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Seed Germination in *Stevia rebaudiana*

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Stevia (*Stevia rebaudiana* Bertoni, Asteraceae) is a non-caloric natural-source alternative to artificially produced sugar substitutes. The sweet compounds pass through the digestive process without chemically breaking down, making stevia safe for those who need to control their blood sugar level (Strauss 1995). There have been no reports to date of adverse effects from the use of stevia products by humans (Brandle and Rosa 1992). Shock (1982) reported that stevia contains eight glucoside compounds, each featuring a three-carbon-ring central structure. Stevioside is the most abundant glucoside produced. An extract of one or more of these compounds may be up to 300 times sweeter than sugar (Duke 1993). Preliminary trials at Davis, California indicate that stevia could produce a sweetener equivalent to 10 t/ha of sucrose (Shock 1982).

The Guarani Indians of Paraguay, where stevia originates, have used it for centuries as a sweetener for maté tea (Brandle and Rosa 1992). Since the 1970s, stevia extracts have been widely used in many countries as a sugar substitute. In Japan, for instance, stevia extracts account for about 5.6% of the sweetener market (Strauss 1995). Stevia usage in the United States is limited at this time because the Food and Drug Administration does not allow its use as a sweetener in manufactured and processed food products. In 1991, the FDA banned stevia, claiming it was an "unsafe food additive." The FDA now allows the sale of stevia, but only as a nutritional supplement (Whitaker 1995).

HORTICULTURE

Stevia is a perennial herb with an extensive root system and brittle stems producing small, elliptic leaves. Stevia will grow well on a wide range of soils given a consistent supply of moisture and adequate drainage; plants under cultivation can reach up to 1 m or more in height (Shock 1982). Stevia is grown as a perennial in subtropical regions including parts of the United States, but must be grown as an annual in mid to high latitude regions, where longer days favor leaf yield and stevioside contents.

The tiny white florets are perfect, borne in small corymbs of 2–6 florets. Corymbs are arranged in loose panicles. Oddone (1997) considers stevia to be self-incompatible and insect pollinated. Additionally, he considers "clear" seeds to be infertile. Seeds are contained in slender achenes, about 3 mm in length. Each achene has about 20 persistent pappus bristles.

Propagation of stevia is usually by stem cuttings which root easily, but require high labor inputs. Poor seed germination is one of the factors limiting large-scale cultivation. Shock (1982), Duke (1993), and Carneiro (1997), all mention poor production of viable seeds. Propagation is a special concern for northern growers who must grow stevia as an annual.

GERMINATION STUDY

A study was undertaken to investigate the low seed germination of stevia seeds. The influence of pollination treatments as well as the effect of light and darkness during germination were evaluated. Rooted stem cuttings of a Chinese clone of stevia obtained from Dr. Ken Rohrback, University of Hawaii were transplanted into 24 cm diameter plastic pots containing silty clay as a soil medium. On Oct. 12, 1997, the plants were placed in two separate greenhouses where temperature, wind, and pollen access could be controlled. At this time, the plants were at the first stage of floral bud development. The plants were subjected to five pollination treatments: (1) cross-pollination by bumblebees in a cage; (2) cross-pollination by hand; (3) cross-pollination by wind from a fan; (4) self-pollination by hand; (5) a control group isolated from other genotypes.

Ten plants of the Chinese clone were utilized (two in each treatment group). Clone SR8 provided cross-pollination in all but the selfing by hand and control treatments (one plant in each treatment group). Pollination treatments were initiated on Oct. 30, 1997 when blossoms were beginning to open and treatments continued for the duration of anthesis (30–40 days). For the bumble bee treatment, a cage (122 cm × 91.5 cm × 183 cm) covered with wire screen was placed over a greenhouse bench. Plants were placed in the cage along with a small hive of bumble bees (*Bombus impatiens*) obtained from Koppert Biological Systems of Ann Arbor, Michigan. A large circulation fan was used for wind pollination. Plants were placed 0.9–1.7 m from the

Table 1. Seed weight and viability (by tetrazolium chloride staining) of stevia.

Seed color	Seed wt. (mg/1000 seeds ± SD)	Seed viability (% ± SD)
Black	300 ± 5	76.7 ± 23.1
Tan	178 ± 8	8.3 ± 10.4

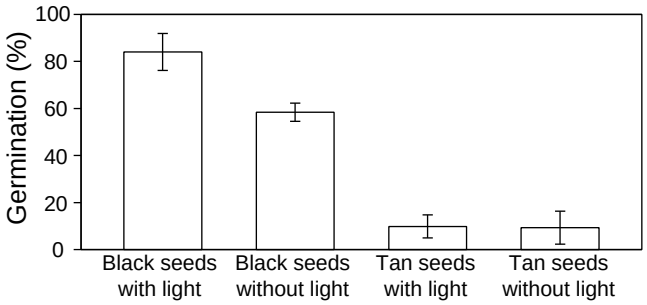

Fig. 1. Average germination rate for cross-pollinated "Chinese" stevia (± SD).

Table 2. Germination of black stevia, seeds under light derived from various pollination treatments.

Plant pollination treatment	Germination (% ± SD)
Selfing (hand)	93.3 ± 0.6
Crossing (hand)	92.0 ± 2.0
Crossing (bumblebees)	78.3 ± 14.5
Crossing (wind)	68.3 ± 22.5
Control[z]	36.3 ± 25.5[y]

[z]Control germination = 62, 36, 11
[y]Plants in isolation

intake side of the fan, providing enough breeze for gentle movement of the blossom 10–15 times daily, for 2–5 min periods. Cross-pollination and self-pollination by hand was accomplished by transferring pollen between blossoms every other day with a bumble bee thorax on the end of a toothpick. Seeds ripened during the period between Nov. 30, 1997 and Jan. 21, 1998. Seeds were judged to be ripe when they fell away with a gentle shake to the plant. Two or three harvests were done for each plant. For the final harvest, branches were cut and shaken vigorously inside a collection box. Debris was removed from the seeds by hand. Black and tan seeds were then separated.

Each germination test utilized 100 seeds placed between paper towels in a nursery flat, covered by a plastic dome. The temperature for all tests was 24°C. Fluorescent lights were placed above the domes for light treatment, 15 cm above the seeds. After 7 and 12 days, the number of seeds exhibiting normal germination were counted. For viability tests, 20 seeds were submerged in a 10% tetrazolium chloride solution at 24°C for 1 h and the stained seed counted. Each germination experiment was carried out with three replications.

There were two types of seed: black and tan. Black seed weighed more than tan seed, 0.300 vs. 0.178 mg, and viability of black seed based on tetrazolium chloride was much higher than tan seed, 76.7 vs. 8.3% (Table 1). Germination in the dark was higher for black as compared to tan seed (83.7% vs. 16.0%) while light increases the germination of black seed but not tan seed (Fig. 1). This suggests that tan seeds represent inviable seed that are produced without fertilization. There was no significant difference in black seed germination among the four pollination treatments suggesting that incompatibility is not a factor in these clones (Table 2). However, all pollination treatments increase seed germination of black seed over the control suggesting that some active manipulation of the blossoms is necessary to achieve pollination. It would appear that many of the black seeds in the control were misclassified tan seed.

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