endocarp showed much better germinabilities and seedling vigour; seeds possessing about 28 per cent moisture and higher, showed germination values of 90 per cent and higher and seedlings with more than 3.5 cm roots and those possesssing 22.4 per cent and 12.7 per cent moisture showed 70 per cent and 65 per cent germination and 2.1 and 2.05 cm roots, respectively. Thus, in neem seeds, endocarp was found to influence the germination and desiccation responses. It delayed the loss of moisture during desiccation and also the uptake of water during imbibition on sowing the seeds for germination. Neem seeds, in the absence of their endocarp, showed desiccation tolerance upto 4 per cent moisture level-extent found in the orthodox seeds. However, it was accompanied by 25-30 per cent loss in viability. Intact seeds on desiccation to about 13-15 per cent moisture showed lower germination values even when sown without their endocarp in comparison to the seeds desiccated to the same level in excised state from the beginning. This was due to the comparatively longer time taken by the intact seeds to achieve this level of moisture which could have caused some physiological deterioration.

Excised seeds possessing moisture contents of about 27 per cent and above on exposure to liquid nitrogen for 24 h showed total loss of viability (Fig. 2). Only seeds desiccated to 18.5 per cent and lower upto 4 per cent moisture content survived liquid nitrogen exposure for 24 h. High survival values between 57-65 per cent were, however, obtained only for seeds possessing moisture contents between 13.5 to 4 per cent.

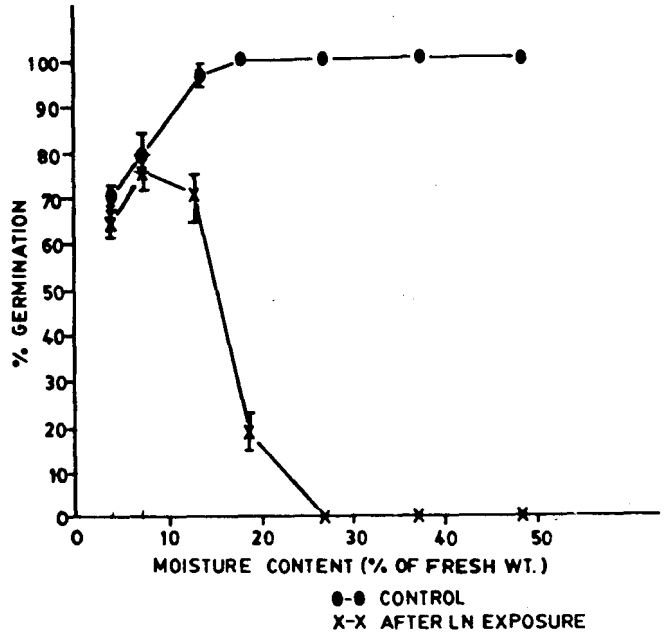


Fig. 2. Germinability of excised seeds of neem after desiccation and liquid nitrogen exposure

Based on desiccation and freezing sensitivity, neem seeds could be clearly categorised as orthodox in nature. It was in the presence of endocarp that the seeds showed recalcitrant nature since desiccated seeds showed germination only when endocarp was removed. Cryopreservation of neem seeds is thus feasible using the simple procedure of desiccation of seeds to 13.5 per cent and lower and freezing them rapidly in liquid nitrogen. This can ensure long term conservation of neem germplasm for future use.

## ACKNOWLEDGEMENTS

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