Chapter 3

Effect of physical factors on growth and development of regenerants
Physical factors contribute greatly to the plant growth in vitro and much progress has been made toward a better understanding of their roles in recent years (Read, 1992). However, the available details on their influence on the physiology and development of orchid plantlets are few and far between (Arditti, 1982). Light requirements which may be subdivided into photoperiod and light intensity, vary considerably in orchids (Arditti and Ernst, 1984; Chow, 1986). Morphogenetic requirements for light in an in vitro system may be satisfied by one or both of these factors. However, only a few studies are available where these factors are considered separately (Hughes, 1981). It is well known that light exerts a marked influence on plant growth independently of photosynthesis (Economou and Read, 1986). Besides, it is important for regulating photomorphogenetic processes in tissue cultures in terms of duration, intensity and spectral quality (Murashige, 1978). Orchids appear to require light for induction or improvement of shoot and/or root formation (Ueda and Torikata,
Werckmeister (1971) working on Cymbidium observed that high light intensities were important for the development of shoots alone, but that darkness was necessary around the roots for the best results.

Early studies on the growth of plant tissues in culture indicated that the optimum temperature range was between 26 and 28°C in a number of cases but the requirements were found to differ considerably (Carew and Staba, 1965; Puchan and Martin, 1971). Though, a range of 32-35°C was found to be optimum for certain plants (Hughes, 1981), a temperature as low as 12°C was reported suitable for Streptocarpus (Appelgren and Hiede, 1972). The optimal temperature for growth and development of most orchid species is 20-25°C with the range extending from 6-40°C for seed germination (Arditti, 1967a,b; Mukherjee et al., 1974; Thompson, 1977; van Waes and Debergh, 1986; Lee et al., 1988). Tanaka and Sakanashi (1978) have reported influence of temperature on the morphogenic events in case of Phalaenopsis, with the flower stalk buds cultured at 25°C exhibiting position effects i.e., development of reproductive shoots from the upper nodes and vegetative shoots from the lower ones. At 28°C, however, all the buds were reported to develop into vegetative shoots irrespective of their positions.

Although little information is available about the influence of pH of a culture medium on in vitro morphogenesis, it is one of the important factors, as the growth promoting properties and the selectivity of the culture media are pH dependent. Changes in pH
in plant tissue culture systems have been reported by various workers (Eriksson, 1965; Butenko et al., 1984; Skirvin et al., 1986). Butenko et al. (1984) have attributed the media pH changes to the release of compounds from the plant material or uptake of particular nutrients such as ammonium, nitrate or phosphate ions. The pH values lower than 4.0 or higher than 8.0 have been found to be inhibitory for orchid growth in vitro (Arditti et al., 1982). No generalizations regarding different physical factors, however, can be made as different orchids show different requirements for their optimal growth and development.

**Materials and Methods**

The plbs subcultured on MS hormone-free medium for 3 passages as described in Chapter II were transferred to fresh MS medium supplemented with 3% (w/v) sucrose, 0.8% (w/v) agar without growth regulators. The cultures were then incubated in white, fluorescent light at a range of 1,000 - 4,000 lux (16 hr photoperiod) and at 8 - 24 hr photoperiod (2,000 lux) at 24±2°C to study the growth and development of D. wardianum regenerants in vitro. Besides, plbs were also maintained in complete darkness. The effect of temperature at light intensity 2,000 lux (16 hr photoperiod) was studied by incubating the cultures at a range from 15 - 35°C. The pH of the MS medium for the above experiment was adjusted to 5.8. To standardize the acidity level for optimum growth and development, the plbs were transferred to fresh MS media (as described above) having pH range of 4.5-7.5.
The cultures were incubated at 24±2°C under 16 hr photoperiod of 2,000 lux light intensity. Growth parameters studied were number and length of shoot and root and their fresh and dry weights. Data were collected every 30 days for 3 months. Five replicates were taken for each treatment and the experiments were repeated twice.

Results

Effect of light

i) Light intensity - Light was promotory in conversion of plbs to plantlets (Fig. 5). Growth and development of plantlets enhanced from 0 to 2,000 lux light intensity, declining thereafter (Fig. 7). Highest fresh and dry weight were recorded at 2,000 lux light intensity, however, extremes of 0 and 4,000 lux were observed to inhibit the growth of the regenerants.

ii) Photoperiod - The increase in photoperiods from 0 to 16 hr resulted in an increase in growth of regenerants with highest shoot, root number, length, fresh and dry weights observed in regenerants formed at 16 hr photoperiod (Fig. 6). Total darkness and continuous illumination were, however, found to be inhibitory (Fig. 8).

Effect of temperature

Growth of regenerants was observed to increase from 15 to 25°C
Fig. 5 Effect of different light intensities (0 - 4,000 lux) on growth and development of regenerants. Bar and line represent shoot/root length and number respectively.
Fig. 5
Fig. 6 Effect of different photoperiods (0 - 24 hr) on growth and development of regenerants. Bar and line represent shoot/root length and number respectively.
**Fig. 6**

The figure shows a comparison of shoot and root length (cm) and shoot number over different days (30, 60, 90) for three conditions: 0-60, 60-90, and 90-120. The data is represented as bars with standard errors. Additionally, the graph includes fresh weight and dry weight data for different time intervals (0, 8, 12, 16, 20, and 24 HR).
Fig. 7 Regenerants developed at different light intensities [0 (a), 1,000 (b), 2,000 (c), 3,000 (d) and 4,000 (e) lux] (after 90 days).

Fig. 8 Regenerants developed at different photoperiods [0 (a), 8 (b), 12 (c), 16 (d), 20 (e) and 24 (f) hr.] (after 90 days).
after which a decline in growth was recorded (Fig. 9). Shoot and root number and length, fresh and dry weights were recorded to be highest at 25°C. Poor growth and development of regenerants, however, was observed at higher temperatures (Fig. 11).

**Effect of pH**

Growth and development of regenerants varied markedly due to different pH levels of the medium (Fig. 10). Optimum growth was observed at pH 6.0 (Fig. 12). Fresh and dry weights were observed to be at their maximum at pH 6.0 with the development of green and healthy plantlets after 90 days of culture. Both pH 5.5 and 6.5 were also found suitable for the growth and development of the regenerants. Low pH levels (>5.0) were inhibitory. At pH 4.5, thick, stout, stunted bodies were developed. A pH range higher than 6.5 was also inhibitory for the growth and development of the plantlets.

**Discussion**

Environment exerts an important effect on the physiology and development of orchids (Arditti and Ernst, 1984). The influence of light intensity seems to be related to species, with some benefitting from high intensities, others responding to intermediate levels, while still others best cultured under low light or darkness (Thorpe and Murashige, 1970; Miller and Murashige, 1976; Papachatzi et al., 1981). Murashige (1974) reported that optimum light intensities for plant tissues in
Fig. 9 Effect of different temperatures (15 - 35°C) on growth and development of regenerants. Bar and line represent shoot/root length and number respectively.
Fig. 9
Fig. 10 Effect of different pH values (4.5 - 7.5) on growth and development of regenerants. Bar and line represent shoot/root length and number respectively.
Fig. 10
**Fig. 11** Regenerants developed at different temperatures [15 (a), 20 (b), 25 (c), 30 (d) and 35 (e)°C] (after 90 days).

**Fig. 12** Regenerants developed at different pH [4.0 (a), 4.5 (b), 5.0 (c), 5.5 (d), 6.0 (e), 6.5 (f), 7.0 (g) and 7.5 (h)] (after 90 days).
culture may differ from the requirements of the plants themselves growing in nature. Though light intensity of about 400-500 lux was reported favourable for the growth and development of Calanthe discolor (Hasegawa et al., 1978), in case of Arundina bambusifolia it was 3000 lux (Mitra, 1971). Werckmeister (1970a,b, 1971) has indicated a range of 400 - 5,000 lux light intensity suitable for the growth and development of Cymbidiums. In our study, 2,000 lux was found to be optimum for the morphogenesis followed by 3,000 lux light intensity.

The effective photoperiod for morphogenesis varies between taxa (Hughes, 1981). In the case of orchids, these have been found to vary from none at all (i.e. complete darkness, Morel, 1971) to continuous illumination (Wimber, 1963). Homes et al., (1971a,b) have reported a photoperiod of as much as 23.5 hrs for the development of Cymbidium protocorms. A range of 12 - 16 hr photoperiod has been observed to be beneficial for the optimum growth and development of a number of orchid species (Mitra, 1971; Hasegawa et al., 1978). Parallel to their study, we have found 16 hr photoperiod to result in optimum growth in D. wardianum. Light-triggered induction of morphogenesis may be related either directly or indirectly to the increased accumulation of starch in specific cells through the photosynthetic processes which may serve as a readily available source of energy for shoot production (Thorpe and Meier, 1972). A concomitant increase in growth with photoperiod could be due to improved metabolic processes besides higher photosynthetic
activity (Ziegler et al., 1985). Also, an alteration in endogenous levels of growth inhibitors and promoters by different light levels are reported to influence growth and development (Economou and Read, 1986).

Optimum temperatures for the growth of plant tissue cultures have been investigated by several researchers and a range of 20-27°C has been employed most often. Influence of temperature on basic physiological processes such as respiration, and on cell and organ formation is well known (Read, 1992). For the growth and development of orchids, a range of 20-25°C is reported to be most suitable (Harvais, 1973; Stoutamire, 1974; Arditti et al., 1982; van Waes and Debergh, 1986). The present study also shows a temperature of 25°C to be optimum for the development of D. wardianum plantlets. An increase in the growth rate in the range 20-30°C could be due to an increase in their metabolic rate (Muire, 1982; Ziegler et al., 1985). Poor performance of the plantlets at extreme temperatures could however, be attributed to the loss of water from the medium as well as the living system. Bazzaz et al. (1970) have reported photosynthesis to be affected negatively above 30°C which may result in poor growth and development.

Plant tissue cultures are known to tolerate a wide range of pH and a value between 5.2 and 5.8 is most often used (Kruse and Patterson, 1973). Murashige and Skoog (1962) reported that a pH value of 5.7-5.8 is suitable for maintaining all the salts in soluble form, even with relatively high phosphate levels, and is
low enough to permit rapid growth and differentiation of the tissue. From the data obtained on the growth of *Ipomoea* suspension cultures under controlled pH regimes, Martin and Rose (1975) suggested that the influence of pH was through its effect on the utilization of ammonia and nitrate rather than from any general effect on cell physiology. Some orchid plantlets can tolerate acidity and grow well even at a pH of 3.3 to 3.7 (Ernst, 1967a,b; Miyazaki and Nagamatsu, 1965). The present study shows optimum growth of *D. wardianum* plantlets at pH 6.0. This could be attributed to better uptake of nutrients and water from the medium (Knudson, 1946; Ito, 1955). Conversely, injury of root cells, depression in the uptake of potassium and calcium at pH 4.5 and precipitation and/or non-utilization of iron compounds at higher pH values could be the reason for the detrimental effect of the extreme pH values on the growth and development of in vitro growing plantlets. Evidently, pH of the medium surrounding the roots can affect the growth of the plants by controlling the availability and rate of uptake of nutrients by plants.