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ASYMBIOTIC GERMINATION AND SEEDLING GROWTH OF *CYMBIDIUM ELEGANS* LINDL. AND *COELOGYNE PUNCTULATA* LINDL. AS INFLUENCED BY DIFFERENT CARBON SOURCES

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Abstract

Lower concentrations (20 gl^{-1} and 30 gl^{-1}) of sucrose, D-fructose, and D-glucose in Knudson C medium were found suitable for germination and seedling growth in *Cymbidium elegans* and *Coelogyne punctulata.* While moderate growth was recorded using trehalose, maltose, D-mannose, and raffinose, the growth was poor when L-glucose and L-mannose were incorporated into the medium. In sugar free medium, very poor germination and seedling growth was noticed.

Introduction

The importance of sugars in promoting orchid germination and growth has been emphasized by several authors (Downie, 1949; Yates and Curtis, 1949; Knudson, 1950; Liddell, 1953a, b; Arditti, 1967, 1982; Ernst, 1967b; Arditti *et al.,* 1972; Nakamura, 1982). Depending upon the species some form of sugar is necessary, either in early stages to promote germination, or later to stimulate the development of protocorms (Downie, 1941; Harvais and Hadley, 1967b). The pentose sugars L-arabinose and L-xylose failed to support growth in *Cattleya trianaei* seedlings while good growth was observed by using the following carbohydrates in order of suitability: D-mannose, D-glucose, maltose, D-fructose, sucrose, and raffinose. However, no growth was observed when media contained D-galactose and L-rhamnose (Ernst, 1967b). The growth inhibition by galactose was observed in many orchids such as *Dendrobium nobile* (Quednow, 1930) and *Phalaenopsis* hybrid (Ernst *et al.,* 1971). Nakamura (1982) also noticed that lactose, melibiose, and raffinose which contain galactose and glucose as constituents allowed poor growth in the orchid *Galeola septentrionalis.* L-series of carbohydrates were unsuitable as energy sources for *in vitro* grown seedlings of *Phalaenopsis* and *Dendrobium* (Ernst, 1967b). However, D-series of sugars were suitable and D-fructose was found to be the best. Withner (1959) stated that sucrose and glucose were, in general, the best carbohydrate sources for the germination and seedling growth in orchids.

The North-Eastern region of India is endowed with ideal climatic conditions for the growth of different orchids.

However, the number of orchids in nature is going down considerably due to unplanned human activities. In an attempt to develop practical methods for propagation of orchids, studies have been initiated. This paper presents the influence of different forms of sugars on seed germination and seedling growth of *Cymbidium elegans* Lindl. and *Coelogyne punctulata* Lindl.

Materials and Methods

As described earlier (Sharma and Tandon, 1986, 1987), about 20 wk-old unopened immature capsules of *Cymbidium elegans* and *Coelogyne punctulata* were surface sterilized with 7% sodium hypochlorite solution and seeds removed under aseptic conditions. These were germinated in Knudson (1946) medium with or without 2-7% of either of sucrose and D-glucose, or maltose, or D-fructose, or D-mannose, or trehalose, or raffinose, or L-glucose, or L-mannose.

The pH of the medium was adjusted at 5.0. Difco-bacto agar (1.4%) was used to gel the medium.

The cultures were incubated at 25±2°C in dark for 60 days and the per cent germination was determined. The cultures were brought to continuous light of 3,000 lx at this stage. 90-day-old protocorms were transferred to media containing different carbon sources for studying their influence on seedling development. To quantify growth, the average number and length of leaf and root/rhizoid, and fresh weight of 180-day-old, seedlings, were recorded. For each experiment ten replicates were taken and experiments were repeated twice.

Results

Effect of Carbon Sources

Sucrose : The seeds germinated very poorly, (Figs. 1a, c) unless subjected to appropriate quality of sucrose in the medium. Optimum germination was achieved by using 30 gl^{-1} and 20 gl^{-1}sucrose for *Cymbidium elegans* and *Coelogyne punctulata,* respectively. A higher concentration of sucrose (70 gl^{-1}), however, proved inhibitory to germination. The influence of sucrose on seedling development alone was also promotive. The optimum seedling growth resulted in medium containing 30 gl^{-1} of sucrose (Figs. 1b, d).

D-glucose : The germination and seedling growth were optimum in the media containing 20 gl^{-1} of D-glucose (Figs. 2a, c). However, both these processes were progressively impaired with a corresponding increase in the level of this carbohydrate source in the medium. A similar growth pattern was recorded in protocorm raised seedlings cultured on media containing different concentrations of D-glucose (Figs. 2b, d)

Maltose : Maltose was moderately useful for germination and seedling growth. Out of its various concentrations used, 20

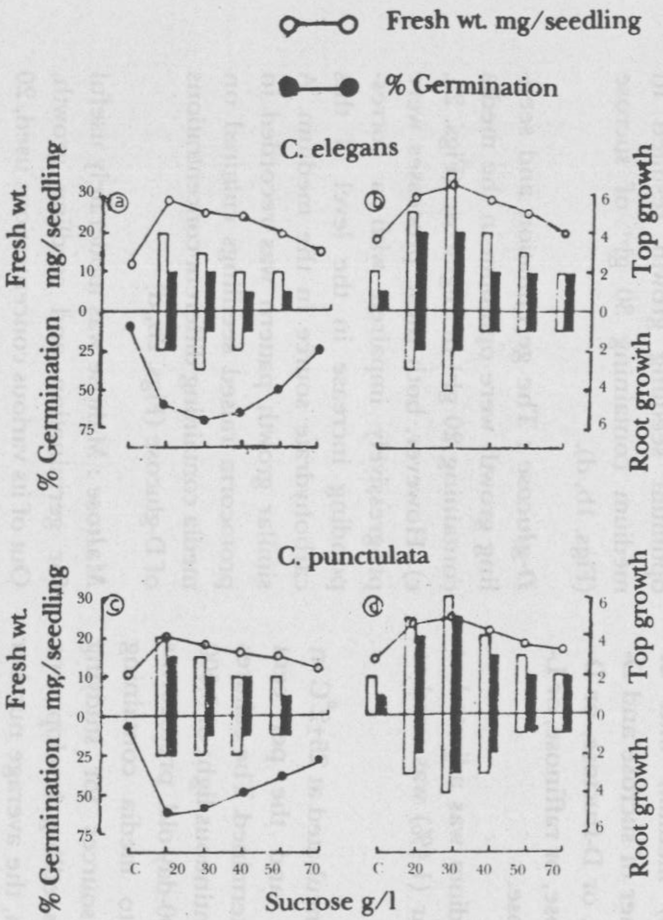

Fig. 1. Effect of sucrose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans* (b) and *C. punctulata* (d).

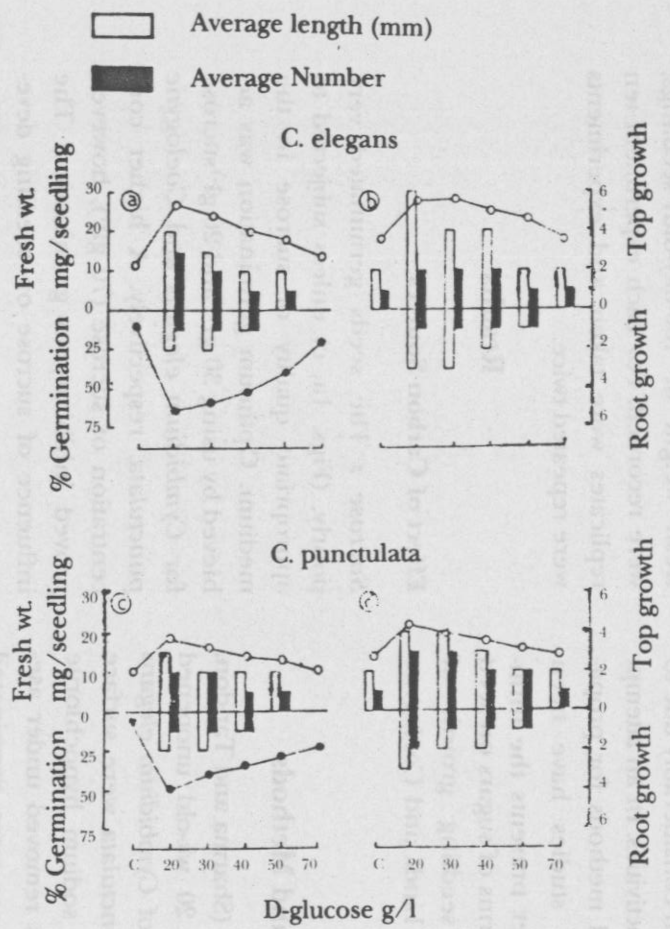

Fig. 2. Effect of D-glucose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans (b) and C. punctulata* (d).

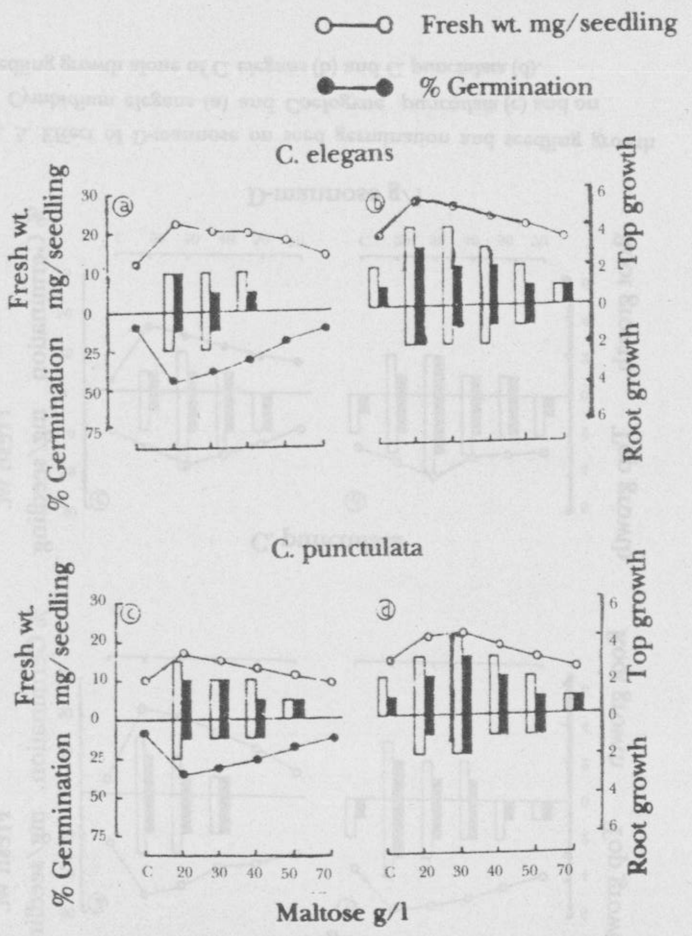

Fig. 3. Effect of maltose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans* (b) and *C. punctulata* (d).

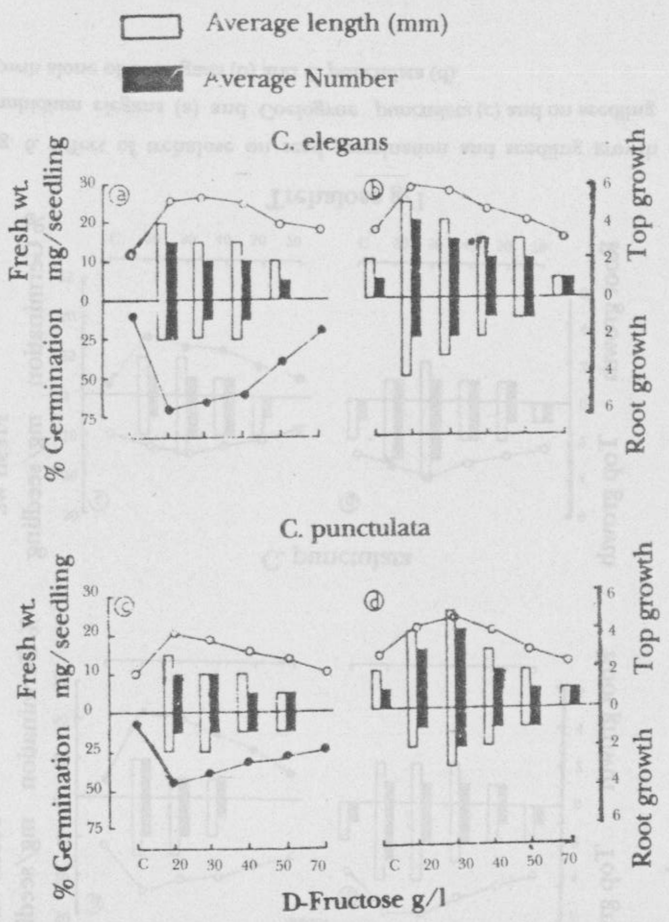

Fig. 4. Effect of D-frutose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans* (b) and *C. punctulata* (d).

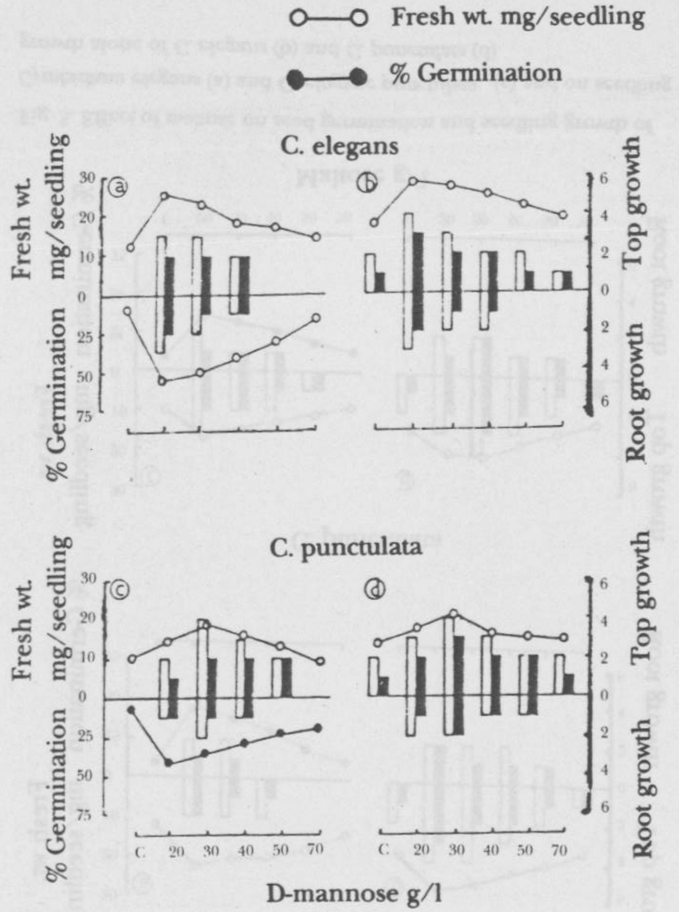

Fig. 5. Effect of D-mannose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans* (b) and *C. punctulata* (d).

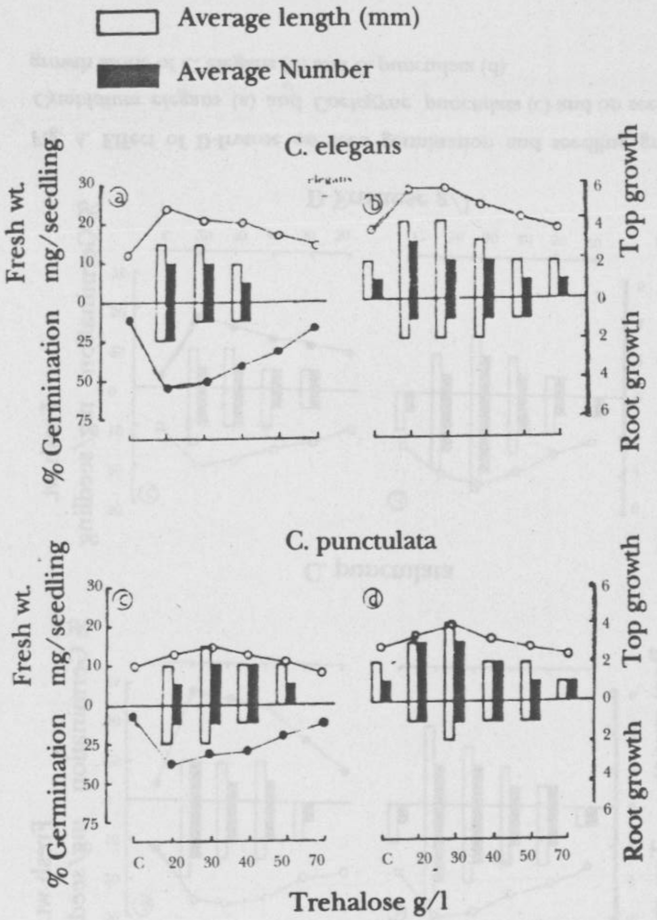

Fig. 6. Effect of trehalose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans* (b) and *C. punctulata* (d).

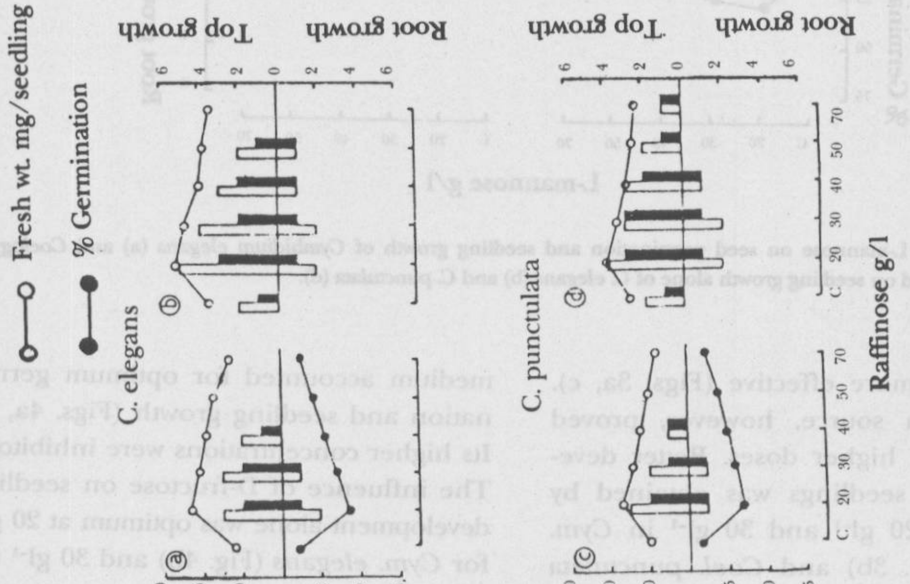

Fig. 7. Effect of raffinose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans* (b) and *C. punctulata* (d).

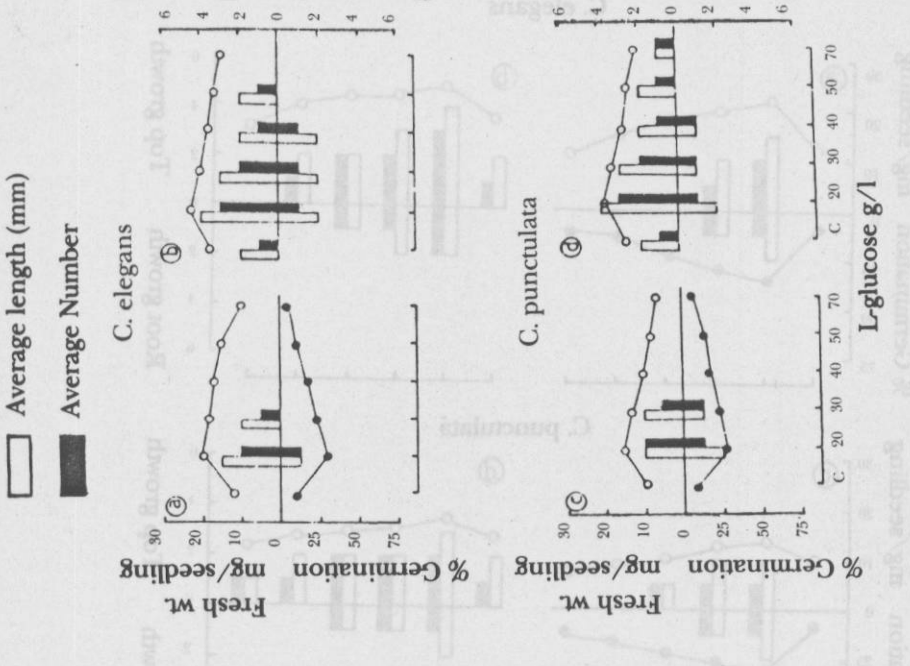

Fig. 8. Effect of L-glucose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans* (b) and *C. punctulata* (d).

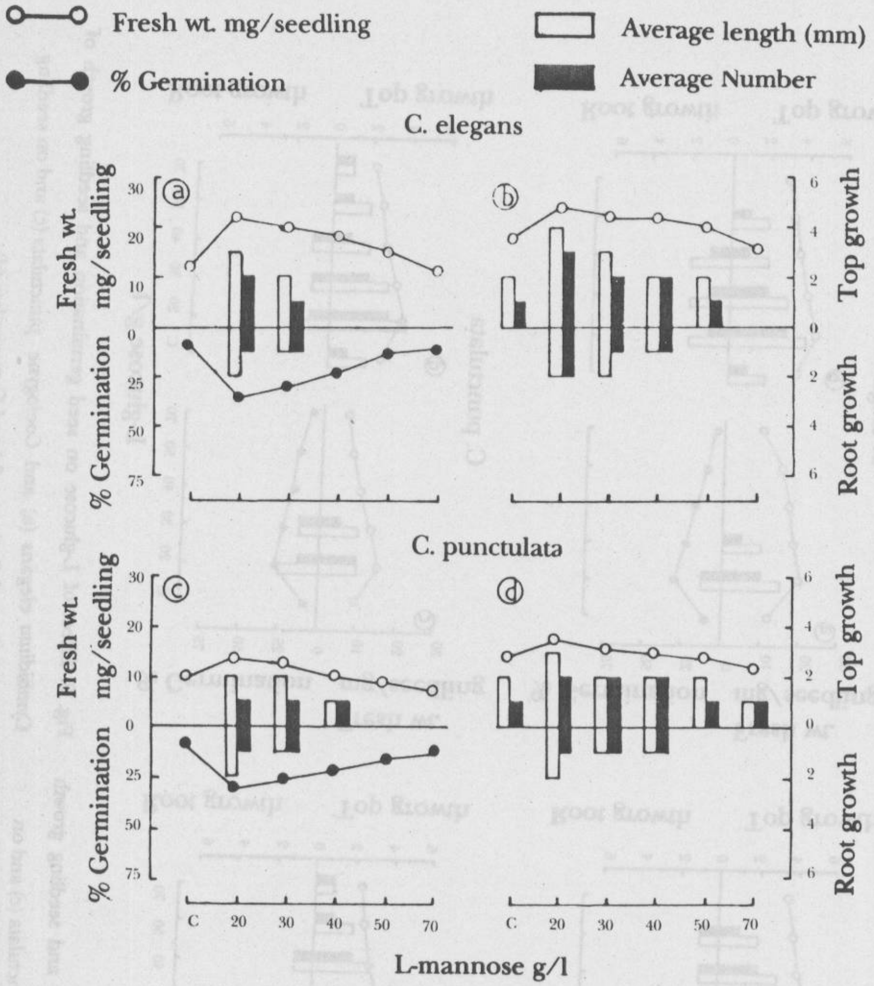

Fig. 9. Effect of L-mannose on seed germination and seedling growth of *Cymbidium elegans* (a) and *Coelogyne punctulata* (c) and on seedling growth alone of *C. elegans* (b) and *C. punctulata* (d).

gl^{-1} proved more effective (Figs. 3a, c). This carbon source, however, proved inhibitory at higher doses. Better development of seedlings was obtained by using it at 20 gl^{-1} and 30 gl^{-1} in *Cym. elegans* (Fig. 3b) and *Coel. punctulata* (Fig. 3d), respectively.

D-fructose : D-fructose at 20 gl^{-1} in the medium accounted for optimum germination and seedling growth (Figs. 4a, c). Its higher concentrations were inhibitory. The influence of D-fructose on seedling development alone was optimum at 20 gl^{-1} for *Cym. elegans* (Fig. 4b) and 30 gl^{-1} for *Coel. punctulata* (Fig. 4d).

D-mannose : The influence of D-mannose on germination and/or seedling growth was optimum at 20 gl^{-1} in *Cym. elegans* (Figs. 5a, b) and 30 gl^{-1} in *Coel. punctulata* (Figs. 5c, d). A decline in growth of seedlings was recorded with further increase in the level of this sugar in the medium.

Trehalose : With 20 gl^{-1} trehalose in the medium, maximum germination was recorded (Figs. 6a, c). *Cym. elegans* seedlings failed to develop in combination containing 50 gl^{-1} and 70 gl^{-1} trehalose whereas similar results were obtained but only at the latter concentration of the sugar in *Coel. punctulata.* In *Cym. elegans* (Fig. 6b), the seedling development alone was maximum at 20 gl^{-1} whereas in *Coel. punctulata* (Fig. 6d) it was highest in the medium containing 30 gl^{-1} trehalose.

Raffinose : Raffinose at 20 gl^{-1} stimulated better germination and seedling growth (Figs. 7a, c) as compared to the controls but the effect was, in general, poor in raffinose supplemented media. At 50-70 gl^{-1} of raffinose, the germination was significantly reduced, and the seedling development completely suppressed. On the other hand, the influence of raffinose was better on seedling development alone as compared to its effect on both the germination and seedling growth collectively (Figs. 7b, d).

L-glucose : L-glucose served as a very poor carbohydrate source during germination and seedling growth (Figs. 8a, c). Its influence on seedling growth alone was, however, maximum at a concentration of 20 gl^{-1}. Higher concentrations of this sugar resulted in poor growth of the seedling in both the species (Figs. 8b, d).

L-mannose : L-mannose was likewise a very poor carbohydrate source for germination and seedling growth (Figs. 9a, c). It, however, promoted development of seedlings, when used at 20 gl^{-1} (Figs. 9b, d).

Discussion

The ability of germinating seeds and young seedlings to utilize various sugars and other carbohydrates in orchids has been tested by several investigators (Downie, 1949; Yates and Curtis, 1949; Knudson, 1950; Liddell, 1943a, b; Ernst, 1967; Nakamura, 1982; Van Waes and Debergh, 1986). While a majority of the species can utilize a wide variety of sugars as carbon source, organic acids and L-series of sugars are of little value for the purpose. Certain species, although able to utilize many different sugars, do show some preferences. On the other hand, some species fail to germinate unless supplied with a specific sugar or combination of sugars in the medium. It is possible that these differences could be due to specific requirements for selected sugars.

Germination, seedling growth, and number and length of leaf and root were significantly affected by the quality and quantity of carbohydrate source in the present cultures of *Cymbidium elegans* and *Coelogyne punctulata.* Sucrose, D-glucose, and D-fructose, at lower concentrations, were found most effective for germination and seedling growth, whereas maltose, mannose, raffinose, and trehalose were moderately useful for the purpose. The processes were variously impaired in sugar free or L-glucose and L-mannose supplemented media. L-series of carbohydrates were similarly reported as unsuitable energy source whereas D-fructose proved best for the purpose in seedling cultures *in vitro* (Ernst, 1967b). Galactose was growth inhibitory in *Dendrobium nobile* (Quednon, 1930) and *Phalaenopsis* hybrid (Ernst *et al.*, 1971). Glucose, if present at an adequate concentrations, eliminated the galactose suppression in tomato-explant growth (Hughes and Street, 1974). Raffinose, which has galactose and glucose as its constituents, allowed poor growth in *Galeola septentrionalis* (Nakamura, 1982). *Goodyera repens* showed comparable growth on glucose, fructose, sucrose and trehalose (Purves and Hadley, 1975). However, it did not germinate or grow on mannitol. Ernst (1967) reported growth of a *Phalaenopsis* cultivar on mannitol but carbohydrate free controls were not used.

Non-infected orchid tissue are capable of using trehalose as a carbon source (Smith, 1967) and *Dactylorhiza purpurella* germinated and grew equally well in trehalose and glucose supplemented media (Smith, 1973). For the obtained results to constitute positive evidence that trehalose is a transfer compound it would have to be shown that it was absorbed without external hydrolysis as suggested by Ernst *et al.* (1971). Smith (1973) demonstrated both uptake and metabolism of glucose and trehalose by leaves of *Bletilla hyacinthina.* Hadley (1984) showed that the non-infected protocorms of *Goodyera repens* continued to take up (^{14}C) glucose at a very low rate.

The present findings revealed that both *Cymbidium elegans* and *Coelogyne punctulata* prefer sucrose, D-fructose, and D-glucose in the medium for better germination and seedling growth. This study alongwith influence of other growth factors will help in obtaining orchid seedlings in large numbers through asymbiotic seed germination.

Acknowledgements

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