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Ultradry Seed Storage: Improved Strategy and Technology for Germplasm Conservation

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ABSTRACT

A research program on ultradry seed storage (supported by IBPGR) was carried out at the Beijing Botanical Garden from 1989～1993. Various kinds of seeds of more than 20 speices were used. Many significant rsults and findings were obtained, and some new concepts were derived from them. Based on a series of experiments, it was concluded that ultradry storage has the potential to be an alternative technique for germplasm conservation in gene banks. Advances of researches in detail were reported in this paper.

INTRODUCTION

Although cold stroage of seeds at -10℃ to 20℃ is presently the most common method for conservation of germplasm in gene banks, and the preferred -15℃ was recommended by International Board for Plant Genetic Resoures (IBPGR), its use in developing countries has been greatly limited because of the high cost for building and operating. It is obvious that mankind today is faced with a challenge for urgent and efficient conservation of biodiversity. It is imperative that a low cost gene bank be used instead of the routine cold storage.

Seed moisture content (MC) and storage temperature are the most important factors affecting seed longevity and vigor during storage. Previous work (Zheng 1980) showed that low MC could substitute for low temperature alternatively during storage of some orthodox seeds. However, the viability and genetic stability after storage of seeds dried below 4～5% have not yet been affirmed (Harrington 1973, Roberts 1972, Tao et al., 1991). The results of IBPGR supported research projects undertaken by University of Reading (Ellis et al. 1986, 1988, etc.) and Beijing Botanical Garden (Cheng et al., 1992 (b), Zheng et al., 1990 (b), 1993) have consistently indicated that the longevity of seeds of many crops, ornamentals and trees could be greatly increased by storing them under ultradry conditions (<5%MC). For example, the increase in longevity by reducing sesame seed MC from 5% to 2% was approximately the same as reducing storage temperature from +20℃ to -20℃ (Ellis et al. 1986). In China, an experiment with short-lived elm seeds has been carried out with MC reduced to <2% under non-controlled room temperature, and it was compared with seeds in conventional long term storage (-20℃) and liquid nitrogen (L N -196℃) conditions. The results

showed that no significant difference was found either in the viability level, or in the isoenzyme electropherogram patterns among the three storage methods (Tao et al., 1993). Thus, it was suggested that refrigeration of gene bank could be reduced or omitted by ultradrying the seeds before storage and they can be stored under non-controlled room temperature conditions. This information supports the possibility of establishing and operating a low cost gene bank with ultradried (UD) seeds in the near future.

EXPERIMENTAL RESULTS

More than 20 species of various seeds, including several cultivars of *Brassica campestris*, *B. oleracea*, *B. pekinensis*, *Beta vulgaris*, *Carthamus tinctorius*, *Cucumis satvus*, *Cucubita pepo*, *Glycine mex*, *Eucommia ulmboides*, *Althaea rosea*, *Arachis hypogaea*, *Asparagus officinalis*, *Oenothera orderata*, *Oryza sativa*, *Raphanus sativus*, *Sesamum indicum*, *Sorghum vulgare*, *Triticum aestivum*, *Ulmus macrocarpa*, *Populus* spp. were tested. Lots of singificant results, findings and new concepts were obtained. Twenty papers have been completed. Most of them have been published (Cheng et al. 1991, 1992, 1993, Jian et al. 1992, Lin et al. 1993, Tao et al. 1993, Zhang et al. 1992, Zheng et al. 1990, 1992, 1993 etc.).

1 Effective methods to obtain the seeds with ultra low MC

Among various methods used to ultradry seeds, using quick lime or silica gel for direct drying in sealed desiccator was the most effective, safe and economic one (Cheng et al. 1991 (b)). Based on it, an equipment for autoblowing was designed and successfully used under a proper orderly warming condition (15℃ ~38℃) (Zhang et al. 1992).

2 Effects of ultradrying on germination, vigor and storability of seeds

Experiments with oil seeds, starchy seeds and some seeds rich in protein have confirmed that ultradrying itself was harmless (Cheng et al. 1991 (a), Lin et al. 1993 (b), Zheng et al. 1992). It did not induce any significant change either in germination percent or in seed vigor. On the contrary, the experimental data indicated that ultradried seeds became more tolerant of aging under accelerated aging condition and more storable under the condition of air open storage (Cheng et al. 1991 (b), 1992 (a)). The following two outstanding examples were that the storability of elm seeds was greatly improved in terms of viability level and seedling emergence, both of which were almost the same for the ultradried seeds stored in ambient temperature, in -20℃ and in LN (-196℃) conditions (Tao et al. 1993). The viability and vigor of UD seeds of eucommia stored in the air open condition were the same as those in the cold storage (-20℃) as control for 2 years simultaneously (Zheng et al. 1993).

3 Sensitivity of some kinds of seeds to ultradrying and how to prevent it

Among the 21 species mentioned above, the seeds of soybean, rice, poplar and big fruit elm were sensitive to ultradrying. Experiments showed that morphological and cell structure damage in soybean seeds during the course of ultradrying did not occur by pretreating them with hydration-dehydration (H-DH) or polyethylene glycol (PEG) priming. This principle and practice of osmotic pretreatment were also effective to prevent rice seeds from ultradrying damage, especially by using gibberellic acid (GA) as a synergist. The two others (poplar and big fruit elm) could be effectively prevented by

PEG coating or polyvinyl alcohol (PVA) pelleting. Pretreatments with some antioxidants such as butylated hydroxytoluene (BHT) or iodination were also effective (Zheng 1992, Zheng et al. 1993).

A significant diversity of sensitivity to ultradrying between cultivars has been found. For example, the cv Heihe V (China) of soybean was more serious in comparison with the cv Fishkeby V (U. K.). Decoating experiment revealed that seed coat was the limiting factor for cracking. Another one was the rice seeds between the common cultivar and hybrid. The latter was more sensitive than the former without protective pretreatment by PEG.

4 Effective pretreatment to avoid imbibitional injury of ultradried seeds beforegermination

The imbibitional injury occurred in a wide range of crops when dry seeds were dipped directly in the water (Zheng et al. 1990 (a)). Especially, It is inevitable to ultradried seeds. Thus, the phenomenon of poor germination of ultradried seeds was often misunderstood as a direct harmful damage from the ultradrying process. Experiments confirmed that some osmotic pretreatment which was based on the membrane repair theory, including gradual moisture equilibrium, PEG priming and H-DH etc., could be successfully used to aviod the imbibitional injury in ultradried seeds (Cheng et al. 1991 (b), Lin et al. 1993 (a), Zheng 1992).

5 Cytological, physiological and biochemical events of ultradried seeds

Scanning electron microscopy (SEM) observation indicated that the ultradried cells showed morphological integrity, and no breaking of cell surface was detected. Transmission electron microscopy (TEM) observation indicated that the ultrastructure of radical cells was perfect after ultradrying. All the organelles were normally developed (Cheng et al. 1991 (b)).

The frequency of various types of chromosome abnormality in anaphase of cell division after ultradrying and that in ultradried seeds after storage were examined. It revealed that ultradrying could effectively retard the decrease of seed viability and the frequency of chromosome damage, although there were some increase in chromosome damage in UD seeds compared with control for any given level of deterioration (Zheng et al. 1993).

A series of physiological and biochemical experiments including conductivity, Na^+ K^+ ratio and Na^+ K^+-ATPase activity, respiration intensity, ability of self-defence against lipid peroxidation (superoxide dismutase (SOD), peroxides, catalase), and products of deterioration (malonaldehyde (MDA) and volatile aldehydes) etc. further confirmed the improvement of storability of UD seeds, at least in some oil seeds (Cheng et al. 1991 (b), 1992 (a) (b)).

6 Relationship between oil content in seeds and its ability of resistance to ultradry

The experiments indecated that the higher oil content in seeds the more rapid dehydration during drying. The vigor level in cultivar with higher oil content was better maintained than that with lower oil content, especially there was a threshold value at 40% oil content (Cheng et al. 1993).

Evidently, strong resistance to dry in oil seeds was due to the hydrophobic colloid as a dominant constituent of them. According to the classical rule, i. e. the lower limit of critical MC for most starchy seeds was 5%, it should be dropped to 2～3% for oil seeds.

7 Genetic stability of ultradried seeds

The experimental results of elm seeds after 6 months of UD storage (2～4% MC) under air open condition, comparing with seed of 9% MC in cold (-20℃) and LN (-196℃) conditions respectively,

have showed not only the uniformity of seedling morphology in greenhouse test but also that of the chromosome behavior of anaphase during early germination (Tao et al. 1993). There was no significant difference in the banding patterns of several isozymes and DNA polymorphism analysis between these treatments. These results demonstrated the genetic stability of UD seeds.

8 Lipid peroxidation in ultradried seeds

The peroxidation of lipids in seeds is facilitated at the lower level of MC, especially $<5\%$, becaues excessive loss of bound water may enhance the access of O_2 to the sensitive sites of membrane lipid and the other macromolecules themselves. The results of experiments with oil seeds mentioned above showed that the lipid peroxidation was slightly depressed in ultradried condition (Cheng et al. 1992 (a)). Besides, another fact was that an additional effectiveness for improving storability of ultradried elm seeds was achieved by using antioxidants or/and O_2 free storage, and a sufficient contents of natural antioxidants in some ultradried oil seeds was maintained for defending lipids and other macromolecules from the access of O_2. Therefore, it seems that the lipid peroxidation would be existent in spite of the contradictory facts which are unable to be explained yet.

CONCLUSION

On the basis of the experimental results, it is confirmed that ultradrying did not influence seed qualities, and no significant harmful effect was found in ultradried seeds. Their storability was greatly improved, which was affirmed by the physiological, biochemical and ultrastructural aspects.

The ultradried seed samples could be rapidly and safely obtained by a procedure of orderly warming dry in the autoblowing hygroscopic desiccator, specially easy in oil seeds. Its practical effect was the same as the freeze-dry vacuum. Moreover, because the nearly same effect of storability as in cold storage, its economic benefits can be very considerable. It is expected that ultradry storage would be in conformity with the IBPGR's primary objectives in seed storage that seeds could be stored at ambient temperature instead of the old routine procedure for long term storage in gene bank.

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