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Responses to dehydration and conservation of the non-orthodox seeds of *Warburgia salutaris*

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Seeds from naturally-ripened fruits of *Warburgia salutaris*, for which viability retention on slow air-drying has not been achieved, survived relatively rapid dehydration in silica gel to water contents at which they could be cryopreserved by immersion in liquid nitrogen. Subcellular events during natural seed maturation were attenuated when premature fruits were harvested and stored, perhaps explaining improved responses to desiccation and cryopreservation presently reported for mature seeds. However, the desiccated mature seeds did not survive for more than a few weeks when stored at 16°C or 25°C, and lost viability far more rapidly at 6 ± 2°C, revealing their chilling-sensitivity. Seedlings were readily established from seeds retrieved from cryostorage and germinated in bottom-heated sand-beds at 25°C.

Introduction

Warburgia salutaris (Bertol. f.) Chiov. (syn. *W. ugandensis*; *W. breyeri*), the only African genus of the Canellaceae, is unarguably among the most endangered plant species in South Africa for two main reasons: the lack of seedling recruitment, and the non-sustainable level of bark stripping which has all but exterminated mature trees in the wild. Lack of seedling recruitment stems from the fact that developing fruits are heavily predated by an (unidentified) insect larva, so that mature seeds can be obtained only following special precautions, as described below. Also, in the unusual event of mature seeds being formed without human intervention, these lose viability after being extracted from the fruit as their originally high water content gradually equilibrates with ambient relative humidity (Kioko *et al.* 1993, Mbuya *et al.* 1994, personal observations).

The bark of *W. salutaris* contains a variety of sesquiterpenoid dialdehydes (Warthen *et al.* 1983, Taniguchi and Kubo 1993), with collective anti-feedant and molluscicidal activity (Hutchings 1996, Clark and Appleton 1997) and broad anti-fungal efficacy (Taniguchi and Kubo 1993). Bark preparations are therefore used for a wide variety of health problems (Watt and Breyer-Brandwijk 1962, Hutchings and Van Staden 1994, Hutchings 1996), which is the basis of the demand for the product in traditional medicinal practice. This has led to the over-exploitation of *W. salutaris*, and its endangered status, despite the species being protected by law in South Africa (Scott-Shaw 1999). The status of *W. salutaris* in Zimbabwe was recorded as 'locally extinct' as a result of its over-exploitation, although some amelioration of the situation has been brought about by the importation of rooted cuttings (from a re-introduction project in South Africa), and their establishment in the home gardens of local farmers (Cunningham 2000).

Faced with the impending extinction of *W. salutaris* in South Africa, which meant the irretrievable loss of its sought-after resources, a sustainable alternative was envisaged by the production of plants via cuttings from as broad a geographic spread of the limited number of mother plants within the country, as was possible (Cunningham 2000). While this project was successful, it could not satisfy *ex situ* conservation ideals for immediately-available planting stock representing a broad genetic base, as is facilitated by seed banking. This situation is exacerbated by the ever-present problem that mature seeds would still not be available, because of the unsolved predation problem and that, even if seeds could be nurtured to maturity by human intervention, apparently they could not be stored.

However, in a seed-screening project jointly sponsored by the IPGRI (International Plant Genetics Resources Institute, Rome) and DANIDA (The Danish Agency for Development Assistance), it emerged that, if dried relatively rapidly, the water content of the seeds of *W. salutaris* of Kenyan provenance could be reduced to surprisingly low levels, without immediate loss of viability (Pritchard and Daws 1997). This was followed by work showing that 30% of desiccated seeds (from the same provenance) survived after cryopreservation in liquid nitrogen (Kioko *et al.* 1999, 2000).

While cryopreservation slows metabolism in the explants to unmeasurable levels (Kartha 1985) and is reported to maintain genetic fidelity (e.g. Fretz and Lörz 1994,