Abstract

The critically endangered *Protea roupelliae* ssp. *hamiltonii* persists as a single population (of 124 individuals in 2005) within the 26ha Dr Hamilton Reserve in Mpumalanga province, South Africa. Between 2000 and 2005, no recruitment had been observed. In order to aid the recovery of this species, aspects of its reproduction and conservation were studied, namely; (a) the relationship between the demographics and the reproductive capacity of the population, (b) the relationship between achene mass and germination, (c) methods of propagating the species *ex-situ*, (d) the *ex-situ* seed storage of the species and (e) methods of establishing seedlings *in-situ* (the reserve) in order to augment the small population. The reason for the decline of the species has been attributed to the reported high levels of herbivory before an antelope proof fence was erected. The survival and reproductive capacity of the species is sensitive to herbivory as browsing decreases canopy size and both achene production and germination were found to be significantly related to canopy size. The number of cones produced per plant (R^2= 0.532; P<0.005), number of achenes filled with embryos (filled achenes) (R^2=0.178; P=0.014), the mass of the achenes produced within a cone (R^2=0.127; P=0.041) and the germination percentage of filled achenes (R^2=0.200; P=0.009) increased with the plant canopy area. While the number of days for 50% of the achenes to germinate (T_{50}) (R^2=0.231; P=0.005), peak day of germination (R^2=0.208; P=0.008) and mean days until germination (R^2=0.270; P=0.002) decreased with plant canopy area. However, the percentage of filled achenes (seed set) did not increase with plant canopy area (R^2=0.044; P=0.241). Each plant had a degree of autonomy in the mass of the achenes produced which was significantly different between plants (P<0.05). The mass of individual achenes from various plants was not related to the germination (rate or viability) of that achene. However, the germination percentage of filled achenes (R^2=0.133; P=0.037), the peak value of germination (Czabator 1962) (P.V.) (R^2=0.403; P<0.001), the T_{50} (R^2=0.209; P=0.0074) and the mean number of days for achenes to germinate (R^2=0.178; P=0.014) were all significantly related to the mean mass of the achenes produced within cones harvested from different plants. The achenes not filled with an embryo had a higher variation in mass and were significantly lighter than filled achenes (P<0.05). Mean seed set (n=33

cones) was 36.08±2.31% (±S.E) filled achenes per cone. By sorting achenes using a simple achene mass based selection method, a sample containing 93.67±1.46% filled achenes was selected per plant. Overall, the filled achenes had high germination percentages ranging from 57.67% to 97.00%, however, germination was sensitive to various pre-treatments. Soaking achenes in water for 48 hours before germination decreased the P.V. from 3.69 to 1.22 and germination percentage from 94.06% to 82.11% compared with un-soaked achenes. However, when embryos were excised from the seed coat after 48 hours soaking, both the P.V. and the germination percentage increased further to 6.03 and 97.00% respectively. Excised embryos did not germinate normally *in-vitro* when plated on various media but dedifferentiated into callus on all media tested (including growth regulator free media). The most effective regeneration via adventitious somatic embryogenesis (but not statistically significant) occurred on a growth regulator free medium (containing 2.21g.l^{-1} Murashige & Skoog (1962) salts with vitamins, 30.00g.l^{-1} sucrose and 3.00g.l^{-1} Gelrite® with a pH of 4.20) producing a mean of 4.66±1.09 (±S.E.) embryos per explant. Secondary somatic embryogenesis also occurred on a growth regulator free medium (containing 2.21g.l^{-1} Murashige & Skoog (1962) salts with vitamins, 30.00g.l^{-1} sucrose and 3.00g.l^{-1} Gelrite® with a pH of 5.20). The somatic embryos developed into plantlets (generally un-rooted) on media containing a high gibberellic acid to cytokinin ratio (3:1 by mass) and a growth regulator free medium (containing 2.21g.l^{-1} Murashige & Skoog (1962) salts with vitamins, 30.00g.l^{-1} sucrose and 3.00g.l^{-1} Gelrite® with a pH of 5.20). Limited success (5.77%) was achieved in rooting shoots (from somatic embryos) and transferring them to a greenhouse environment. Other vegetative propagation techniques were attempted, including the rooting of stem cuttings and direct shoot organogenesis but these were unsuccessful. As the achenes of *Protea* species are not long lived *in-situ*, the *ex-situ* conservation of achenes (and therefore the species) was tested. Achenes had low water contents (9.27±0.10%) and were therefore categorised as orthodox seeds (Roberts 1973). Germination percentages were high in all tested storage regimes (Ambient, 25°C, 4°C, -70°C and -196°C), including those at ultra-low storage temperatures and after 18 months ranged between 86.81% and 92.23%. Evidence of loss of germination vigour was found in achenes from the Ambient and 25°C storage regimes. After 12 months of storage, the

quality of stored achenes under all storage regimes was thoroughly tested. Those achenes germinated successfully and produced seedlings that grew well under greenhouse conditions. Those seedlings were of high enough quality to be transplanted back into the Dr Hamilton Reserve, where growth and survival continued successfully. This showed convincingly that seed storage is an effective tool in the future *ex-situ* conservation of *Protea roupelliae* ssp. *hamiltonii.* Population augmentation was effective when planting achenes directly *in-situ* or transplanting *ex-situ* propagated seedlings *in-situ* (transplants), but varied between the two methods. Planted achenes had a low seedling emergence (less than an estimated 10% of planted, viable achenes emerged) and survival after one year was 76.41% and 69.81% after two years. However, 96.44% of the transplants survived after the first year and 95.37% after the second year. Additionally the leaf production rate of transplanted seedlings was superior to seedlings that originated from planted achenes. Through this project 1707 *Protea roupelliae* ssp. *hamiltonii* transplants have been planted into the Dr Hamilton Reserve. Although these individuals have not yet reached a reproductive size, the population numbers *in-situ* have increased considerably. Intensive intervention has worked in aiding the recovery of the *Protea roupelliae* ssp. *hamiltonii* population *in-situ,* however new threats to the population have been identified and still need to be mitigated.

Key words: critically endangered species, propagation, reproductive output, population augmentation, seed storage.