Effect of Boron and Agar on Germination of Pea Pollen in Sucrose Media

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Since the discovery of the pollen tube in 1824 by Amici, numerous studies on the in vitro germination of pollen have been reported. Extensive reviews of the literature on pollen germination have been made by Brink (1, 2, 3, 4), Doroshenko (6), Visser (18), and Johri and Vasil (9). Sucrose and agar, individually and together, have been among the most common and effective components of artificial germination media. Concentrations within the range of 0.5 to 2% agar, and 10 to 80% sucrose have been used almost exclusively (18).

Tsao (16) found that a medium containing 1% agar and 10% sucrose was satisfactory for chemotropie studies on pollen tubes of peas (Pisum sativum L.). Warnock and Hagedorn (19) reported best germination of pea pollen on media containing 1% shredded agar and 15% sucrose adjusted to pH 7.

Schmucker (11, 12, 13, 14) was the first to discover the significance of boron in pollen germination experiments. Numerous reports reviewed by Gauch and Duggar (7) have emphasized the importance and often the essentiality of boron for pollen germination. Visser (18) listed 52 genera in which pollen of many species gave improved pollen germination by addition of boron to the medium. Most pollen studied required 10 to 150 ppm boric acid for optimum germination (9).

The effect of boron on the germination of pea pollen has not been reported. The purpose of this work was to study the separate and combined effects of boron, sucrose, and agar on the germination of pea pollen.

Materials and Methods

Cleaning of glassware. In experiments where boron was studied all glassware was first soaked 12 to 24 hours in N HCl, rinsed at least 3 times in single-distilled water and 5 times in double-distilled water, then covered to protect from dust. All stock solutions of boron and sucrose were stored in polyethylene containers to protect from boron contamination from glassware. Stock solutions containing agar were stored in Pyrex glass containers to withstand autoclaving.

Preparation of stock solutions. A stock solution containing 100 ppm boron and 15% sucrose was prepared by weighing out 0.5716 g. HoBO3, 150 g. analytical grade sucrose, and making up to 1000 ml. with double-distilled water. The diluent was prepared in the same manner with boric acid excluded. Both solutions were adjusted to pH 7 using a few drops of 1N NaCl or 1N NaOH as required. Appropriate dilutions of the stock solution could give a range of boron concentrations from 0 to 100 ppm at the same sucrose concentration. Similar procedures were used to prepare other stock solutions containing 200 ppm boron and 20% sucrose, and 200 ppm boron without sucrose.

Preliminary attempts to germinate pea pollen on media containing 1% Difco Bacto-Agar and 15% sucrose gave consistently poor results. Few germinating grains were observed but pollen grains were frequently ruptured. Agar media for more detailed studies were prepared from leached Difco Bacto-Agar. Leaching was accomplished by 6 washings of 50 g. agar using about 500 ml. of double-distilled water for each washing. Following the final washing the suspension was filtered and the agar residue dried at 35° C.

Counts were made under low magnification in 3 to 4 microscope fields per slide. Pollen grains with intact or punctured germ tubes longer than the diameter of the parent pollen grain were counted as germinated, but those with shorter germ tubes or lacking them were counted as not germinated.

Experimental design. Preliminary experiments with varying levels of boron and one level of sucrose were designed as Randomized Complete Blocks. Differences in treatment means were tested with Duncan's method, and missing plots estimated following procedures in Steel and Torrie (15). Splits Plots based on Randomized Complete Blocks were used when several levels of boron and sucrose were studied with pollen from two varieties. Whole plots were the varieties by sucrose or varieties by agar interactions and subplot levels were the levels of boron.

Results

Germination on boron-sucrose media. Stock solutions were diluted to provide concentrations of 0, 3, 6, 9, and 12 ppm boron in 15% sucrose. Germination of Alaska pea pollen on these media were studied after 1½-hour incubation at 27° C. Germination data are shown in Table 1.

Boron had a highly significant (p = .01) effect on pollen germination. Differences between replications were not significant. Comparison of treatment means showed that pollen germination was lowest when boron was absent, but when it was present optimum germination occurred at 9 ppm. Germination was better at 6 ppm than...