Tetraploid Bell Pepper Shows High In Vitro Pollen Germination at 15 °C

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In *Capsicum*, the normal development of pollen grains was disturbed at low temperature, inducing seedless fruits, which were deformed in size and shape (Polowick and Sawhney, 1985). Mercado et al. (1997) reported that exine developed at low temperature was thinner than that developed at optimum temperature. However, polyploids are generally characterized to have large flowers and pollen grains (Watts, 1980). We hypothesized that the pollen grains of tetraploid pepper, compared with diploid, would be larger with thicker exine, and that the viability of the pollen at low temperature would be higher. Based on this hypothesis, we tested tetraploid plants of pepper and investigated the rates of pollen germination at low temperature.

Tetraploid plants of *Capsicum annuum* L. 'Chigusa' (Nihon Horticultural Production Institute) were obtained by colchicine treatment of seeds (Ishikawa et al., 1997). Flow cytometric analysis (Partec PA flow cytometer, Partec, Munster, Germany) showed that ≈20% of the seeds treated with colchicine were tetraploid, comparable with our previous report (Ishikawa et al., 1997).

The tetraploid plants had larger flowers than did plants of the diploid counterpart.

While diploid flowers typically had six petals and stamens, tetraploid flowers typically had seven petals and stamens. Tetraploid plants had 20% larger ovaries and 25% larger diameter pollen grains than diploids.

In order to investigate in vitro germination rates of the pollen, flower buds were excised one day before anthesis and incubated at 25 °C in petri dishes with wet filter papers. After 24 h, pollen grains were put into 50 µL of liquid Brewbaker and Kwack's medium (100 mg·L⁻¹ boric acid; 300 mg·L⁻¹ calcium nitrate; 200 mg·L⁻¹ magnesium nitrate; 100 mg·L⁻¹ potassium nitrate and 50 g·L⁻¹ sucrose, Brewbaker and Kwack, 1963). After 5 h incubation at 10, 15, 20, 25, and 30 °C, the pollen grains were observed under a microscope (×100, Olympus IX70, Japan) and were scored as germinated when the pollen tubes had elongated equivalent to the diameter of the pollen grains.

The mean rates of in vitro pollen germination of diploid 'Chigusa' at 20, 25, and 30 °C

were 27.4% to 36.3% (Table 1) and agreed with reports by Kato (1989) and Mercado et al. (1994). The germination rate was reduced to 6.5% at 15 °C, reflecting the high sterility level observed in diploid 'Chigusa' at low temperatures. However, the pollen germination rate (22.1%) of the tetraploid plants at 15 °C was significantly ($P < 0.05$) higher than that of the diploid plants. There was no significant difference among the pollen germination rates of diploid plants at 20, 25, and 30 °C, nor among those of tetraploid plants at 15, 20, 25, and 30 °C (by Tukey, $P < 0.05$).

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Table 1. In vitro pollen germination rates^a of diploid and tetraploid plants of *Capsicum annuum* 'Chigusa' at different temperatures.

Plants	Temperature (°C)				
	10	15	20	25	30
Diploid	0.1 ± 0.1	6.5 ± 2.2	27.4 ± 2.9	36.3 ± 6.4	29.6 ± 5.3
Tetraploid	1.4 ± 0.4	22.2 ± 2.2***	31.3 ± 3.1	21.9 ± 2.4	19.5 ± 2.6

^aGermination rates = (number of germinated pollen grains / number observed) × 100. The experiments were performed two times using separate preparations of tetraploid plants with comparable results. Data show one set of results. Values represent means ± sd (n = 6 to 19).

***Significant within columns by Tukey's Studentized range test ($P < 0.05$).

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