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Effect of ultra dry seed storage on longevity of onion (*Allium cepa*) and china aster (*Callistephus chinensis*) under ambient and controlled temperatures

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ABSTRACT

Ultra dry seed storage was studied in onion (*Allium cepa* L.) and china aster [*Callistephus chinensis* (L.) Nees] to find out its effect on seed longevity under ambient and controlled low temperatures. Seeds of onion cultivars Arka Kalyan and Arka Niketan ultra dried to 2.8 and 2.6 %, respectively, and seeds of china aster cultivar Arka Kamini ultra dried to 3.0% were compared with seeds of respective cultivars having moisture content close to recommended levels for packing in moisture proof containers. These seeds were hermetically sealed in aluminium pouches and stored at ambient and controlled (constant 15°C) temperatures. The initial germination in onion was 90.0 and 88 % in Arka Niketan and Arka Kalyan, respectively, and it remained unaffected after ultra drying. During storage, ultra dry seeds and seeds with ~5% moisture content of both varieties showed no significant reduction in seed germination, first count, seedling vigour and field emergence even after 54 months of storage both under ambient and controlled temperatures. In china aster, seeds with 5.9% moisture showed decline in seed viability and vigour under ambient temperature after 18 months of storage and rapid decline was noticed after 24 months of storage, reaching zero at 36 months but at 15°C showed no decline in viability up to 48 months of storage. Ultra dry seeds maintained higher viability and vigour at both ambient and at 15°C even after 48 months of storage. Genetic fidelity tests on onion seed clearly demonstrated that neither the profiles of soluble proteins and enzymes nor the DNA were affected by ultra drying.

Key words: China aster, Onion, Seed storage, Ultra dry

Seed moisture and storage temperature are the two important factors that determine the longevity of seeds in storage. Hitherto, seed researchers have given more importance to storage temperature than to seed moisture for extending seed viability during storage. But several studies have shown that seed moisture could be more important than temperature in extending viability of seeds especially under tropical conditions (Justice and Bass 1978). For long-term storage the seeds are dried to 5-6% moisture content and stored at sub zero temperature (FAO/IPGRI 1994). Drying seeds to below 4-5% was considered as detrimental to seed viability. But studies have shown that seeds can be dried to below 4-5% and in some species even below 1% without affecting the viability as well as genetic fidelity of the species (Ellis *et al.* 1986). Such ultra dry seeds can be stored for more than 8-10 years under ambient temperature (sub zero condition in temperate region in winter to 50°C in tropical region during summer). The longevity of ultra dry seeds can be further extended by keeping at lower temperature (10-15°C). This method will eliminate or avoid the requirement for low temperature storage. Low temperature storage in tropical developing countries cannot work efficiently and effectively because of frequent power cuts. As a result risks of losing valuable seed material is very high. Ultra low dry seed storage offers a great hope under such situations with minimum cost. However, before subjecting valuable germplasm/commercial seed to storage at such low seed moisture, the potential benefits and risks of ultra drying to seeds longevity must be evaluated as some reports have indicated that drying to very low water content damages seeds. Hence, the study on ultra low dry seed storage was taken up in onion (*Allium cepa* L.) and china aster (*Callistephus chinensis* (L.) Nees) which are known to lose viability quickly under ambient conditions.

MATERIALS AND METHODS

Onion and china aster each representing vegetable and flower crops respectively were selected for this study mainly because these crop seeds are known to lose viability quickly under ambient room temperatures. In onion, two cultivars, viz. Arka Kalyan and Arka Niketan were chosen. Fresh, cleaned and graded seeds of these cultivars were obtained from Breeder Seed Production unit of Indian Institute of Horticultural Research (IIHR), Bengaluru, India. These seeds were further cleaned and graded using seed blower. In china aster, only one cultivar, i.e. Arka Kamini was chosen and seeds were collected from seed crop raised during *rabi*-

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summer 2009 at IIHR. Seeds were hand threshed, cleaned and graded by using seed blower.

Seed quality parameters such as germination and vigour of fresh seeds with initial moisture content of 5-6% in onion and china aster, were assessed before subjecting them for ultra low drying. Seeds were ultra dried using activated blue silica gel in 1:3 ratio (seed:silica gel) kept in glass desiccators. Partially saturated silica gel was replaced by activated silica gel 2-3 times during drying. In onion, seed moisture was brought down to 2.6% in Arka Niketan and 2.8% in Arka Kalyan. In china aster, the seed moisture was brought down to 3.0%. This drying process took around 30 days to achieve ultra low moisture levels. These ultra dry seeds and seeds with moisture content of around 5% in onion and 5.9% in china aster were packed in poly aluminum pouches, heat sealed and stored at ambient room temperatures ranging from (average min. temperature 23°C and average max. temperature of 28°C) and at controlled temperature of constant 15°C. In order to avoid exposure of seeds to outside humidity at the time of sampling seeds for quality tests at regular intervals, as many packets as the number of observations were made so that each time one packet of seed could be used completely.

The seed viability, seedling vigour, field emergence, protein and DNA stability, and seed moisture of ultra dry seed were monitored along with control seeds at regular intervals for 54 months in onion and for 48 months in china aster. Hot air oven method as described by ISTA (1993) was followed for seed moisture estimation. Two replicates of 2 g each were dried at 103°C for 17 hr as onion and china aster seeds are rich in oil content. Moisture content was expressed on a fresh weight basis. Germination test was conducted using paper towel method with 4 replications of 100 seeds each. Before keeping the ultra dry seeds for germination, the seeds were brought to equilibrium moisture with the surrounding air RH of 75-80% by exposing the seeds for 48 hrs to avoid imbibition damage. For seedling vigour estimation mean seedling length of 10 normal seedlings (cm) obtained randomly at the end of germination test period, was measured and this value was multiplied by germination per cent. Field emergence test was done with 4 replicates of 50 seeds each in plastic plug trays containing decomposed coco peat and expressed in percentage. Biochemical and molecular changes in control and ultra dry seeds that were stored at two different temperatures (15°C and ambient room) were studied in onion cultivars. Soluble proteins, enzymes and DNA were isolated from 15 day old seedlings using 0.5 M phosphate buffer pH 7 and 2% CTAB, respectively, and stored at -20°C till further use. Tris soluble proteins and enzymes were extracted from 15 day old seedlings raised from different treatments. One gram tissue was fine powdered using liquid nitrogen and homogenised with 0.5 M phosphate buffer pH 7.0 under chilled conditions. The crude extract was left at -20°C overnight and spun at 10 000 rpm for 10 min to collect supernatant. The supernatant was run on 12.5% SDS PAGE (Laemeli l970) and 10% native PAGE (Glaszman *et al.* 1988), respectively, to obtain protein and enzyme profiles. Protein gels were stained with 0.1% CBB solution over night and destained using 10% acetic acid till the background is clear. Enzymes gels were stained with specific stains. For esterase, gels were stained for one hour in staining solution containing 50 mg fast blue RR salt and 50 mg of alpha napthyl acetate dissolved in 100 ml of 0.5M sodium phosphate buffer. In case of peroxidise, gels were incubated in 100 ml of 50mM sodium acetate buffer (pH 4.2) solution containing 50 mg of 3-9-AEC (dissolved in 3ml of dimethyl formamide) for five minutes and later stained with 1 ml of 30% H2O2 till brownish red bands appear. DNA was isolated using 2% CTAB solution (Cao and Oard 1997) and stored at -20°C till further use. SSR primers were used for amplification of DNA isolated from seedlings raised from different treatments. PCR was performed using 20 µl reaction mixture containing 2.5 µl of 1mM dntp, 2.5 µl of 15mM MgCl2 with 50ng dna,1 unit of taq polymerase, 5 picomles of both forward and reverse primers and required amount of DNAse and RNAse free water. PCR was run for 40 cycles (Initial denaturation at 94°C for 4 min; Denaturation: 94°C for 1min; Primer annealing: 55°C for 1 min; Primer extension: 72°C for 2 min; final extension: 72°C for 7min and held at 4°C) and the amplified products were run on 3% agarose gel with 1X TBE running buffer at 100 v for 1 hour.

Statistical analyzes were performed separately for each cultivar and observation using two way analysis of variance and means were compared using critical difference at <0.01 level of significance.

RESULTS AND DISCUSSION

Seed quality evaluated at six months interval during storage starting from initial evaluation just before storage, are presented crop wise.

Onion

There was no reduction in seed quality of ultra dry seed compared to control seed (seeds with ~5% moisture content) when tested immediately after drying. The initial germination was 90.0 and 88% in Arka Niketan and Arka Kalyan, respectively, and it remained unaffected after ultra drying. During storage ultra dry and control seeds in both varieties showed no significant reduction in seed germination and seedling vigour even after 54 months of storage both under ambient and controlled temperatures (Fig 1-4). In fact, little increase in germination percentage was noticed during initial storage period and then remained same till 54 months. At the end of 54 months of storage, the germination was around 95% in both the cultivars irrespective of seed moisture and storage temperature. The same trend was noticed with respect to field emergence as well. The field emergence was above 90% in both ultra dry and control of both varieties irrespective of storage temperatures. SDS-PAGE of protein profiles of control and ultra dry seeds in both the cultivars of onion showed similar pattern without any noticeable change indicating no effect of ultra drying on proteins. Similarly, isozyme profiles of

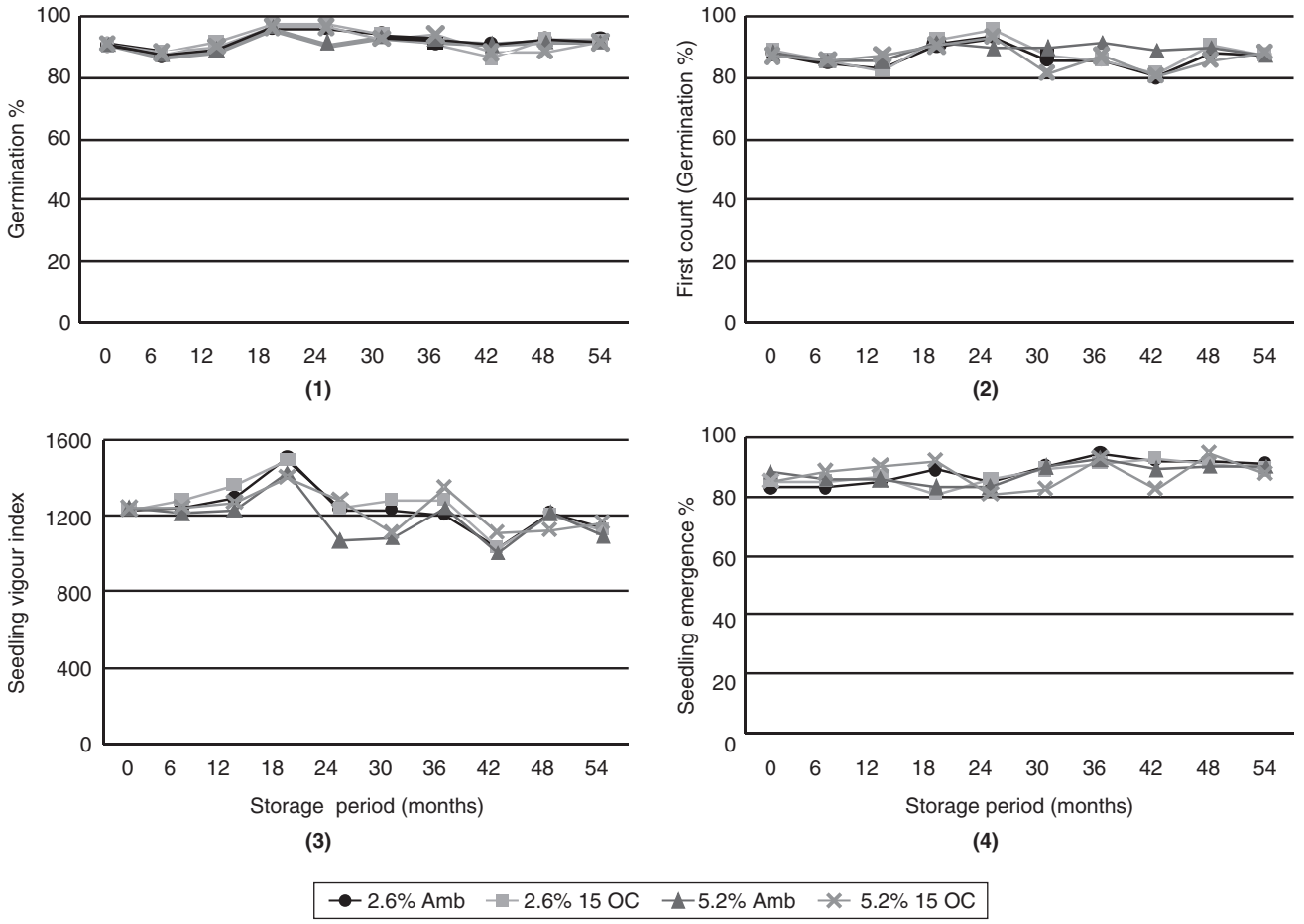

Fig 1-4 Germination, first count, seedling vigour index and field emergence as affected by seed moisture and storage temperature over 54 months of storage in onion cv. Arka Nikethan (The means were found non significant at all storage periods for all the parameters)

esterase and peroxidase did not show any variation between control and ultra dry seeds in both the cultivars indicating that the enzyme activities remain unaltered. Studies on DNA polymorphism using two SSR markers also showed no variation between ultra dry and control seeds in both cultivars.

China aster

Seeds of aster cv. Arka Kamini ultra dried to 3% tested for seed quality before and after storage at 15°C and ambient condition along with control seeds with moisture content of 5.9% showed no reduction in seed quality of ultra dry seed compared to control (Fig 5-8). The initial germination was 80.5% and 82.2% in ultra dry and control seeds respectively. Until 12 months of storage there were no significant differences between moisture contents as well as between storage temperatures for germination percentage. There was no drop in seedling vigour as reflected in first count and seedling vigour index when compared to initial level. After 18 months of storage, seeds with 5.9% showed decline in seed viability and vigour under ambient temperature and rapid decline was noticed after 30 months of storage, reaching zero germination at 36 months. At 15°C both ultra dry and seeds with 5.9% moisture showed no decline in viability up to 48 months of storage. However, marked decline in seedling vigour index was noticed in ultra dry seeds stored under ambient temperature as well as at 15°C after 18 months compared to the original level. The decline was also noticed in seeds with 5.9% moisture at 15°C. The field emergence percentage followed similar trend as lab germination percentage till 36 months of storage but showed reduced emergence percentage from 42 months of storage in ultra dry seeds at both temperatures and also in seeds with 5.9% moisture at 15°C. Field emergence percentage in seeds with 5.9% stored at ambient temperature declined rapidly at 30 months reaching zero at 36 months.

Drying seed beyond a critical moisture level provided no additional benefit to longevity (Ellis *et al*. 1988, 1989, 1990a, and b, Vertucci and Roos 1993) and many even accelerate seed aging rate (Ellis *et al*. 1989, 1990a, b, Chai *et al*. 1998, Hu *et al*. 1998; Kong and Zhang 1998). In our study with onion it may be partly correct as the seeds both ultra dry and control maintained very high germination and vigour under ambient temperature for 54 months and it is on par with the seeds stored at low temperature, i.e. constant 15°C. This shows that further reduction from the recommended moisture level of 5% in onion does not provide any benefit if the seed is intended to be stored for

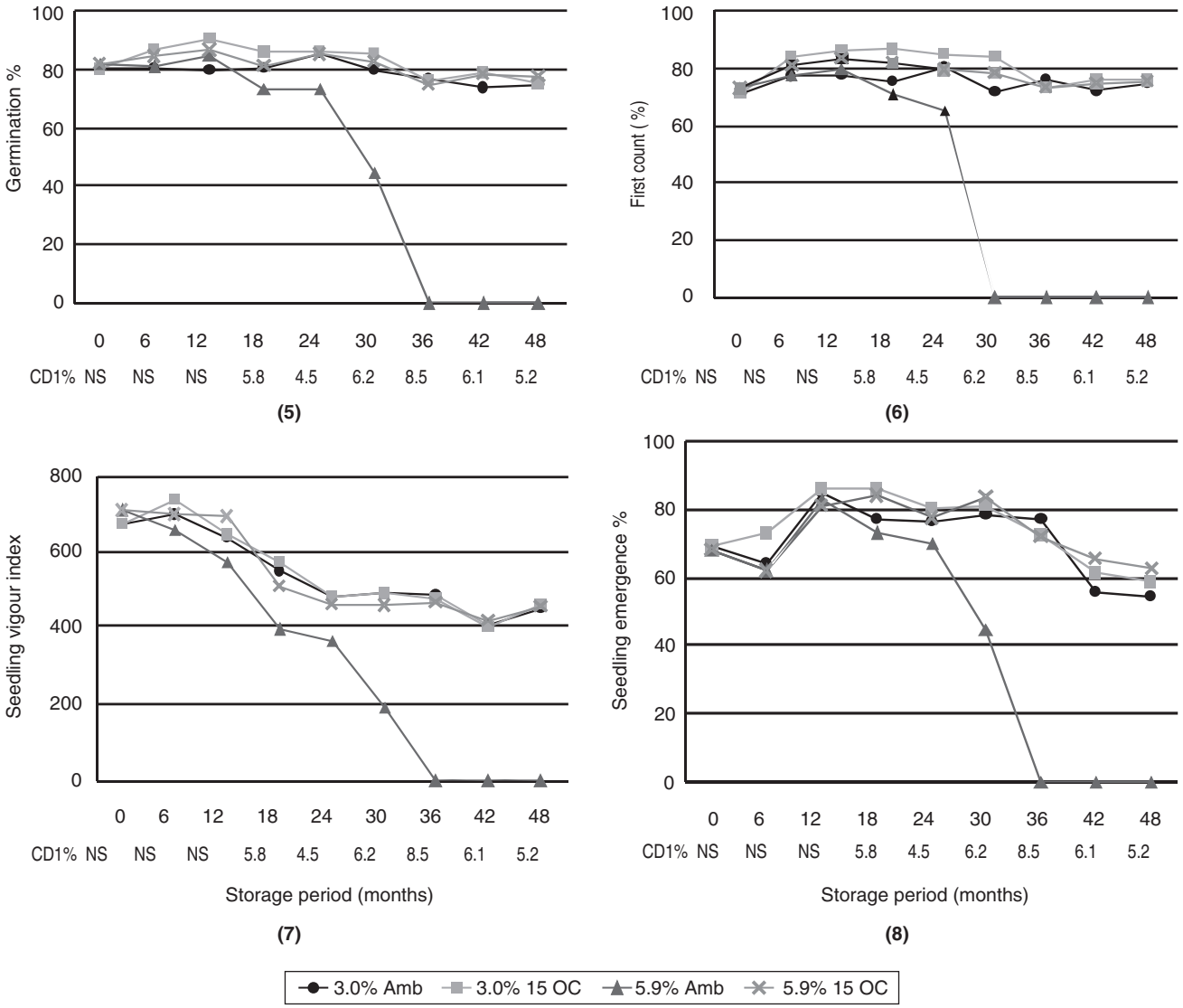

Fig 5-8 Germination, first count, seeding vigour index and field emergence as affected by seed moisture and storage temperature over 48 month of storage in China aster cv. Arka Kamini (CD values at 1% level of significance are given below X-axis {each value corresponds to storage period})

4-5 years. As this study was planned for 54 months only, the benefits of ultra dry storage in extending longevity beyond this period could not be ascertained, hence cannot be said unambiguously that ultra dry storage has no beneficial effect on longevity in onion. Nevertheless, ultra drying had no harmful effect on longevity. Seed moisture of 2.5 – 4.5% at ambient temperature was recommended for long term storage of leak, Chinese cabbage, tomato, radish, eggplant and cucumber (Zheng *et al*. 2001). However, in case of china aster it is very clear that ultra dry storage offered potential benefits in extending longevity of the seed under ambient temperatures. Ultra dry seeds of china aster maintained high viability and vigour even after 48 months. Contrarily seeds with moisture content of 5.9% showed decline in viability and vigour after 18 months and reaching zero at 36 months of storage under ambient temperature. Lettuce seed stored at room temperature with 6-7% moisture content gave 91% germination after 9 year but only 2.5% after 15 years; but seed with 2.5% moisture content gave 40% germination after 20 years (Nakamura 1975). Vigour level and antioxidant activities in ultra dry seeds of *Ammopiptanthus mongolica* were higher than control seeds indicating the beneficial effect of ultra dry storage (Yi *et al*. 2010). Seed moisture content of the ultra dry and control seeds did not change during storage with variation of + 0.5 % indicating moisture vapour proof characteristic of aluminium foil used in this study as packing material.

Doubts were expressed by Walters (1998) and Walters *et al.* (1998) pertaining to maintaining the genetic fidelity of seed that was under ultra dry storage as the main objective of germplasm preservation is to maintain genetic purity in addition to viability. Our study with onion clearly demonstrated that neither the profiles of soluble proteins and enzymes nor the DNA were affected by ultra drying. This clears the apprehension of loss of fidelity in ultra dry storage and substantiates the claims made by Zheng *et al.*

(1998) and Cheng *et al.* (1997).

Then the next question is whether the onion and china aster seed can be further dried below the levels tested in this study without affecting the viability. This question cannot be answered as we could not reduce the moisture content further using silica gel. However, in continuation of this work another study initiated with several other horticultural crops and the preliminary results of which indicated that extreme desiccation lead to no effect on viability in many species but in few starch rich species it had detrimental effect (data not published). Critical moisture contents for some species were reported previously by Ellis *et al.* (1989), Kong and Zhang (1998), Hu *et al.* (1998), Shen and Qi (1998), Mira *et al.* (2015). But in some other species ultra low moisture was neither detrimental nor beneficial to seed longevity (Ellis *et al.* 1989, Hong *et al.* 2005, Yi *et al.* 2007 and Mira *et al.* 2015). Several other studies have reported that some species have no critical water content as they were dried to even below 1% moisture level with beneficial effect on longevity under varied temperatures (Ellis *et al.* 1986, Ellis *et al.* 1996, Zheng *et al.* 2001; Yi *et al*. 2008, Tong and Hang 2009, Sastry *et al.* 2007). These studies have clearly shown that some species can withstand extreme desiccation with or without beneficial effect on longevity whereas some species show sensitivity to extreme desiccation with detrimental effects below a critical moisture level. Ultra dry seeds of both onion and china aster showed initial viability as similar to control seeds indicating desiccation tolerance to the lowest moisture content tested in this study.

The results clearly demonstrated that seeds of onion and china aster which are known to lose viability rapidly under ambient storage can be dried to as low as 2.6 and 3.0% moisture levels using silica gel, respectively without affecting seed quality. And such ultra dry seeds could be stored under ambient conditions for more than 48- 54 months without affecting seed viability, vigour and fidelity. This technique will completely avoid use of cold storage facility in short to medium term seed storage.

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