

Studies on Micropropagation of *Odontoglossum* Alliance

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Odontoglossum alliances are south American native, epiphytic, cool-temperature-growing, tropical orchids consisting of more than 200 species. There are many nice hybrids with beautiful flowers, but it is difficult to cultivate them in Japan because of the hot summers. Recently, however, heat-tolerant hybrids have been bred and production of these has started in Japan. Mericlinal plants are available and on sale now, but there is only limited information on the micropropagation of these orchids. In the present experiments, sterilization methods, culture media, and culture conditions were investigated.

MATERIALS AND METHODS

Sterilization Methods. Shoot tips were excised from young immature unrooted shoots. These shoots were cut from stock plants, washed in tap water, dirty parts cut away with a scalpel, and any leaves surrounding the stems were removed. These stems with exposed buds were sterilized in sodium hypochlorite solution with moderate shaking, washed three times with sterilized water, 2 or 3 scale leaves removed, and the shoot tips excised. The isolated shoot tips were rinsed with 0.5% sodium hypochlorite solution, soaked in sterilized water, placed on new phalaenopsis medium (NP; Table 1) supplemented with coconut water (150 ml liter⁻¹), and cultured at 25C with a constant illumination of about 650 lux provided by Plantlux (Toshiba) fluorescent lamps.

Times of sterilization, concentration of sodium hypochlorite, ultrasonic treatment during sterilization, and the effects of pH adjustment of the sodium hypochlorite solution were investigated.

Culture Media. Sucrose concentrations and organic supplements were investigated using protocorm-like bodies (PLB) of *Odontoglossum* 'Lovely Morning' and *O.* 'Spring Dress'.

Culture Temperature. Protocorm-like bodies of the above two cultivars were cultured on NP medium at 20, 22.5, and 25C.

RESULTS AND DISCUSSION

Sterilization. The contamination rate decreased with the increase in sterilization time in 0.5% sodium hypochlorite solution, but the rate of dead shoot tips also increased. The maximum survival rate without contamination was obtained with 20 min sterilization. In 1.0% sodium hypochlorite solution, the sterilization rate was also the best (90%) when sterilized for 20 min.

No beneficial effects from ultrasonic treatment and pH adjustment were observed.

Culture Conditions. The addition of sucrose, sorbitol, and coconut water (CW) was effective for PLB growth. The effect of sorbitol was inferior to that of sucrose and CW.

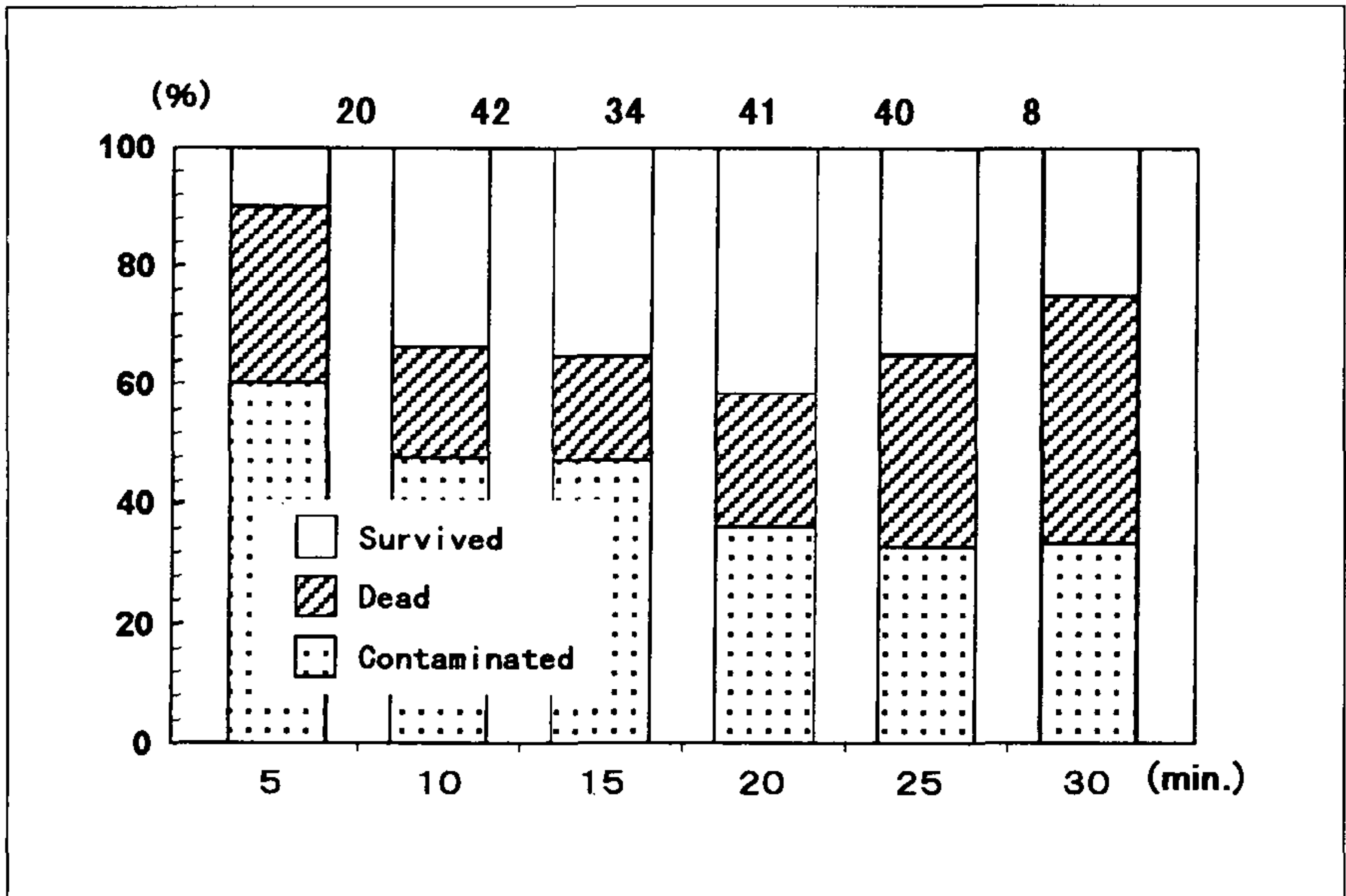


Figure 1. Effects of sterilization times with 0.5% sodium hypochlorite. Numbers on the top indicate number of shoot tips used.

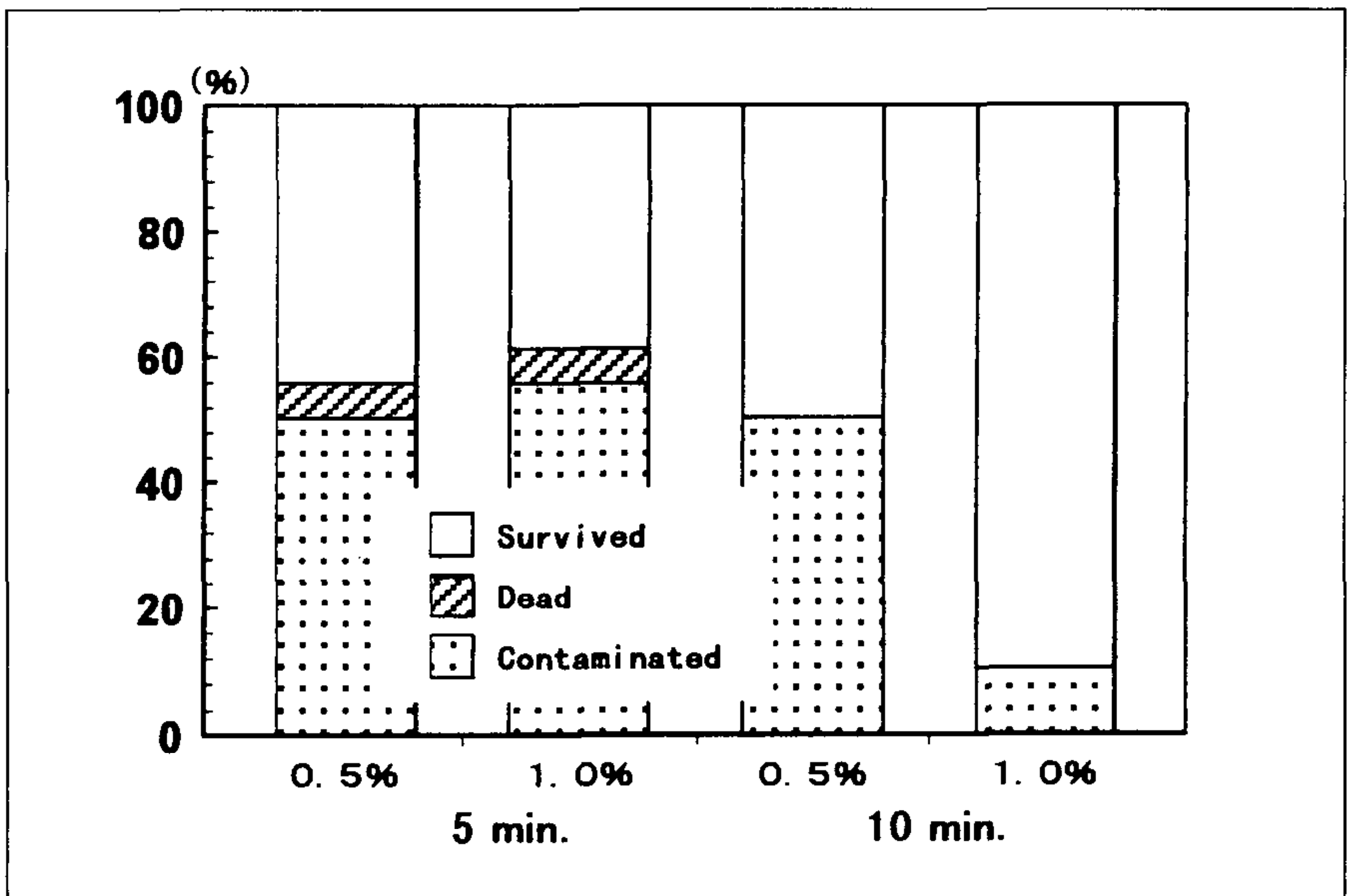


Figure 2. Effects of sterilization times and concentration of sodium hypochlorite. Ten shoot tips used for each treatment.

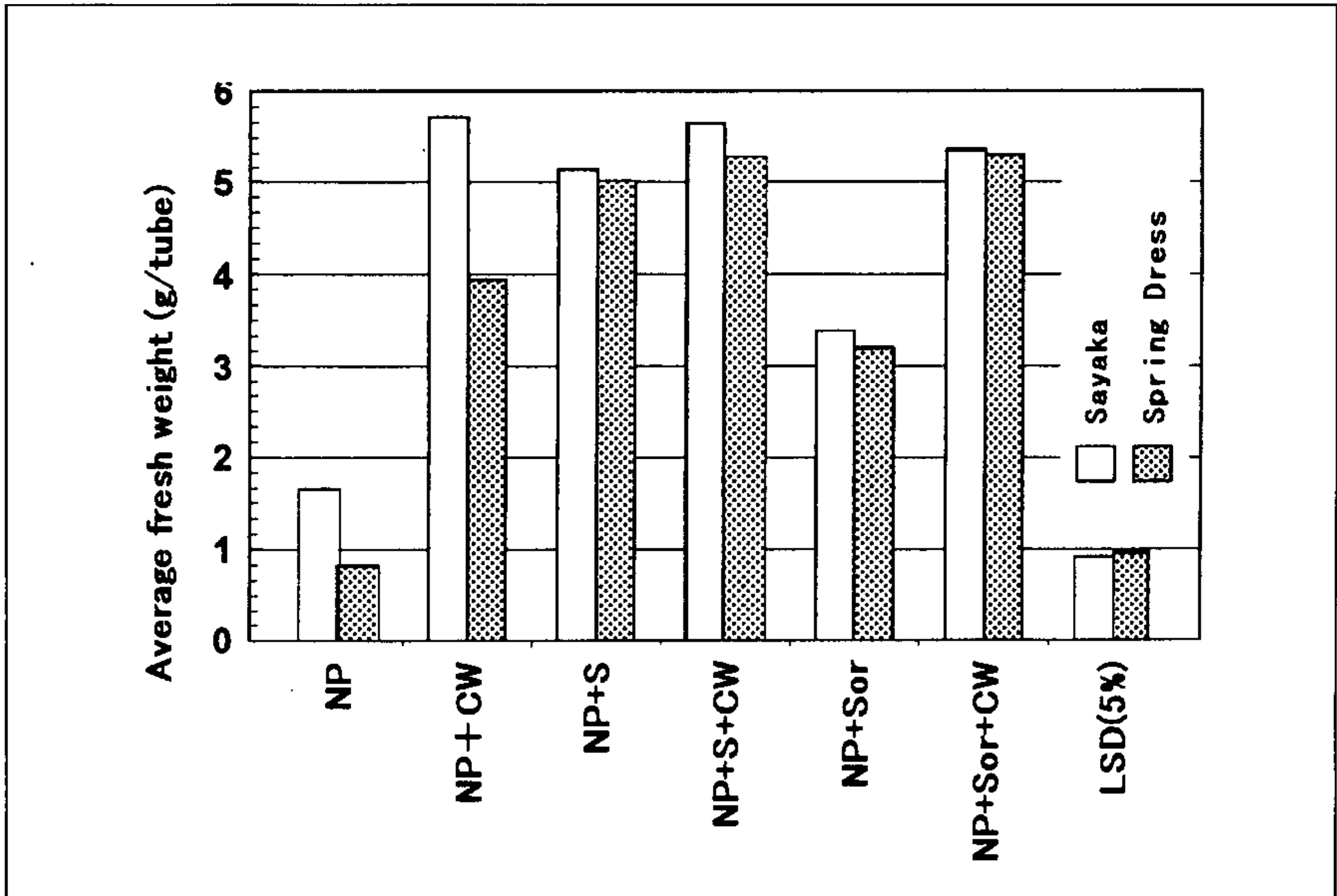


Figure 3. Effects of sucrose (S) and sorbitol (Sor) with and without coconut water (CW) on the growth of protocorm-like bodies (PLB).

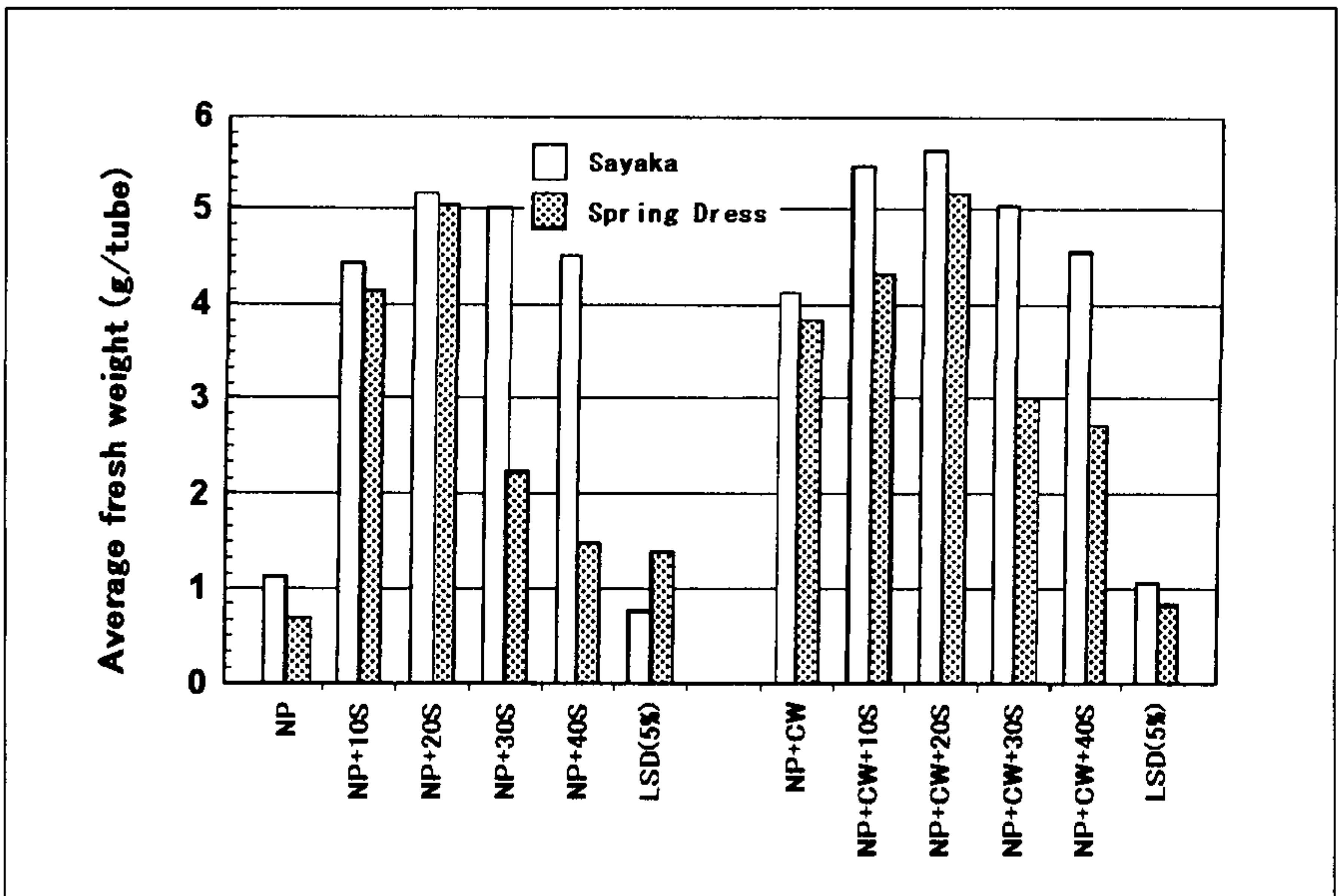


Figure 4. Effects of sucrose concentration with and without coconut water (CW) on the growth of protocorm-like bodies (PLB).

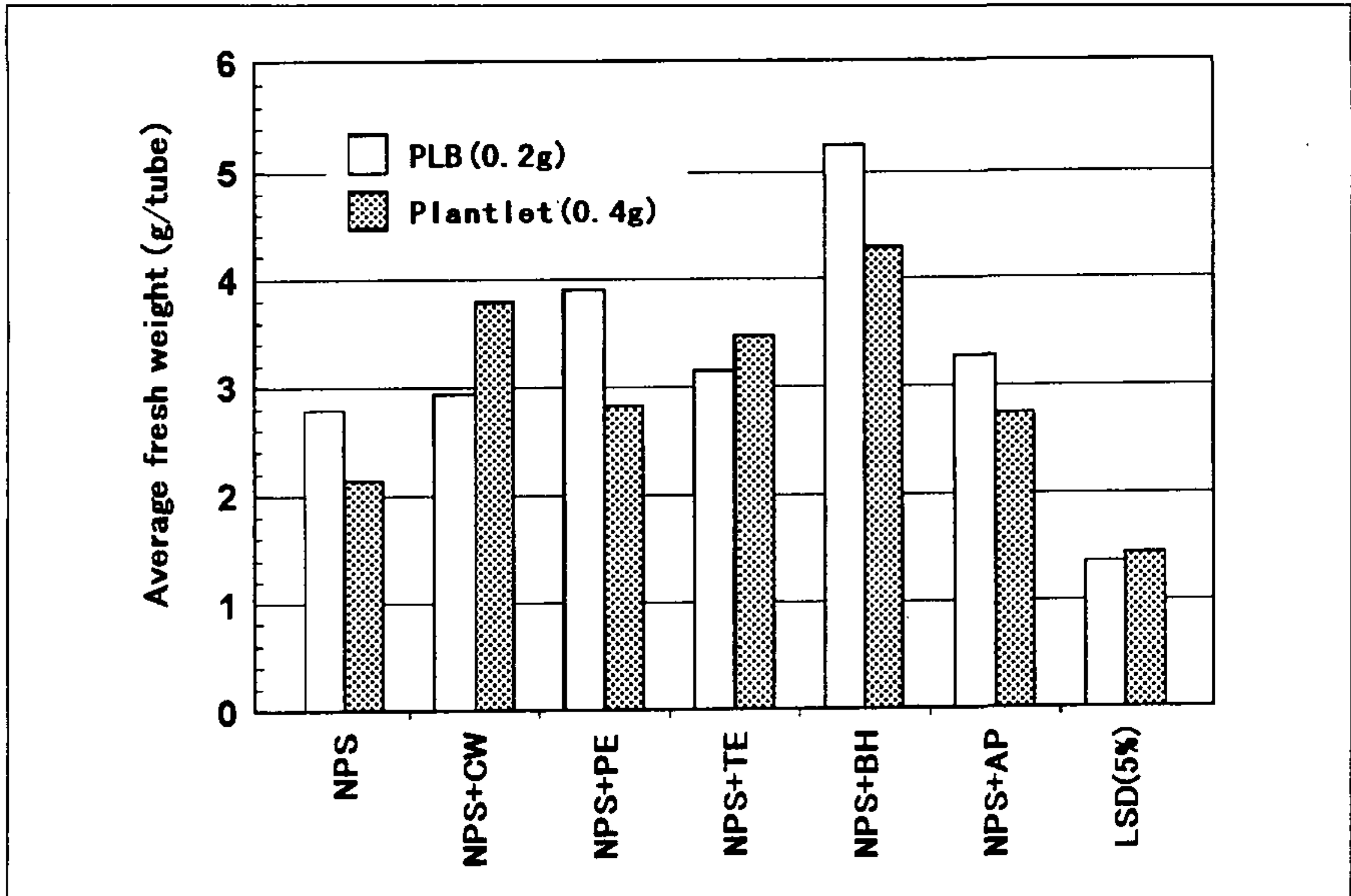


Figure 5. Effects of organic additives on growth of *Odontoglossum* 'Lovely Morning'. Coconut water (CW) 150 mg liter⁻¹, potato extract (PE) 150 g liter⁻¹, taro extract (TE) 150 g liter⁻¹, banana homogenate (BH) 75 g liter⁻¹, and apple extract 150 g liter⁻¹.

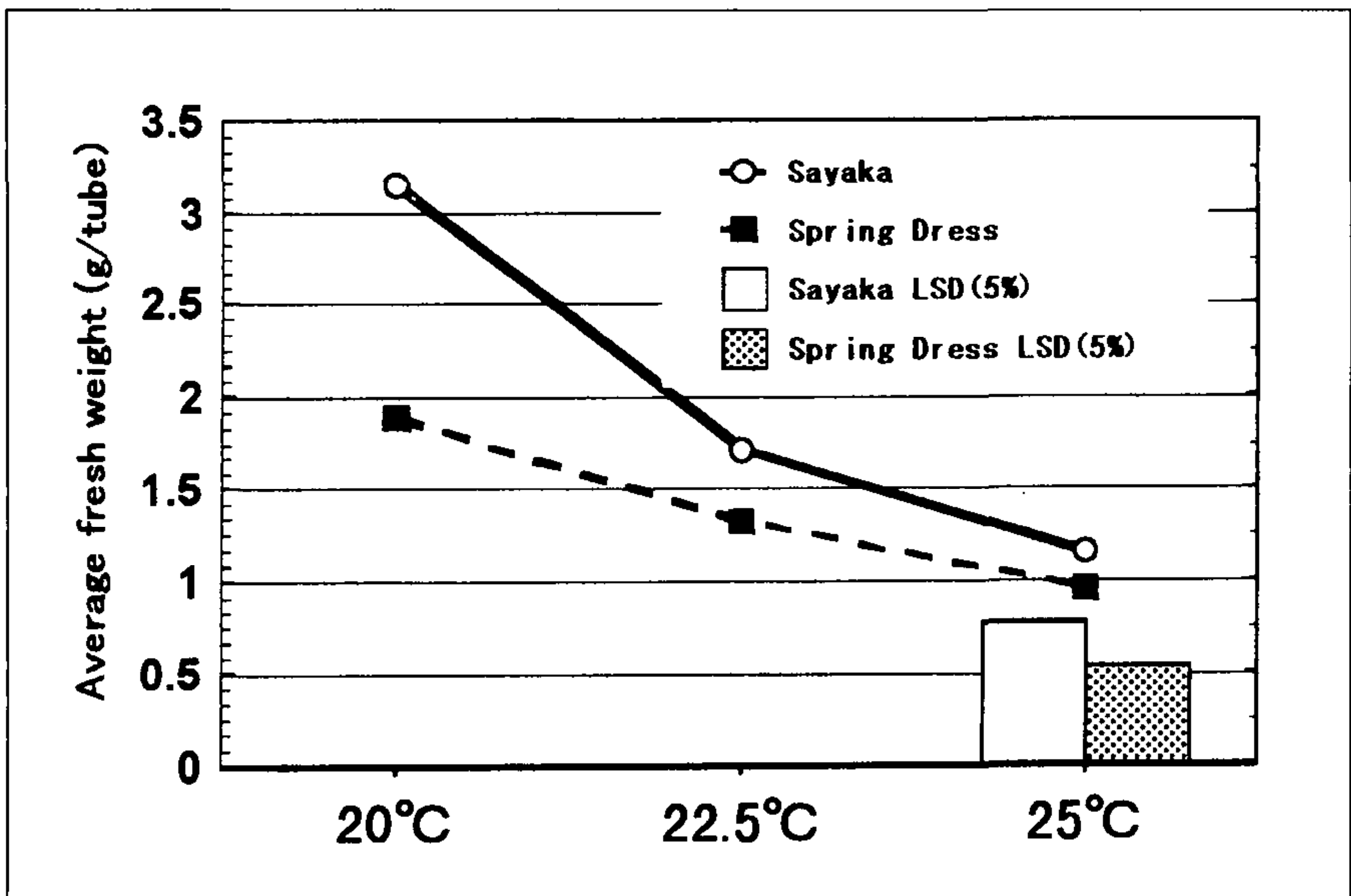


Figure 6. Effect of temperature on protocorm-like bodies (PLB) growth.

The optimum concentration of sucrose was 20 g liter⁻¹ both with and without CW (Figs. 1, 2, 3, and 4).

The addition of organic additives to the NP medium supplemented with sucrose (20 g liter⁻¹) stimulated PLBs and plantlet growth of *O.* 'Lovely Morning'. Among the additives tested, banana homogenate (75 g liter⁻¹) was the best (Fig. 5).

Of the temperatures tested 20C gave the best results, however, a temperature below 20C would appear to be the optimum temperature for PLB growth of these two cultivars (Fig. 6).

Table 1. Composition of new phalaenopsis (NP) medium.

Components	mg liter ⁻¹
Major elements*	
(NH ₄)SO ₄	303.9
KH ₂ PO ₄	462.7
NH ₄ NO ₃	32.0
Ca(NO ₃) ₂ ·4H ₂ O	637.6
Mg(NO ₃) ₂ ·6H ₂ O	256.4
Fe-EDTA**	
Minor elements**	
Organics/vitamins**	
Solidifier	
Gelrite	3000
Sugar	
Sucrose	20000

* The balance of cations and anions are NH₄⁺ : K⁺ : Ca²⁺ : Mg²⁺ = 25 : 38 : 27 : 10; NO₃⁻ : H₂PO₄⁻ : SO₄²⁻ = 60 : 17 : 23; and the total ionic concentration of cations and anions is 20 me liter⁻¹.

** The amount of Fe EDTA, vitamins, glycine, and myo-inositol are equivalent to those in Murashige and Skoogs's (MS) medium. The minor elements were reduced to + of those of the concentration in MS. The pH was adjusted to 5.6±0.1.