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Loss of Seed Viability and The Induction of Genetic Damage in Aged Seeds

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SUMMARY

There is a close relationship between loss of seed viability during storage and the induction of genetic damage, which is evaluated in two categories, chromosomal aberrations and phenotypic mutations.

Historical background of this relationship was given in a wide range of species and, especially, recent studies in this field were also reviewed in detail to update the knowledge on some conflicting aspects.

Key words: Seed viability, chromosomal aberrations, phenotypic mutations.

ÖZET

Yaşlanmış Tohumlarda Canlılık Kaybı ve Genetik Bozulmanın Teşvik Edilmesi

Depolama esnasındaki tohum canlılığının kaybı ile kromozomal bozulmalar ve fenotipik mutasyonlar olarak iki kategoride değerlendirilen genetik bozulmanın teşvik edilmesi arasında yakın bir ilişki vardır.

Bu ilişkinin tarihsel geçmişi geniş bir tür aralığında verilmiştir. Özellikle, bazı tezat görüşler üzerine bu bilgilerin güncelleştirilmesi için bu alanda son yıllarda yapılan çalışmalar da detaylarıyla incelenmiştir.

Anahtar sözcükler: Tohum canlılığı, kromozomal bozulmalar, fenotipik mutasyonlar.

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INTRODUCTION

The term "genetic damage" is used to designate two types of damage, chromosomal aberrations and gene mutations. Previously, following Kostoff (1935) reviewers have suggested that the history of induction of genetic damage in seeds as they age began with de Vries (1901). He observed a high proportion of mutant phenotypes in five-year old seeds of *Oenothera erythrosepala,* and pointed out that the mutant seeds have greater longevity. However, Priestley's (1985) review has clarified the view that mutants observed by de Vries were not actual gene mutations but manifestations of trisomics, polyploids or other complex genetic segregations peculiar to *Oenothera.* Therefore, the historical pioneers of the discovery that genetic damage accumulates as seeds age are Navashin (1933a, b), working on seeds of *Crepis tectorum,* and Peto (1933), investigating seeds of *Zea mays.* Both researchers found that high frequencies of visible chromosome aberrations occurred in roots produced from old seeds.

It is now well known that there is a correlation between loss of seed viability during storage and the accumulation of chromosome damage. Some of the damage is microscopically visible and some, like point mutations, is not but is manifest in subsequent generations as heritable phenotypic mutations (Roberts, 1988).

1. CHROMOSOMAL ABERRATIONS IN AGED SEEDS

Since Peto (1933) and Navashin and Gerassimova (1936), the work on the accumulation of chromosome damage with period of seed storage has been confirmed in a wide range of species. These are briefly described below.

Navashin and Gerassimova (1936) found an increased frequency of chromosome aberrations assessed by cytological observations, in root-tips from old seeds of other species of *Crepis,* i.e. *Crepis dioscoridis* and *Crepis capillaris.*

In work on several varieties of onion, Nichols (1941) pointed out that the frequency of chromosomal aberrations increased with an increase in the age of seed and a decrease in germinability, in root tips at first mitosis.

Increased frequencies of chromosomal aberrations due to seed ageing were reported for *Nothoscordum fragrans* (D'Amato 1948) and for pea (D'Amato 1951).

Gunthardt et al. (1953) found that the frequencies of chromosomal aberration (chromosomal bridges and fragments in mitotic anaphases of root-tip cells) increased with the age of the seeds of common wheat, durum wheat, barley, rye and peas.

Harrison and McLeish (1954) and Harrison (1966) confirmed that seed ageing promotes the accumulation of chromosomal aberrations in lettuce seeds stored up to five years. However, they also reported that the frequency of aberrations was poorly correlated with decreasing germinability in six-year old onion seed lots.

Abdalla and Roberts (1968), working with seeds of barley, broad bean and pea, found that the frequency of aberrant cells (anaphase figures with bridges and/or fragments per anaphase cells observed) increased with seed ageing. When the percentage aberrant cells was plotted against seed viability the response curves were essentially identical for each storage condition. Therefore, they concluded that as the percentage viability decreased the percentage of chromosomal abberrations increased. Recently, this conclusion was confirmed by Murata et al. (1980, 1982, 1984) and Dourado and Roberts (1984a) in aged barley seeds, and by Dourado and Roberts (1984a) and Sivritepe (1992) in aged pea seeds.

Furthermore, as the first attempt to investigate the validity of this relationship in land races, Sivritepe (1992) has shown that there is also a negative relationship between loss of viability and accumulation of chromosome damage in land race pea seeds. This relationship was examined within the range of 96.5 % and 21 % normal germination in cultivars, and 100 % and 20 % normal germination in land races. In addition, these results are also compatible with Dourado and Roberts (1984a), showing that significant increases in the percentage of aberrant cells (during the first mitotic divisions) can occur in association with very small losses of viability.

However, the seeds of land race peas showed greater longevity compared with the cultivated pea seeds. This might be due to the higher initial seed viability (i.e. greater Ki value) and or different genotypic characteristics (which tolerate adverse storage conditions) of land races. The results of the IBPGR sponsored project (by the Boyce Thompson Institute, Cornell University, USA, the National University of Mexico and CIMMYT, Mexico) on genetic control of storage characteristics in pea suggest that the storage life of the seeds produced from white flowered plants is shorter than those of coloured-flower plants (IBPGR, 1991). Similarly, phenotypic observations carried out by Sivritepe (1992) show that cultivated pea plants produce white flowers whilst land race pea plants produce pink flowers.

Previously, Rao (1986) concluded that in lettuce, the frequency of chromosomal aberrations in the surviving seeds in relation to a given loss of seed viability, increased with decrease in the seed moisture content (within the range

5.5-13.0 %). However, Sivritepe (1992) has shown that there is no significant difference between high and low moisture content pea seeds in terms of the accumulation of chromosomal aberrations at an identical viability level, i.e. low moisture content seeds do not exhibit a greater frequency of aberrations than high moisture content seeds of equivalent viability. Land race pea seeds showed the same trend to that of pea cultivars. Rao (1986) also concluded that lipid peroxidation-mediated-free radical injury might be mainly responsible for the increased chromosome damage at low moisture contents. However, since pea seeds are high in starch (52 %) and low in lipid (6 %) content (Bewley and Black, 1985), unlike lettuce seeds, it is probable that lipid peroxidation-mediated-free radical injury in pea seeds is not as effective as in lettuce seeds. Therefore, this might be a reason why the negative relationship between seed moisture content and the amount of chromosome damage for a given loss of viability found in lettuce seeds did not occur in pea seeds.

The findings of Sivritepe (1992) are compatible with those of Abdalla and Roberts (1968), working with barley, broad beans and peas, in that for a given percentage survival of seed, the mean frequency of aberrant cells in the survivors is the same, irrespective of how rapidly the loss of viability occurred, or what combination of environmental conditions led to it. They showed this relationship in pea seeds under different storage conditions, i.e. storage temperatures between 25 and 45°C, and seed moisture contents between 13.0 and 18.3 %. Sivritepe (1992) also suggests that in cultivated and land race pea seeds this relationship also applies to storage temperatures varying between 50 and 65°C, and seed moisture contents between 4.7 and 15.1 %.

Moreover, Roberts et al. (1992) indicated that in barley seeds ultra dry storage results in less rapid chromosomal damage than dry seed storage while the frequency of chromosomal aberrations accumulated in the former is greater than that in the latter for a given loss in viability.

Thus, there is now no doubt that the production of chromosomal aberrations during ageing is related to loss of seed viability but the relationship at ultra low moisture contents needs further work.

2. PHENOTYPIC MUTATIONS IN AGED SEEDS

In seed ageing studies, chromosomal aberrations have received the most attention because of their ease of detection. Another advantage is that they are seen immediately after germination, at the first mitotic divisions of the root tips. There are several indications that point mutations, particularly those involving

recessive genes, accumulate as seeds age but they can only be detected in the following generations. Besides, large number of progenies have to be observed to detect significant differences between aged and control seeds. Two types of study have been commonly investigated in this situation: pollen abortion analysis and the observation of chlorophyll mutant phenotypes (Roos 1982, Priestley 1986, Roberts 1988).

Pollen viability is one of the best ways for detecting age-induced point mutations. Cartledge and Blakeslee (1933, 1934) were the first to demonstrate that pollen infertility in A_1 plants of jimson weed arose because of point mutations as well as chromosomal aberrations. Furthermore, they showed that these defects were promoted by increased temperature and hydration during storage of the seeds from which the A_1 plants were grown (Cartledge et al. 1936). These conclusions have generally been confirmed by later studies, e.g. in rye-grass (Griffiths and Peglar 1964), barley, broad beans and peas (Abdalla and Roberts 1969), pea and wheat (Purkar and Banerjee 1979, 1983). However, Murata et al. (1984) were unable to confirm that pollen infertility was linked to seed ageing in barley.

Recessive chlorophyll mutations induced by seed ageing have been a convenient subject of study because of their distinctive appearance in the A_2 generation (Gunthardt et al. 1953, Floris and Meletti 1972). However, only a few mutations produce obvious phenotypic changes, in particular those affecting pigmentation or gross morphology. Thus, since only a small range of mutations are easily observed, it is necessary to examine large numbers of plants to detect these. Abdalla and Roberts (1969) have presented extensive analyses of ageing induced chlorophyll mutations, in barley, broad beans and peas. They concluded that under conditions leading to a 50 % loss of viability in A_1 seeds, approximately 1 to 3 % of the surviving A_1 plants yielded A_2 seeds segregating for chlorophyll mutations. Dourado and Roberts (1984b), working with peas and barley, confirmed and extended these conclusion by examining segregation of A_3 progenies. They found that heritable mutations are induced by seed ageing, and even small losses of viability of a few percent are also associated with significant increases in the frequency of point mutations. More recently, Rao (1986) examined the relationship between loss of seed viability and induction of phenotypic mutations to establish if especially large amounts of mutations occur in the storage of very dry lettuce seeds. Although increases were observed, they were not statistically significant. Dourado and Roberts (1984b) suggested that it is impossible to avoid the problem entirely when storing seeds for genetic conservation. Even before seeds are stored some deterioration will inevitably

have taken place. Such observations support the view that for genetic conservation, seeds should be stored under conditions which minimise loss of viability.

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