Advances in Ultra-dry Seed Storage

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Abstract: The research progress on seed ultra-dry storage was reviewed. The research status and existing problems on the optimum seed moisture content, the physiological and biochemical basis of the ultra-dry seed storage, the seed ultra-dry method, the ultra-dry seed to suitable humidity were clarified. In addition, perspective of research on seed ultra-dry storage has been made in this paper.

1. Introduction

Ultra-dry storage of seeds has attracted the attention of the peers in the seed industry for its high efficiency, low consumption and broad application prospects since it was explicitly proposed in 1986. Ultra-dry storage of seeds, also known as ultra-low water content storage, refers to the storage of seeds under 5% moisture content (depending on different kinds of seeds), sealed and stored at room temperature. Scholars at home and abroad have studied a large number of plant seeds such as crop, vegetable, tree. It shows that most plant seeds can improve seed vigor and storage performance by ultra-dry storage. In this paper, the research progress of Ultra-dry seed storage and some problems in its application are discussed.

2. Optimum Moisture Content of Ultra-dry Seeds

The optimum storage moisture content of seeds varies with the chemical composition of seeds and the differences among varieties. The lower the moisture content of Ultra-dry seeds, the better. Many experiments have proved that the optimum moisture content of different types of seeds varies greatly. Seeds can be divided into high oil seeds, starch seeds and protein seeds according to their nutritional components. Walters [1] et al. studied the effects of temperature and seed moisture on the aging rate of 20 common crops and vegetable seeds. The results showed that the critical relative humidity of seeds was related to the material composition of seeds. The ultra-dry storage effect of seeds with high oil content was higher than that of seeds with high protein and starch content. Yunlan Zhang et al. [2] studied the ultra-low water content of mung bean and millet. It was found that cereal seeds with more starch were more resistant to drying than those with more protein, and their lower limit of safe water content was lower. The optimum moisture content for seed storage varies with temperature. Ellis [3] believed that the optimum moisture content was obtained when seeds reached equilibrium at relative humidity of 10%-11%. The lower critical moisture content of seeds could not change significantly at different storage temperatures. Vertucci [4] considered that temperature affected the optimum water content of seeds. Lin Jian et al. [5] Stored wheat seeds with different moisture content in 50, 35, 20 and 5℃. The optimum moisture content was 4%-5%, 5%-6% and 7%-8% respectively. The optimum moisture content of wheat seeds stored at 5 ℃ was relatively wide. It was also found that the optimum moisture content of wheat decreased with the increase of storage temperature. Chenglian Hu et al. [6] studied the effects of different drying

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methods and drying rates on the viability of rice seeds with silica gel as drying agent. It was found that the optimum moisture content of Indica and japonica seeds stored at 45 ℃ was 4%-5%, while the optimum moisture content of Indica and japonica seeds stored at room temperature was 5%-7%. Yuzhu Han et al. [7] studied the effects of storage temperature and moisture content on the seed vigor of tall fescue. The results showed that both storage temperature and moisture content could affect the seed vigor of tall fescue. With the prolongation of storage period, the seed vigor of tall fescue showed a downward trend. The ultra-dried tall fescue seeds had different moisture content (1.77%-8.11%). There were no significant differences in germination rate, germination index, vigor index and seedling weight between the two groups. Hu et al. [8] found that the optimum moisture content of rice, wheat, peanut and millet were 4.5%, 5%, 2% and 4% respectively at 45 ℃. High or lower water content accelerated seed aging. The results of room temperature storage at the same time showed that too low water content had harmful effects on seeds. Kong, et al. [9] found long cowpea, chives, broccoli, green vegetables such as seeds in 40 ℃ storage after 52 months, except the leeks didn't find out the optimum water content security limit, long cowpea optimum moisture content of 6%, the rest of the seeds in the range of 2.0% - 3.0%. Therefore, it is worthwhile to explore a general and simple method to determine the minimum threshold of safe water content for different seed types.

3. Physiological and Biochemical Basis of Ultra-Dry Storage Seeds

3.1 Effect of Ultra-drying on Cell Structure.

Cell structural integrity is the basis of seed vigor. With the prolongation of storage time, seed vigor began to decrease, cell membrane structure damage, organelle and genetic material distortion and disintegration, a large number of storage materials exosmosis, various enzyme activities decreased. When the water content of seeds decreased, the arrangement of phospholipids on the cell membrane changed, and the continuous interface of the membrane could not be maintained [10]. When the water content of seeds decreases to a certain level, phospholipids and protein molecules turn away because of the loss of water film protection, and the continuous interface of the membrane is destroyed. The membrane is transformed from the mobile phase to the gel phase, and the osmoregulation function is lost by. Mingfang Zhang [11], Wang [12] and other studies found that after ultra-dry treatment, the structure and function of cell membranes were stable, and the ultrastructure of cells remained intact, thus preventing the leakage of storage materials. The results of Hongyan Cheng et al. [13] show that ultra-dry treatment may affect the configuration of the membrane system to some extent, but the effect is slight and reversible, and can be completely eliminated by appropriate pretreatment (such as backwater). Hongyan Cheng et al. [13] studied the ultrastructural changes of super-dried seed cells of Chinese cabbage. The results showed that the surface cells of super-dried seed cotyledons were contractile, but the cell morphology was intact. The ultrastructure of radicle apex meristem cells of super-dried seeds after aging was normal, while the damage of radicle apex meristem cells of non-aged super-dried seeds was serious, and the double-layer membrane of mitochondria could not be recognized. The endoplasmic reticulum is swollen, the nuclear membrane is blurred, and the bilayer membrane structure of cells is unrecognizable. It can be seen that the internal structure of seeds was not damaged and the anti-aging performance of seeds was enhanced by ultra-dry treatment with moderate water content.

3.2 Effects of Ultra-dry Storage on Antioxidant Enzyme System and Membrane Peroxidation of Seeds.

In the course of seed aging, membrane peroxidation produces many potential toxicants, such as free radicals, superoxide radicals and malondialdehyde. Free radicals can destroy the membrane system of macromolecule polymers and cells, thus causing a series of complex chain reactions, which eventually lead to seed deterioration, and the vigor of seeds also declines rapidly. Malondialdehyde (MDA) is one of the end products of lipid peroxidation. The increase of MDA content can indicate the degree of lipid oxidation in seeds. Ural Guli et al. [14] studied the ultra-dry storage of Isatis indigotica seeds. It was found that the content of MDA in seeds increased gradually with the decrease of water content of seeds. Zhao Li et al. [15] showed that the content of oxygen free radicals and malondialdehyde in ultra-dry soybean seeds increased less than that in non-ultra-dry soybean seeds, and the scavenging ability of oxygen free radicals in ultra-dry aging soybean seeds was higher than that in non-ultra-dry aging soybean seeds. Zhu [16] Studies on ultra-dry storage of peanut seeds showed that moderate drying inhibited lipid peroxidation directly or indirectly, and excessive drying promoted lipid peroxidation to a certain extent. Some studies also showed that the vigor of Ultra-dry seeds was significantly higher than that of non-ultra-dry seeds, and the maintenance of high-vigor seeds was related to the integrity of free radical scavenging system.

Antioxidant system (enzymatic or non-enzymatic protection system) is a set of scavenging active oxygen system in plants. It can maintain the level of active oxygen metabolism and the structure and function of membrane. Enzymatic protection system mainly includes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbic acid peroxidase (APX), glutathione reductase (GR), etc. [17,18]. Non-enzymatic protection system mainly refers to ascorbic acid (AsA), alpha-tocopherol (VE), reduced glutathione (GSH), etc. It also includes some secondary metabolites (such as polyphenols, tannins, flavonoids, etc.) and osmotic regulators (such as proline, betaine, mannitol, etc.), which quench reactive oxygen species directly or indirectly through multiple pathways. In the process of seed drying and dehydration, antioxidant system can prevent, tolerate or repair free radical attack on cells, and protect cell membrane from free radical damage. Zhao Li et al. [15] found that the strong anti-aging ability of ultra-dried seeds was also related to their relatively high antioxidant enzymes (SOD, POD, CAT) system. The decline of SOD, POD and CAT activities in embryos and cotyledons of ultra-dried seeds was smaller than that of non-ultra-dried seeds. Xiaoyun Duan et al. [19] compared the effects of ultra-dry storage and conventional storage on the vigor of celery seeds. It was found that the activities of SOD, POD and CAT of Ultra-dry Storage celery seeds were higher than those of conventional storage seeds, indicating that ultra-dry treatment was beneficial to remove harmful substances produced during storage and improve the storage resistance of seeds. Hongmei Sun et al. [20] found that after super-drying treatment, the AsA content of Brassica japonica seeds increased slightly compared with the control seeds. The ASA content of Ultra-dry seeds was negatively correlated with the water content of seeds to some extent. Hongyan Cheng [21] found that the content of VC and GSH in ultra-dry rapeseed seeds decreased by the same extent during storage. The content of VE in ultra-dry seeds was higher than that in non-ultra-dry seeds before and after storage. The same result was obtained in Priestley [22]. He also found that the content of reduced VE in ultra-dry seeds was significantly higher than that in non-ultra-dry seeds during the course of absorption.

It can be seen that the preservation of membrane lipid peroxidation resistance is one of the physiological reasons for the storage resistance of Ultra-dry seeds. Previously, it was thought that lipid peroxidation was aggravated by water loss in ultra-dry seeds, which led to seed deterioration. With the in-depth study of scholars, it is now believed that the structural water in ultra-dry seeds is still retained when dehydrated to a suitable low water content, while the harmful biochemical reactions with water as the reaction medium are inhibited due to the decrease of water content. Storage tolerance of Ultra-dry seeds may be the result of the combination of the positive effects of maintaining a high quality free radical scavenging system and the toxic effects of peroxidation increased by ultra-dry seeds [23]. Exploring the optimum moisture content for ultra-dry storage of seeds is still a subject for further study.

4. Drying Methods of Ultra-dry Seeds

Ultra-dry seed drying methods mainly include vacuum freeze-drying, desiccant drying, heating drying, drying with different saturated salt solutions, etc. Vacuum freeze-drying can remove water quickly, and can deal with large quantities of seeds. The effect is better within 24 hours, and the effect is slight after a long time. Moreover, the equipment is expensive and the energy consumption is high. Vacuum freeze-drying machine is suitable for treating oilseeds and small seeds, but the effect is not obvious for protein and starch seeds. Xiaoying Zheng et al. [23] compared the effects of four drying methods on ultra-dry storage, including silica gel room temperature drying, vacuum freeze dryer drying, low temperature and low humidity drying (relative humidity 30%, 12 ℃) and electric heating blast drying box heating drying (45 ℃). The results showed that only heating drying could damage seeds, and some studies showed that heating drying might damage seeds. Harm or inhibit the activity of some enzymes in seeds and reduce the resistance of seeds to germination. Different methods of saturated salt solution treatment are seldom used in seed drying due to inconvenient use. At present, the commonly used desiccants for ultra-dry seeds are silica gel, quicklime, $CaCl_2$ and so on. The drying speed of quicklime is higher than that of silica gel. Although the drying speed of silica gel lasts a long time, it causes less damage to seeds. The effects of types, proportion, drying time and temperature of desiccants on ultra-dry storage of seeds are one of the research directions in the future.

5. Rewetting of Ultra-dry Seeds

Membrane system is one of the primary sites of cell damage and death caused by dehydration. Maintaining the integrity of cell membrane system is crucial to the normal germination of seeds after ultra-dry storage[24]. Ultra-dry seeds absorb water too quickly during germination, which affects the permeability of cell membrane of seeds and causes the damage of seed imbibition. The effective way to prevent the Imbibition Damage of Ultra-dry seeds is to wet the ultra-dry seeds. Different methods are used to wet the seeds before imbibition, which can improve the selective permeability of the membrane and enhance the vitality of the seeds. Juzhen Zhi et al. [25] found that the main reason for the decline of rice seed vigor after ultra-dry treatment was the imbibition damage. Saturated water vapor humidification treatment for 2 days could significantly improve the vigor of Ultra-dry rice seeds. Yushan Zheng et al. [26] studied the effect of osmotic adjustment treatment with different concentration of PEG and different treatment time on the viability of Ultra-dry seeds of Cunninghamia lanceolata, Pinus massoniana and Pinus thunbergii. The results showed that different PEG concentration and treatment time had different effects on seed vigor. Osmotic adjustment treatment with 20% PEG could significantly improve the germination rate and vigor of super-dried Chinese fir and Pinus massoniana seeds. The lower the moisture content of seeds, the more obvious the effect of rewetting treatment.

In conclusion, ultra-dry storage has great potential in Germplasm conservation. Although scholars at home and abroad have done a lot of research on ultra-dry storage of seeds and made important progress, there are also some controversial issues, such as the limit water content of seed dehydration and the most suitable storage temperature, the transformation of genetic integrity of Ultra-dry seeds, the mechanism of controlling the toxicity of free radical reactive oxygen species in ultra-dry seeds during storage, and the repair mechanism of Ultra-dry seeds after imbibition. The problem remains to be further studied.

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