REPRODUCTIVE BIOLOGY, PROPAGATION AND *EX SITU* CONSERVATION STRATEGIES OF A CRITICALLY ENDANGERED KENYAN HARDWOOD SPECIES: *IXORA SCHEFFLERI* K. SCHUM. & K. KRAUSE SUBSP *KENIENSIS*. BRIDSON

Peter Kariuki Njenga

A thesis submitted in fulfilment for the degree of doctor of philosophy in Botany in the Jomo Kenyatta University of Agriculture and Technology

2008

ABSTRACT

Ixora scheffleri K.Schum.& K.Krause subsp. *keniensis* Bridson is a critically endangered Kenyan hardwood species on the brink of extinction in its area of endemism. The pollination ecology and breeding system of *I. scheffleri* subsp *keniensis* was investigated at the singular natural population at the Mount Kenya region of Kenya in an attempt to unravel the causes of of its decimation. *I. scheffleri* subsp *keniensis* was in flower from November to March (Fruits April to July) with a peak flowering in February. All the 8 insect species regularly visiting the flowers frequently made contacts with the stigmas and they carried copius amounts of pollen. Three Lepidoptera, two Diptera, one Coleopteran one Hymenoptera and one Thysanoptera were the visitors that frequently visited the flowers. *I scheffleri* subsp *keniensis* produced fruits through both self and cross-pollination. The tree species showed high fruit production under natural, open pollination conditions. The control, wind and/or insect pollination treatment resulted in 79.5% fruit set. The spontaneous autogamy treatment resulted in 36.5% fruit set. No germination of *I. scheffleri* subsp *keniensis* seeds was observed either in the field or in the laboratory experiments. The effect of Indole butyric acid (IBA), Napthalene acetic acid (NAA), Propagation media, season, leaf area, cutting position on mother plant and cutting length on rooting was evaluated. The Forest soil: Sand (FRS: S) rooting medium recorded the highest percentage of rooting after 8 weeks (69%). This was significantly different ($p<0.01$, ANOVA) between FRS: S and the rest of rooting media tested. Both IBA and NAA influenced maximum rooting at a concentration of 50-55 μg ($p<0.05$). The rooting of the cuttings did not differ significantly ($p=0.779$) across the

seasons (rainy and dry season). Percentage rooting of the cuttings from the various leaf areas increased with increase in leaf area from 0-80 cm^2 but reduced as the leaf area increased to 100 cm^2. Cuttings obtained from the upper third of the mother plant significantly rooted better ($p<0.01$, cumulated ANOVA) than the ones obtained from the lower third.

An *in vitro* propagation method of *I. scheffleri* subsp *keniensis* by means of axillary buds proliferation was also developed. The nodal explants were cultured on full strength Murashige and Skoog's salt medium supplemented with different concentrations of cytokinins. The concentration 25 μM BAP: 0.5 μM TDZ had the highest effect on the elongation of the *in vitro* shoots and on the mean number of microshoots. There was a significant difference ($p=0.039$, log linear modeling procedures for count data) in the number of microshoots that emerged under a concentration of 25: 0.5 and the rest but no significant difference ($p>0.05$) between 20:1.0, 20:1.5 and 25: 0.1 BAP: TDZ μM combination. Based on these results, one nodal explant can give rise to 12 plantlets in one year. Results showed that both IBA and NAA achieved their optimum *in vitro* rooting at a concentration of 50 μM. There was a significant difference in the mean percentage of the microshoots that rooted ($p<0.001$) between the two hormones where IBA performed better than NAA. The *ex vitro* rooting results showed that there was significant difference ($p=0.030$, ANOVA based on mean rooting percentage, s.e.d=2.98) between the two hormones in inducing rooting where IBA performed better than NAA. Somatic embryogenesis was also attempted using leaf discs as explants. The discs were cultured in MS medium supplemented with various concentrations of 2,4-D and TDZ. Callus initiation was evident in all cases but there was no embryogenesis recorded

RAPD analysis was carried out for 20 individuals in the single population of *I scheffleri* subsp *keniensis.* From a set of 40 primers screened, eight produced clear RAPD patterns consisting of a

total of 213 scorable markers, 177 of which (80.3%) were polymorphic. The mean level of genetic diversity within the population based on Nei's (1973) gene diversity measure was 0.2438. The mean based on Shannon's information index was 0.3776.

The scientific findings recorded herein clearly indicate the possibility of mass propagation of rare and critically endangered plant species using micropropagation and macropropagation protocols when factors affecting these processes are clearly optimized.