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Germination and seedling growth requirements for propagation of *Dioscorea dregeana* (Kunth) Dur. and Schinz — A tuberous medicinal plant

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Abstract

Intensive collection of underground tubers of the medicinal plant *Dioscorea dregeana* from indigenous forests is a threat to the wild population. Domestication of this plant is the only possible alternative to fulfill the demand of formal and informal medicinal markets in South Africa. This study reports fundamental requirements for seed germination and seedling growth of *D. dregeana*. Germination responses of seeds were tested at different temperature regimes, light conditions and smoke treatments. The highest percentage germination (≥95%) and mean germination time (MGT) of 10 days was at 30/15 °C, followed by 25 °C. No germination was observed at 10 °C. Different light conditions did not significantly affect percentage germination at 25 °C; however, under constant dark conditions the MGT was reduced. Smoke-water (1:500 v/v) and a butenolide (10^{-7} M), isolated from smoke, stimulated germination and improved seedling vigour. Seedling growth was best at 25 °C and 30/15 °C, with large underground tubers forming on all seedlings. This indicates that temperature plays a significant role in regulating growth of tubers. Seedlings watered once a week with half-strength Hoagland's nutrient solution showed best growth performance, whereas, a deficiency of either nitrogen, phosphorous or potassium negatively affected seedling growth. Thus, we recommend application of moderate fertilizers and watering once per week at 25 °C for raising healthy *D. dregeana* seedlings.

Keywords: *Dioscorea dregeana*; Medicinal plant; Propagation; Seed germination; Seedling growth; Smoke; Tubers

1. Introduction

The majority of the species of genus *Dioscorea* are perennial, herbaceous climbers that form rhizomes and tubers as storage organs (Burkill, 1960; Purseglove, 1972; Van Staden and Fowlds, 1992) and are distributed in tropical regions of Africa, America and Asia (Terui and Okagami, 1993). *Dioscorea dregeana* is found in coastal and midland forests of South Africa, particularly along the forest margins of the eastern parts (Van Wyk et al., 1997; Diederichs et al., 2002). It is a dioecious climber having large underground hairy and fleshy tubers with a diameter of up to 300 mm (Van Wyk et al., 1997). This plant is widely utilized in KwaZulu-Natal, Mpumalanga and the Northern Province of South Africa and remains one of the most popular medicinal plants (Mander, 1998). The tubers are mainly used for their sedative properties to treat ailments such as hysteria, convulsions and epilepsy (Watt and Breyer-Brandwijk, 1962; Watt, 1967; Pujol, 1990). The tubers are also administered to treat sores, wounds, fits and to facilitate childbirth (Kelmanson et al., 2000; Diederichs et al., 2002). Ethno-pharmacological studies have shown that the leaves and stems of *D. dregeana* have antibacterial properties (Kelmanson et al., 2000). In spite of having medicinal properties, the tubers contain some poisonous ingredients that are highly toxic to humans (Van Wyk et al., 2002).

Several species of *Dioscorea* are amongst the principle sources of diosgenin, which can be converted to medicinally important steroids (Van Staden and Fowlds, 1992). These steroids are used as contraceptives and anti-inflammatory agents (Bruneton, 1995). Recently Mulholland et al. (2002) reported the isolation of alkaloids of both the isoquinoline and isoquinuclidine types from *D. dregeana*. Commercially, Central American, Chinese and Indian *Dioscorea* species are used for

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extraction of steroidal saponins, but this is not practiced in South Africa (Van Wyk et al., 1997).

Due to the increasing rural population of South Africa, the demand for medicinal plants is increasing and sustainable harvesting can no longer meet these needs. Consequently, exploitation of popular indigenous plants is increasing (Mander, 1998; Van Staden, 1999). The tubers of *D. dregeana* are illegally uprooted from the indigenous forests (Cocks et al., 2000) and are traded at informal markets across the country (Cunningham, 1993). Such circumstances have suggested the necessity for commercializing many *Dioscorea* species in Africa (Okole and Odhav, 2004). Although micropropagation protocols for many *Dioscorea* species have been developed, there are a few disadvantages that make them expensive to grow (Van Staden and Fowlds, 1992).

Currently the moisture availability and supply rate of macronutrients (N, P and K) is not well understood for forest soils of South Africa (Louw and Scholes, 2002). At the same time, the demand for *D. dregeana* is high, and there is very little to no information readily available on propagation practices. Consequently, at this stage, seed propagation remains the most feasible and the cheapest option. This study therefore aims to establish the optimum conditions for seed germination and seedling growth of *D. dregeana*.

2. Materials and methods

2.1. Seeds

2.1.1. Seed collection

Dry fruits of *D. dregeana* were collected in May 2005 from the University of KwaZulu-Natal Pietermaritzburg, Botanical Garden and kept in a brown paper bag until they completely dehisced and released winged seeds. The seeds were removed, de-winged and stored in brown paper bags for a month at room temperature before being tested for germination.

2.1.2. Seed moisture content and imbibition

Moisture content of seeds was determined by drying them at 110 °C in a preset incubator until there was no further loss in seed weight (Probert and Hay, 2000). In imbibition studies, the seeds were placed in 9 cm disposable Petri dishes on two layers of filter paper (Whatman No.1) moistened with 4.5 ml distilled water and allowed to imbibe at room temperature (25±0.5 °C). The increase in seed mass was determined after 2, 4, 6, 8, 12, 24, 48 and 96 h. Seeds were blotted dry before weighing and returned to wet filter paper. The amount of water imbibed by the seed is graphically represented as the percentage increase over the initial seed mass.

2.1.3. Seed germination

Optimal germination conditions were determined by subjecting seeds to different temperature regimes, light conditions and by treating seeds with smoke. The seeds of *D. dregeana* were decontaminated with 0.1% mercuric chloride for 2 min and then rinsed with distilled water prior to germination tests. All treatments consisted of four replicates with 20 seeds in each. The seeds were placed on two layers of Whatman No.1 filter paper in disposable plastic Petri dishes (9 cm). The filter paper was wet through with 4.5 ml distilled water or smoke solutions and kept moist by adding respective solutions, when required, till the end of the experiment. Seeds were incubated with smoke-water (dilution of 1:500 v/v) or a butenolide (3-methyl-2*H*-furo[2,3-c] pyran-2-one) (10^{-7} M) isolated from smoke. The smoke-water was prepared by the methods outlined in Baxter et al. (1994), and the butenolide was isolated from plant-derived smoke-water, according to the method described by Van Staden et al. (2004). To determine the effect of different temperatures, the seeds were incubated at 10, 15, 20, 25, 30, 35 and 30/15 °C. The experiments were conducted at a 16 h light and 8 h dark photoperiod, with cool-white fluorescent lamps, which provided a photosynthetic photon flux density (PPFD) of 80.6±7.8 μmol m^{-2} s^{-1}. For continuous dark conditions and smoke treatments, the Petri dishes were placed in light proof boxes at 25±0.5 °C, and the seeds were inspected daily under green "safe light" with a PPFD of 0.3 μmol m^{-2} s^{-1}. In continuous light at 25 °C, PPFD was 80.4±3.5 μmol m^{-2} s^{-1}.

Germination was recorded daily and was considered complete once the radicle protruded about 2 mm in length. The experiments were continued for 21 days. Mean germination time (MGT) was calculated by using the equation: $MGT = \sum(n \times d)/N$, where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment (Ellis and Roberts, 1981).

2.2. Seedling development

2.2.1. Influence of smoke on seedling vigour

To test the response of *D. dregeana* seedlings to smoke constituents, seeds that were germinated with smoke-water and butenolide under continuous dark conditions were allowed to grow in the same Petri dishes for 21 days. The water-treated seeds served as a control. Seedling vigour index was calculated as SVI = seedling length (mm) × percentage germination.

2.2.2. Effect of temperature, watering and nutrient application on seedling growth

Seedling growth primarily relies on light, temperature, water and nutrient availability. To evaluate the specific requirements for the growth of *D. dregeana* seedlings, these factors were investigated under controlled conditions. Three-week-old seedlings grown in Petri dishes were transplanted in 15 cm (diameter) pots filled with sterile quartz sand and moistened with Hoagland's nutrient solution (HS) (Hoagland and Snyder, 1933) of various strengths depending on the treatment. Each pot consisted of four seedlings with six replications per treatment. Pots were arranged randomly in plant growth chambers under 16:8 h light and dark conditions at 25 °C (PPFD of 80.4±3.5 μm^{-2} s^{-1}).

To test the effects of different temperatures on seedling growth, the pots were placed at constant (10, 15, 20, 25, 30, 35 °C) and alternating (30/15 °C) temperatures. At 15 day intervals, 100 ml half-strength HS was added to each pot. The