云 南 植 物 研 究 2007, **29** (1): 43~50
Acta Botanica Yunnanica

植物遗传资源的种子基因库保存*

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摘要: 一个物种的灭绝是与其受生物因子和非生物因子的威胁程度相关的。随着物种的加速绝灭, 保护生物多样性受到广泛地关注。保护生物多样性的最有效的生物技术之一是建立种子基因库, 进行迁地保护。种子库理想的贮藏条件主要取决于种子含水量、贮藏环境 (如温度和湿度) 和贮存种子的容器。进行种子贮藏, 了解种子生命力和活力的影响因子的作用机理是十分重要和必要的。除了种子自身的生理特征外, 种子的贮藏寿命与种子成熟度、收获技术、加工处理方法也是息息相关的。即使在最适的库存条件下, 种子也会随时间发生劣变。因此, 必须根据种子特定的贮藏行为, 加以考虑影响种子存活的 3 个主要方面 (贮藏环境、贮藏期和植物种类) 而选择有效的贮藏方案。本文试图讨论种子贮藏生理的几个重要方面及其需解决的技术问题, 以便更好地通过种子基因库, 长期有效地保存植物种质资源。

关键词: 生物多样性; 种子库; 植物种质; 种子贮藏行为; 种子生命力与活力; 贮藏寿命
中图分类号: Q 948 文献标识码: A 文章编号: 0253-2700 (2007) 01-043-08

Ex Situ Conservation of Plant Genetic Resources through Seed-Gene Bank

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Abstract: With the acceleration of extinction of species, biodiversity conservation is extensively concerned. The extinction of species is concerned with the degree of threat by biotic and abiotic factors. So, taking action to preserve plant species is very necessary and paramount before their extinction. One of the most effective biological techniques to conserve the biodiversity is the establishment of genebanks, i. e. *ex situ* conservation. The elucidation of various factors that regulate seed viability and vigor in storage is essential. An ideal condition to prolong the longevity is mainly depended on seed water content, temperature, humidity and types of containers used during storage. The optimum stage of seed maturity, harvesting techniques and processing, in addition to physiological features such as degree of dormancy, also play key roles in seed storage. Certainly, desiccated seeds deteriorate with time even under extremely good genebanking conditions. According to seed storage behavior, it is necessary to consider three principal factors: storage environment, storage duration and plant species which will affect seed survival under good genebanking conditions. The present review is an attempt to discuss the importance of the aforementioned aspects of seeds in detail in order to conserve plant germplasms (especially wild rare and endangered plants) for *ex situ* conservation through seed-gene bank.

* 基金项目: 中国科学院知识创新工程重要方向性项目 (KSCX2-SW-117)、科技部国家科技基础条件平台工作项目 (2004DKA30430) 和 (2005DKA21006)

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Received date: 2006-03-10, Accepted date: 2006-04-30

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Key words: Biodiversity; Seed bank; Plant germplasm; Viability and vigor; Storage behavior; Storage longevity

That biodiversity is sharply decreasing is beyond dispute. In many regions, especially, tropical regions, complex and species-rich ecosystems, are being rapidly destroyed or changed, and some fragile environments, such as wetlands, arid and semi-arid regions, are threatened by the increasing stress from human disturbance and fluctuating climates. At the current rate of deforestation, extinction of species must be imminent. Moreover, a considerable amount of genetic diversity within plants that can survive is likely to be lost. Fragmentation of habitats is bound to affect the fate of plants, especially, rare and valuable plants. If some plants in fragmented habitats may be reduced to such low numbers that they cannot constitute viable populations. In such populations, genetic drift and inbreeding may result in inbreeding depression. Thus, a combination of demographic and genetic factors may hasten the extinction of some plants in small, isolated fragments. Generally, extinction is a consequence of the mutually accelerating demographic and genetic decline of a population. Appropriate scientific information is required to design an effective conservation plan for any threatened species. There has been a general recognition in recent years that the genetic variation present in a kind of species is a very valuable resource for genetic engineering (Lu, 1998). The loss of genetic diversity represents a type of partial extinction that often presages its total extinction. Conservation and evolutional inferences about any impacts of environmental changes on plant populations should consider not only both demographic and genetic processes, but also the complicated interactions of these processes (Reed *et al*. 2002). So it is necessary to understand the certain biological features of species to find out the causative factors which lead to reproductive and regeneration failure. These days, there is a general awareness that is necessary and crucial to conserve natural plant resources worldwide. Studies have shown that many plant species are in danger of extinction, while some have already become extinct. On a global basis, the global red list, 2000 IUCN Red List of Threatened Species (SSC, 2000), includes 5611 threatened plant species. In compiling the red list, only approximately 2% of an estimated vascular plant flora of 270 000 species worldwide was assessed (SSC, 2000). Thus, the proportion of species listed as threatened is more than 20% of species that were assessed (SSC, 2000). Especially, there are many plants distributed only in single country or a special region. Thus, in order to build a relatively small amount of relevant work on rare and threatened (plants) species priorities should be determined so as to make conservation strategies. (http://www.wri.org/biodiv/in-situ.html).

Facing the conservation biodiversity of a problem, conservation biologists are scrambling to devise methods and techniques for plant species protection and preservation. One of most goals of many preservation programmes, in addition to habitat preservation, is maintaining the existing level of genetic variation in species. Presently, there are two strategies commonly used in conserving genetic resources. One is *in situ* conservation, and the other is *ex situ* conservation (Cheng, 2005). In reality, we think, both *in situ* conservation and *ex situ* conservation are complementary and should not view as alternatives. On the contrary, this complementary conservation should be described as *inter situ* conservation.

In agriculture, most crop species are conserved by *ex situ* accesses, such as seed-gene banks, field-seedling banks, tissue culture and cryopreservation gene-bank. However, in forestry, because of the long regeneration time required by trees, the perfect conservation approach is likely to incorporate *in situ* conservation principles into sustainable using and management. But, if *in situ* conservation is difficult for some tropical ecosystems, *ex situ* conservation through seed-gene bank will become an effective approach. To fulfill the goal, we need basic knowledge on seed biology and technology such as seed mature index, seed harvesting, processing, germination, dormancy, viability, vigor and storage physiology for various seeds. Though much knowledge of different seeds has been documented, it is still quite inadequate. This article focuses on all the above aspects of seeds of temperate, subtropical and tropical plant species.

1 Categories of Seed Storage Behavior

Seeds are the most convenient forms by which to store and distribute plant germplasm. In other words, seed storage plays a complementary role in germplasm conservation in sustainable development of social economy. To fulfill the conservation roles, seed-storage life must be maintained in a viable condition from the time of collection until the time of the next generation. Successful long-term storage is depended on continuous viability monitoring with re-collection or regeneration, whenever the viability drops to a limited minimum level. The longevity of seeds varies from species to species, though they are provided identical storage conditions. Practically longevity of seeds in storage depends on their sensitivity to temperatures and tolerance to desiccation.

In the 1970s, Roberts (1973) divided seeds into two major groups, i.e. orthodox and recalcitrant seed depending on their inherent nature. In other words, "orthodox" and "recalcitrant" are used to describe storage behaviors of different seeds. Orthodox seeds can be dried without damage to low moisture contents and, over a wide range of environments, their longevity increases with decrease in seed storage moisture content and temperature in a quantifiable and predictable way (Roberts, 1973; Ellis, 1988; Dickie *et al*. 1990). The latter is defined by the seed viability equation:

$$V = K_i - p/10^{K_E - C_w \log_{10} m - C_H t - C_Q t^2}$$

Where V is probit percentage viability after p days in storage at m percent moisture content (w.b.), t ℃, K_i is a constant specific to the seed lot, and K_E, C_H and C_Q are species viability constants (Ellis and Roberts, 1980; Cromarty et al. 1982). Seeing from the above equation, many researchers have found that the longevity of orthodox seeds varies with different seed water content and temperature.

Generally, orthodox seeds acquire desiccation tolerance during development and may be stored in the dry state for predictable periods under defined conditions. Unless debilitated by zero-tolerant storage fungi, orthodox seeds should maintain high vigor and viability from harvest until the next growing season. Over the years, the techniques, such as ultra-dry storage, for conserving orthodox seeds have been developed (Cheng, 2005). These involve drying seed to low moisture content (MC) (3–7% fresh weight, depending on the species) and storing them, in hermetically-sealed containers, at low temperature, preferably at −18 ℃ or cooler (FAO/IPGRI, 1994). These procedures have been widely adopted by seed-banks worldwide.

Many species of tropical, subtropical or temperate origin have seeds that are sensitive to drying and chilling and cannot be stored in conventional genebanks. Seeds of this type are termed recalcitrant seeds (Roberts, 1973; Berjak and Pammenter, 2001). Recalcitrant seeds aren′t equally sensitive, and the variable degrees of dehydration are tolerated depending on the plant species. This implies that the processes and mechanisms that course desiccation tolerance are variably developed or expressed in the nonorthodox conditions. Differential desiccation sensitivities among recalcitrant seeds of various species are clearly shown by their different responses when subjected to the same drying regime (Farrant *et al*. 1989; Berjak and Pamenter, 2001). Thereby, the recalcitrance of seeds can be also classified into three types (although be further subdivided): minimal, medium and high recalcitrance make up a continuum of seed recalcitrance (Farrant *et al*. 1988). As is known, the response to dehydration depends upon the metabolic activity of the seed and the rate of drying. Thereby, this makes it difficult to measure desiccation tolerance—there is no such thing as "critical water content" that is characteristic of a species (Pammenter and Berjak, 2000). Based on it, Song et al. (2003) suggested a new working approach be to quantify the degree of seed recalcitrance that does not depend on an absolute specific water content related to desiccation damage but depends on storage lifespan of seeds. If seeds of this type are stored under conditions by means of traditional methods, their life spans are limited to a few weeks, occasionally months (Berjak and Pammenter, 2001).

In the 1990s, seeds of woody plants were again classified into four categories based on the length of their viability and tolerance to freezing temperature, i.e. true orthodox, sub-orthodox, temperate recalcitrant and tropical recalcitrant (Bonner, 1990). Temperate recalcitrant seeds of plants from genera such as *Quercus* cannot be dried at all, but they can be stored for several years at near-freezing temperatures. By maintaining high water content and necessary gas, seeds of *Quercus* species can be stored for 3–5 years (Bonner, 1996). But, given the same environment, tropical recalcitrant seeds such as *Dipterocarpus* (Yap, 1986; Tompsett, 1987) and Jackfruit (Chin and Roberts, 1980) cannot survive.

Besides, a third seed storage behavior had been identified as intermediate seed (Ellis *et al*. 1990, 1991). Seeds of many plants show intermediate storage behavior, surviving desiccation to fairly low moisture content, but suffering injury due to low temperature. In comparison with truly recalcitrant seeds, partial drying can prolong the storage life of these intermediate seeds, but it remains impossible to achieve the long-term conservation, which has been realized for orthodox seeds. So the long-term maintenance of viability of intermediate seeds resembling recalcitrant seeds is a vexed problem, provided the storage environment is defined well and controlled. Therefore, if an accession of seeds of a particular plant is to be conserved, it is necessary to determine whether these seeds show orthodox, recalcitrant or intermediate storage behavior. At present, however, the information of seed storage behavior is fairly inadequate. Remarkably, the same plant species of some genera that are distributed in different regions have many individuals impacted by different ecological factors for many years. Thus, they can produce different seeds with different features, in particular, their storage behaviors. In other words, seeds of the same species from different provenances may show diverse storage behavior (Hong and Ellis, 1998).

To summarize, before being stored in seed banks, storage behaviors of seeds collected have to be identified according to complex physiological properties. Only after seed storage behavior is confirmed, can suitable methods and technologies be selected. For storage techniques, besides ultradry storage and low-temperature storage, cryopreservation is thought of as a perfect approach, but how to carry it out successfully for recalcitrant seeds is still a vexed problem (Walters *et al*. 2004; Panis and Lambardi, 2005).

2 Seed maturity, viability and vigor test

High quality seed is a prerequisite for higher and reliable yield of crops and establishments of healthy seedlings for forestry. Don′t forget, seed maturity is close related to seed viability and vigor. The mature stage of seeds has a critical effect on seed vigor. Generally speaking, vigor of mature seeds is higher than that of half-mature and immature seeds. So collection period for conservation purposes should be ideally selected. Only in this way, can seed vigor and viability tested reflect truly the quality of seed lots.

Under certain environments, it is impossible to estimate the viability of seeds by a standard laboratory germination test. Seed researchers have been interested in indirect methods of assessing the viability of seeds without the necessity of a routine germination test, particularly when dealing with the deep dormant seeds or seeds requiring a rather long period for the completion of germination. Indirect tests can be performed within a few hours and are thus a great favor in cases where results of the tests are required as soon as possible. The triphenyl tetrazolium choride (TTC), electrical conductivity of seed leachates, excised embryo test, X-ray cutting test, electrical impedance spectroscopy (EIS) and fluorescein diacetate (FDA) are some of the indirect, reliable, routine viability tests (ISTA, 1993). In the past, the results of storage research were evaluated primarily in terms of germination and/or viability percentage. Now, all well-planned storage works incorporate some type of vigor test as an integral part of the evaluation. It is worth pointing out the value of seed leachate conductivity. Loss of viability and increase in seed leachate conductivity indicate that the changes in thermodynamic properties of seed water reflecting the seed deterioration during storage under accelerated ageing conditions (Krishnan *et al*. 2004).

The importance of vigor as an important aspect in seed quality is clearly indicated by the trends in recent seed storage research. Loss in vigor can be thought of as an intermediate stage of seeds, occurring between the onset and termination of ability of germination. Presently, no general accepted and satisfactory method has been found to measure the vigor of a particular plant, but some vigor test methods have been used for different purposes. These methods include germination value, accelerated aging test, cool temperature test, germination rate, mean germination time, excised embryo test, and germination index (Song *et al*. 2005). Except for methods mentioned, liquid nitrogen quick test is also a good tool (Becwar *et al*. 1983). In short, all the methods play key roles in testing physiological quality of seeds, especially, in testing their viability during long-term storage.

3 Seed germination and pretreatment

The standard for judging seed quality is always a germination test under suitable conditions. Moisture content, temperature, media and light are the critical factors affecting seed germination. Optimum temperature varies with ecotype; seeds are biochemically active at this temperature above and below which any fluctuation retards the rate of biochemical activity, which in turn results in inhibition or slowing of the germination rate (Kebreab and Murdoch, 1999). Similarly, the light and media requirement for optimum germination percentage varies with plant species. Corbinean and Côme (1982) found that the intensity and duration of light at various temperatures had profound effects on the germination of *Oldenlandia corybosa* L. seeds. During the past years, considerable progresses have been made on the quantification of germination responses to temperature and the model of thermal time of seed germination has developed (Moot *et al*. 2000). Seed germination is to calculate the thermal time (Kebreab and Murdoch, 1999; Alvarado and Bradford, 2005). Besides, several researchers showed that the cardinal temperature and thermal time for the rate of germination depend on species and may vary significantly among genotypes (Mohamed *et al*. 1988). Determination of the cardinal temperature and thermal time for seed germination rate will facilitate conservationists or seed-gene bank managers to select a suitable sowing season and agro-climatic zone for introduction of plants species in field for regeneration and as *in situ* conservation stand.

In some mature seeds of woody trees or crops, seeds fail to germinate promptly even under the optimum germination conditions. The absence of germination of an intact viable seed under favorable germination conditions within a specified time lapse is termed as dormancy (Bewley and Black, 1984). Seed dormancy was divided into three types depending on how each of them arises: viz., as innate, enforced and induced (Harper, 1977). Baskin and Baskin (2004) brought forwards a current classification system, including physiological dormancy, morphological dormancy, morphophysiological dormancy, physical dormancy, and combinational dormancy. Of course, the dormant conditions vary even within a species, depending on the differences between individuals, location, climatic conditions, time of collection, as well as nature and duration of seed storage after collection. Depending on the dormancy type and its degree, the pretreatment is different from species to species. The most common requirements are exposure to periods of warmth and/or cold, soaking of in hot and cold water. Generally, there are three types of stratifications, which include warm-moist stratification, cool-moist stratification and warm-moist-cool-warm stratification (Steadman, 2003; Baskin and Baskin, 1991). In other cases of hard seeds, soaking in concentrated sulfuric acid and scarification are also effective methods for breaking dormancy of hard seeds (Nasreen *et al*. 2002; Morris *et al*. 2000). In addition, a biochemical change controlled by the interaction between the inhibitor and growth promoter does have a major role in actual breaking of dormancy (Khan, 1977; Duan *et al*. 2004; Rinaldi, 2000; Jacobsen *et al*. 2002). Currently, it is recognized that smoke-stimulated germination is not limited to species from fire-prone habitats, and a variety of species from fire-free habitats also respond positively (Light and van Staden, 2004). As are aforementioned aspects, knowledge of optimum germination and pretreatment conditions is essential prior to routine viability test during seed storage, even before being sowing.

4 Conclusion

While scientific and sociopolitical communities around the world are aware of the natural and economic importance of biodiversity, we are facing with an ever-increasing number of plant species under threat of extinction. Conservation is thus a vital part of the plant scientist′s work, in the field, in the botanic gardens, in institutes and in universities.

The diverse genetic information of plants is scientific material in the era of genetic engineering, as well as material base of mankind. But, large-scale destroying of forests and wetlands are accelerating species extinction, so conserving them through in situ conservation and ex situ conservation is highly necessary for social economic sustainable development. Moreover, we feel strongly that it is high time to explore the storage physiological knowledge for their cost-effective long-term conservation. Here, we recommended that efforts should be made to develop post-harvest technology for proper handling of various seeds. At the same time, we must understand the relationship between seed vigor and desiccation tolerance. Studies of the premature harvest on seed vigor and viability suggest that maximum desiccation tolerance is achieved step by step (Galau *et al*. 1991). Indeed, desiccation tolerance is acquired continuously during seed maturation, many orthodox seeds acquire maximum vigor and viability after maximum dry matter accumulation, at time, in dry storage (Demir and Ellis, 1992; Welbaum and Bradford, 1989). Recalcitrance appears to be a product of either postvascular separation (PVS) stage or an early termination of development (Finch-Savage, 1992).

According to their storage behaviors, seeds collected are classified into a certain category. Once seeds of a particular species are classified, it is essential to develop complementary strategies for their conservation according to their storage physiology. For instance, ultradry of orthodox seeds and cryopreservation of various seeds can offer effective and economically viable alternatives for long-term *ex situ* germplasm conservation through seed-gene bank. Although the technology of seed ultradry storage is promising in plant germplasm conservation, its application in seed banks has still many challenges such as various safe water contents and methods of their obtaining. In addition, plant cryopreservation technologies have been evolving rapidly, opening the door to the possibility of long-term storage of valuable genetic resources of many plant species, but there are two important aspects (freezing safe water content and procedures) to be solved in academic studies. Perhaps additionally, the damaging consequences could be realized once seeds are removed from either cold- or cryo-storage. As observed by Benson and Bremner (2004), Levitt (1962) originally hypothesized that four potentially injurious phases exist in freezing injury: the moment of freezing; in the frozen state; the moment of thawing; and during the post-thaw period. Of course, to facilitate the development of even more efficient cryopreservation protocols, a better knowledge of the physio-chemical background of cryopreservation is needed. This can only be unraveled through fundamental studies that involve both thermal analysis and a thorough examination of the different parameters that can influence the cryo-behavior, like endogenous sugars, membrane composition, oxidative stress and cryoprotective proteins. So it is essential for seed biologists to investigate these parameters for different seeds, particularly recalcitrant seeds.

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