Chapter 2

Literature review

Pecteilis sagarikii Seidenfaden is a deciduous terrestrial orchid which has botanically been classified in subfamily Orchidoideae, tribe Orchideae and sub-tribe Habenariinae. It is an endemic species of Thailand, is distributed in various regions, i.e. in the north, west, lower part of north-east and the east (Chaithanu, 1998; Thaithong, 2000). Recently, it has been reported that *Pecteilis sagarikii* have two forms which are different in the lip color, i.e. lip white with yellow at base and yellow lip (Thaithong, 2000).

General morphology, it has a short stem, 0.4 – 0.8 cm in diameter; 2–3 radical leaves laying on the soil surface, ovate or obtuse, 6 – 10 cm in width, 10 – 12 cm in length, succulent, dark green and glossy; raceme inflorescence with 6 – 12 florets (Figure 1), 12 – 18 cm in height, covered with 6 – 16 enveloping leaf bracts, 0.3 – 1.9 cm in width, 1.2 – 3.8 cm in length, flower 2.5 – 3.5 cm in width, petal and sepal white, lip white with yellow at base or yellow lip, 1.6 – 1.8 cm in width, 2.1 – 2.4 cm in length, spur cylindrical, white with green at tip, 0.3 – 0.4 cm in width, 3.9 – 4.3 cm in length, fragrant; tuber oval shape and brownish, 3 cm in diameter (Seidenfaden, 1973; Chaithanu, 1998; Thaithong, 2000), 4 – 12 light brown roots, 0.15 – 0.28 cm in diameter, and 2.9 – 8.1 cm in length (Chaithanu, 1998).

Chaithanu (1998) reported that the life cycle of *Pecteilis sagarikii*, yellow lip-form sprouted during March to April and continued to grow in vegetative period during April to July, flowered during July to September and then set pod during September to mid December, and rested during December to March.

Tubers are modified organs that have nutrient storage for growth of new plants in the next growing season. Some authors termed tuber in another name, i.e. sinker (Arditti, 1992; Kumazawa, 1956). For tuber formation, Kumazawa (1956) observed on the sinker development of *Pecteilis radiata*. It showed that the sinker originated as a lateral bud in the axils of the fourth and fifth leaves of a monopodial stem. The hypopodium of the bud elongates by a rib meristem and resembles an absorbing root without root hair. Finally a meristem organizes in the periblem near the second node and develops into a mature tuber that comprises three distinct regions, i.e. 1) the stalk, which is cauline in organization; 2) the neck, lying between the stalk and tuber having a cavity at the base of which is a terminal bud; and 3) the tuber, an adventitious root with root hairs on the apical rhizoderm. Chaithanu (1998) reported that *Habenaria rhodocheila, H. carnea and Pecteilis sagarikii* planted from tubers in the early sprouting stage, formed new tubers at the basal part of stem closed to the old tubers during the vegetative growth before their flowers occurred. Chanaken (2007) reported that new tubers of *Habenaria rhodocheila* and *H. malintana* developed from lateral buds of the first or the second node at the basal part of a stem that covered with small leaf bracts.

Figure 1 *Pecteilis sagarikii* Seidenfaden inflorescence.

Rasmussen (1995) has elaborated on tuber formation of the terrestrial orchids originated in the temperate zone. In *Spiranthes* species, all roots are tuberous when young; these tubers are formed like other adventitious roots that occurred below a node. They have their anatomy similar to other roots, but serves as storage organs. This thickened fleshy root with no stem tissues incorporated was termed root tuber (Lavarack, 1971). Whereas, the tubers formed in the orchidoid orchids such as *Orchis, Platanthera, Dactylorhiza* and *Ophrys* have different anatomy and are almost entirely storage organs. This tuber has first originated as an axillary shoot and follows by geotropic extension of the basal tissue below the axillary shoot meristem resembling an adventitious root growth to form a complete tuber. Thus, the tuber is always formed in the axil of a leaf or scale leaf and has an apical bud. It is termed root-stem tuber or tuberoid. Because most of the root-stem tuber is anatomically root-like but the proximal pole has the organization of a stem, or it comprises a sheath of root structure around a core of stem structure with a bud (Pridgeon and Chase, 1995). The young tuberoid has produced an extended structure called tuber stalk, which serves both to adjust the optimal depth for the developing tuber and new plant in the next growing season, and also shifts a new tuber to new soil at some distance from the mother plant. The tuber stalk has been termed in another name, i.e. attachment-tube. The ontogeny of the tuber stalk varies, being formed either by an expansion of the basal tissue between the mother stem and the bud to generate a solid stalk, e.g.

Dactylorhiza, *Platanthera and Galearis spectabilis;* or by the bud meristem becoming concave to form a deep cup at the bottom of which the youngest leaf primordia of the bud are located to form a hollow stalk, e.g. *Aceras anthropophorum, Himantoglossum hircinum, Ophrys holoserica, Orchis mascula, Orchis militaris, Orchis pallens* and *Serapias*. In addition, an unusual anatomical characteristic of the tuberoid usually has more than one stele. Slender tubers such as those formed by *Galearis spectabilis* probably never contain more than one or two, but some species can form up to about 50 steles. There is no direct correlation between the number of steles and the number of finger-like extensions in the palmately shaped tuber. However, the first tuber is formed in the young plant contains only one stele, and more steles will be added annually in those species that develop polystelic tubers (Rasmussen, 1995). Pridgeon and Chase (1995) explained anatomy of steles in different plant organs that the stele of root comprises a one-layered pericycle, alternating groups of xylem and phloem, and a parenchymatous pith; while the stele of stolonoid root is either a dictyostele with two or three rings of collateral vascular bundles enclosed by the pericycle; and the stele of root tuber comprises a one-layered pericycle of elliptical to polygonal cells surrounding alternating groups of xylem and phloem.

According to Pridgeon and Chase (1995) concerning the studies on the morphological and anatomical nature of the terrestrial orchids in Australia and New Caledonia, including 145 species in 37 genera in the tribes Diurideae and other two genera, i.e. *Spiranthes* and *Disa*, revealed that their underground organs which were called "root-stem tuberoid" were described as root tubers, except for the stem tuber of *Rhizanthella*. The root tubers are formed on either droppers or stolonoid roots which are bounded by 1 – 4 layered velamen and exodermis, whereas the droppers and stolonoid roots may have velamen-exodermis or a simple epidermis depending on the taxon, and often bear multiseriate or uniseriate trichromes.

Rasmussen (1995) has elaborated on life cycle and underground organ formation of some terrestrial orchids. They were divided into 2 groups according to their types of storage organs, i.e. rhizome and root-stem tuber, and then divided into subgroups depending on organ development sequence from a protocorm to a mature plant. The tuber of *Galearis spectabilis* appeared in May, at the same time as the leaves, and reached its full size in late autumn, when the leaves and the old roots were disappearing. In *Orchis mascula*, the young tuber began to develop as bud when the slender roots emerged and the leafy shoot died down in autumn. After that, it broke through the leaf sheath in January. When the new rosette leaf sprouted in spring, the young tuber had reached its full length but continued to increase in girth while the plant produced leaves until its flowers opened. In a similar manner, the young tuber of *Aceras anthropophorum* became visible in winter and attained full size in about 6 months, and then served as a storage organ until the following summer. In many terrestrial orchid species, the tubers swelled after the leafy shoots appears, and presumably are filled with the products of photosynthesis; while some species, the first tubers are produced before the shoot emerges above ground.

Both excavation of plants in natural populations and observation of cultivated specimens have their limitation in methods of investigating the life history. Studies on growth and development of the cultivated plants have advantages that the exact age of the seedlings is known. Moreover, growth conditions can be provided to stimulate seasonal changes in different light and temperatures, resembling the

conditions which organogenesis is regulated in nature. However, this approach also has its shortcomings, since the cultivated seedlings are in a protected environment, so the growth rate and organogenesis are probably much faster than they would be under natural conditions. The life history as described on the basis of the cultivated material is usually shorter than that estimated from the studies of excavated material. Moreover, during the early life stages of *in vitro* cultivation, the development of shoot and root can be altered by the addition of growth regulators into the culture medium. In addition, developmental events such as the termination of the protocorm stage, the unfolding of leaf, the emergence of roots and the development of tuber, largely coincide in all individuals, and at least some of them must be synchronized by external signals. However, in the regulation of events that lead to progression in the life history, there must be an interaction between environmental stimuli and endogenous conditions such as chronological age, size and past history. Many tuberous orchids in various genera such as *Anacamptis, Barlia, Dactylorhiza, Gymnadenia, Orchis, Ophrys* and *Serapias* tend to produce tubers from more than one of the basal buds, both *in vivo* culture and under good conditions in nature. The extra daughter tubers may form at some distance from the mother plant. If the daughter tubers are separated from the mother plant, this serves to stimulate the proliferation further (Rasmussen, 1995).

The physiology of terrestrial orchids especially in the tropical terrestrial orchids has not been studied extensively, but the available information indicates that basically it is similar to the other orchids.

Sugar is very important component in any nutrient medium and essential for *in vitro* growth and development, because the photosynthesis *in vitro* is insufficient to support their growth and development (Pierik, 1977 and Rasmussen, 1995), due to the growth taking place in conditions unsuitable for photosynthesis, or without photosynthesis when cultured in a dark condition (Pierik, 1977). In addition, the carbon dioxide concentration in the test tube can be limiting for photosynthesis (Pierik, 1977). The light energy is absorbed by chlorophyll pigments in chloroplasts and converted into chemical energy through a process called photophosphorylation. The products of photophosphorylation, adenosine triphosphate (ATP) and reduced niacinamide dinucleotide phosphate (NADPH) are used as energy to fix carbon dioxide via three pathways, i.e. C_3 pathway, C_4 pathway and Crassulacean acid metabolism pathway (CAM). Only C_3 and CAM are found in orchids (Arditti, 1992; Hew and Yong, 2004), e.g. C_3 in *Habenaria platyphylla, Arundina graminifolia, Eulophia keithii*, or CAM in *Calanthe vestita*. The important product of photosynthesis is sucrose, and the intermediate substances during these reactions can be utilized in the synthesis of other compounds or used as sources of energy and respiration (Arditti, 1992).

Asymbiotic orchid seedlings cultured on the medium without any kind of sugar do not grow, or the seedlings may just reach beyond the stage of germination, the need for exogenous sugar arises after the seeds have germinated (Rasmussen, 1995). However, at a certain stage in their growth, orchid seedlings have no longer required an exogenous source of sugar. From the work of Arditti and Ernst (1984) *Cattleya aurantiaca* seedlings on the medium without sugar grew to the protocorm stage, but did not produce roots and leaves. But when the protocorms had been cultured for 28 to 30 days on the medium with sugar, and transferred to the medium

without sugar, they could form leaves and developed into larger plantlets. In addition, the chloroplasts of the plantlets cultured in the sugar-free medium were in thick clusters, peripheral vesicles, thylakoids that formed misshapen grana, and numerous osmophilic globules, but had no starch grains. When sugar was added, the structure of the chloroplasts became normal within 24 hours and maximal starch accumulation occurred within four days. The most commonly used sugar in orchid seed and seedling culture is sucrose. It is important mobile form of sugar in plant that can be transported via phloem of vascular tissues to other organs (Arditti, 1992). However, sucrose is partially decomposed into glucose and fructose if the medium is heat sterilized during preparation (Rasmussen, 1995), and changing in pH during autoclaving (Pierik, 1977).

The osmotic potential of a nutrient medium is the sum of the osmotic potentials of the agar and other constituents such as minerals and sugars. Sugar undoubtedly has relatively higher influence on osmotic potential compared to macro elements. If the osmotic potential is greater than approximately $3x10^5$ Pascal or 3 bar, growth and organ formation are stopped, due to the cessation of water uptake (Pierik, 1977). Proliferation of protocorms is enhanced by supraoptimal levels of sucrose, whereas organogenesis is enhanced at suboptimal concentrations (Arditti and Ernst, 1984). Concentration of sucrose at 1–5 % is generally used for *in vitro* culture although glucose and fructose may also be used instead, but differently resulted in its osmotic potential. The sucrose concentration used is depending on the type and growing age of plant material. Very young embryos require a relatively high sucrose concentration (Pierik, 1977). In orchid tissue culture media, sucrose at 2–5 % are generally added; but in some procedures, intermediary steps are even carried out without added sugars (Arditti and Ernst, 1993). Growth and development generally increase with increased sugar concentration, until an optimum is reached, and then decrease at high concentration. The growth of whole plants such as orchid seedlings is also greatly influenced by the sugar concentration (Pierik, 1977). In addition, the uptake of ammonium, nitrate and phosphate by the roots of young *Dendrobium* Multico White, *Aranda* Tay Swee Eng and *Oncidium* Gower Ramsay seedlings, growing on the Vacin and Went (1949) solid medium are enhanced when the culture media are supplemented with sucrose; and the uptake of ammonium is faster than the uptake of nitrate. However, the studies in some orchid tissue culture, i.e. *Aranda, Cymbidium and Dendrobium,* showed that the peripheral layers of cells are involved in sugar uptake and overall rate is determined to a large extent by their surface areas to volume ratio (Hew and Yong, 2004). A study on photoautotrophic *in vitro,* under CO_2 enrichment of *Dendrobium* seedlings cultured on Vacin and Went (1949) medium with or without sucrose at 2 %, in ambient air or CO_2 enriched atmosphere at 40 g/m^3 provided almost equally in shoot length and the number of leaves; whereas the fresh weight and dry weight were higher in the cultures grown in sucrose containing medium, or under CO_2 enrichment condition (Mitra *et al., 1998)*. According to Debeljak *et al*. (2002), the ten-week-old seedlings derived from *in vitro* symbiotic germination of a tuberous terrestrial orchid, *Pterostylis sanguinea* cultured in an oatmeal agar medium supplemented with 0.5 % sucrose and jasmonic acid at 5 μM or 1.0 % sucrose and jasmonic acid at 10 μM gave the highest frequencies of *in vitro* tuber formation. The accumulation of starch after germination is a characteristic of orchids, occurred particularly in asymbiotic seedlings provided with external

sources of sugars. In symbiotic seedlings of *Goodyera repens*, starch deposits in smaller quantity than in asymbiotic seedlings, while the growth rate of the symbiotic seedlings is considerably higher (Rasmussen, 1995).

Coconut water or liquid endosperm of the coconut is important complex substances that have been used extensively to add into *in vitro* culture medium for many plants to promote obvious growth, especially in orchid culture. It contains a wide spectrum of biochemical substances that can act individually as growth factor or synergistically. It is widely used at concentrations about 5–20 % by volume (Arditti and Ernst, 1993; Pierik, 1977). The ability of coconut water to promote remark growth in orchid embryo culture has been known for many years. It is now generally recognized as a rich source of cytokinin which has been identified as 9-β-D-ribofuranosylzeatin. This compound appears to be large proportion of the cytokinin activity in *n*-butanol extraction (Letham, 1974). One such substance isolated from coconut water, was 1,3-diphenylurea, showing cytokinin-like activity (Arditti and Ernst, 1993); but it exhibits very weak cytokinin activity (Letham, 1974). In addition, coconut water is a source of important sugars as sucrose, glucose and fructose, and also sugar alcohols as mannitol, sorbitol and myo-inositol. Moreover, it contains many kinds of amino acids and vitamins (Arditti and Ernst, 1993). Coconut water has produced some striking results. If used together with auxins it strongly induces cell division in general plant tissue cultures (Pierik, 1977). In orchid cultures it has been reported to stimulate the development of tubers and roots (Rasmussen, 1995). Coconut water can induce protocorm proliferation in *Phalaenopsis* seedlings (Arditti and Ernst, 1993). Asymbiotic media with high content of coconut water, thiamine, pyridoxine or nicotinic acid have a tendency to induce excessive branching in the seedlings of European terrestrial orchids (Rasmussen, 1995). However, it has been reported that most plants are able to synthesize vitamin *in vitro* (Pierik, 1977).

Plant growth regulators are organic compounds naturally synthesized in higher plants; they also include synthetic compounds, which influence growth and development. They are usually active at different sites in plants, apart from where they are produced and are only present and active in very small quantities (Pierik, 1977), e.g. auxin produced by shoot apices and transported down to inhibit the growth of lateral buds, known as apical dominance (Arditti, 1992). In addition, auxin plays a minor role in the initiation of flowering (Wikipedia, 2007). In aseptic culture of higher plants, growth regulators whether auxin and/or cytokinin added to a nutrient medium to obtain cell extension and/or cell division is completely dependent on type of explant and the plant species. The natural auxin, IAA is added in a concentration of 0.01–10 mg/l. Auxins generally induce cell elongation and enlargement, adventitious root formation and sometimes in the promotion of seedling growth. In contrast, they inhibit adventitious and axillary shoot formation (Pierik, 1977). Most orchid tissues require auxin and/or cytokinin for growth, formation of callus or protocorm-like bodies, proliferation, and plantlet development. IAA at 1.8 mg/l has helped to produce optimal fresh weight and vigorous protocorm-like bodies in *Cymbidium* tissue culture. The bee orchid, *Ophrys apifera* seedlings cultured on a medium containing IAA at 0.5 mg/l and kinetin at 0.5 mg/l formed callus and protocorm-like bodies, and developed to plantlets on the medium without growth regulators. Tuber sections of *Ophrys fuciflora* and *O. apifera* were taken during the rest periods in July, cultured on a modified Murashige and Skoog medium containing

NAA and kinetin, each at 0.0005 mg/l formed callus masses which subsequently produced protocorm-like bodies. Tuber slices of 6-month-old *in vitro* seedlings of terrestrial species distributed in India, Sri Lanka and south China, i.e. *Pachystoma senile* formed callus in the Mitra-Prasad-Roychowdhury medium containing 1 g/l peptone and 1 mg/l NAA, or only 2,4-D at 1 mg/l, whereas protocorm-like bodies were formed in the medium without growth regulator, but supplemented with yeast extract at 1 g/l. They subsequently developed to plantlets when transferred to the medium containing IAA at 1 mg/l (Arditti and Ernst, 1993). In addition, indole acetic acid and potassium ions can stimulate the production of *in vitro* mini-tuber of *Ophrys lutea* (Rasmussen, 1995). Studies on *in vitro* symbiotic germination of some terrestrial orchids showed that many mycorrhizal orchid endophytes are able to produce some indoleacetic acid. After an isolate of *Rhizoctonia* from *Ophrys lutea* had been grown in pure culture, the presence of IAA and indole-ethanol could be detected in both its mycelium and the substrate. Symbiotic protocorms of *Dactylorhiza incranata* contained 10 times as much auxin and cytokinins as asymbiotic protocorms after 10 days in culture; seedlings that were parasitized by a number of fungi contained even greater amounts of auxin, but less cytokinin (Rasmussen, 1995).

Cytokinins are used to stimulate cell division, growth and development. In higher concentrations at 1–10 mg/l, they can induce adventitious shoot formation. In addition, they promote axillary shoot formation by decreasing apical dominance (Pierik, 1977). In asymbiotic cultures cytokinins tend to stimulate shoot development and inhibit seedling root growth. Seedlings of a tropical *Cymbidium* hybrid were much more sensitive, root and rhizoid development being affected by low concentration at 1 mg/l, and at 10 mg/l it generally inhibits seedling development, including that of the shoot (Rasmussen, 1995). The synthetic N^6-benzyl aminopurine (BAP) is most widely used in orchid tissue culture. Leaf tissues from *Phalaenopsis* plantlets derived from flower-stalk nodes can be induced to produce protocorm-like bodies on a medium containing 1–5 mg/l BAP (Arditti and Ernst, 1993). It also enhances protocorm multiplication in *Cymbidium* (Arditti and Ernst, 1984). The addition of cytokinins such as BAP or kinetin has been found to improve seed germination of *Cypripedium calceolus, C. reginae* and *Epipactis helleborine*. However, browning and mortality in the seedlings of *Epipactis helleborine* increased, at a concentration of 2.2 μM (Rasmussen, 1995).

For another plant growth regulator, Hollick *et al*. (2002) observed on seedlings of southwestern Australia terrestrial orchids, i.e. *Diuris laxiflora, Microtis media* and *Pterostylis sanguinea* cultured symbiotically *in vitro* with their specific mycorrhizal fungi, on the media containing paclobutrazol at 0, 0.5, 1.0, 3.0 and 5.0 mg/l. It showed that paclobutrazol at all concentrations helped to increase tuber stalk production in *D. laxiflora*, but had no effect on *M. media* and *P. sanguinea*.

Abscisic acid (ABA) has a negative influence in plant growth. It induces dormancy, abscission, and senescence. In orchids, ABA can cause senescence and anthocyanin production in *Cymbidium* flowers. At concentrations of 250–500 ppm, it inhibits the development of new growth in *Cymbidium* and accelerates leaf senescence and abscission. ABA had been detected in the seed of some terrestrial orchids, where it could act as a germination inhibitor (Arditti, 1992).

Plantlets growing *in vitro* are similar to seedlings in that they require light for normal development as well as root and/or shoot production, and also their growth. However, plantlets grown under continuous darkness do not produce chlorophyll but turn green when moved them to culture in light (Arditti and Ernst, 1993). Epiphytic and some terrestrial orchid species can germinate both in light and in the dark, although they appear to require light for induction or improvement of shoot and/or root formation (Arditti and Ernst, 1984). Newly emerged seedlings of most terrestrial species are extremely light sensitive. They required complete darkness for the first few months after germination, or during the initial stage of culture. Seedling mortality above 85% was obtained in *Orchis morio* if the cultures were subjected to bright illumination within 24 weeks after germination, although they had already developed shoots (Rasmussen, 1995; Arditti and Ernst, 1993). Darkness is required for the cultures where protocorm proliferation is artificially induced, which is to be expected; since in the terrestrial species, the natural vegetative reproduction by budding usually occurs below ground. In addition, darkness promotes rhizoid formation on seedlings. Polarity and a response to gravity in terrestrial orchid seedlings can be established by using a substrate that is darkened with charcoal, and illuminating the cultures (Rasmussen, 1995).

The study on factors influencing *in vitro* tuber formation of the tropical terrestrial orchids has not been studied extensively. Apavatjrut and Phornsawatchai (1995) reported that the seeds from 5 – 9 weeks old pod of *Brachycorythis helferi* could germinate well on the modified Vacin and Went (1949) medium after sowing for 12 weeks, after that protocorms further developed to produce shoot, tuber and root, respectively. Nualkaew (2004) studied on seed germination and development of *Brachycorythis* sp., a small-size flower type, found that seed germination and also tuber formation could be obtained when cultured at 25 degree Celsius on the modified Vacin and Went (1949), the CMU_1 medium, supplemented with sucrose at 2 % and coconut water at 15 %. For growth regulator supplement, it was found that addition of NAA at 0.5 – 2.0 mg/l gave malformed growth of tuber while adding 0.5 mg/l BAP gave better tuber width but decreased in length. When BAP concentration was increased to 1.0 mg/l, tuber growth was suppressed, and BAP at 2.0 mg/l and higher concentration affected death of the seedlings after culturing for 7 weeks. Chaithanu (1999) reported that seeds of *Habenaria rhodocheila* and *Pecteilis sagarikii* sown on Vacin and Went (1949) liquid medium varying in the basal medium concentrations at 0.25x, 0.50x, 0.75x and 1x, and combined with glucose or sucrose at 0, 1 and 2 % had no significant difference on germination after culturing in darkness for 4 months. In additional, *Habenaria rhodocheila* seedlings having 2 leaves with 2x6 mm in tuber size, when transferred onto Vacin and Went (1949) medium having 15 % coconut water, 100 g/l potato extract, 2 % homogenized ripe banana, 0.2 % activated charcoal and 0.6 % agar, showed that adding sucrose at 2, 4 or 6 % with paclobutrazol at 0, 0.001, 0.01, 0.1 or 1.0 mg/l had no significant effects on number of tuber and tuber size after culturing for 3 months.

According to *in vitro* tuberization of another tuberous plant, i.e. potato plantlets, Ulloa *et al.* (1997) reported that the nodal explants of potato could produce some stolons when cultured on Murashige and Skoog (1962) medium supplemented with Gamborg B5 vitamins, 2 mg/l calcium panthothenate, 0.1 mg/l GA_3 and sucrose at 80 g/l. When the stolons were subsequently transferred onto the above medium

adding BAP at 5 or 10 mg/l, and 2-chloroethyltrimethyl-ammonium chloride (CCC) at 500 mg/l and cultured in darkness at 21±2 degree Celsius, complete micro-tubers could be induced. Gopal *et al.* (1998) found that the *in vitro* potato seedlings when cultured in short day photoperiod and low temperature conditions gave more number of tubers and fresh weight than those cultured in a long day photoperiod and high temperature. In addition, adding BAP at 10 mg/l into a culture medium gave more tuber yields in both tuber fresh weight and total fresh weight of the tubers per plant, after culturing for 60 days. Yu *et al.* (2000) reported that sucrose was a suitable carbon source for starch accumulation in potato micro-tuber. Moreover, both low and high concentrations of sucrose have affected in delaying micro-tuber formation and also decreasing in number of tubers and tuber size. Kefi *et al.* (2000) found that the nodal explants of potato cultured in a short day condition on the medium adding either thidiazuron at 0.1 mg/l or kinetin at 2 mg/l gave no significant difference in tuber inducing time and number of tubers.

In plant nutrient, the orchid requires various essential elements for normal growth. Nutrient deficient symptom may develop when the concentrations of the elements drop below the levels necessary for optimal plant growth. The element concentrations in most plant tissues have been extensively studied. The element compositions in plant tissues are determined by tissue analysis. The level of elemental content varies in different plant parts and stages of plant development. It has a relationship between plant growth and the mineral content in plant tissues (Hew and Yong, 2004). Chanaken (2007) found that the concentrations of mineral, starch and also sugar in different plant parts of four terrestrial orchids, i.e. *Phaius tankervilleae, Eulophia andamanensis, Habenaria rhodocheila* and *Habenaria malintana* had fluctuated depending on plant species and also their stages of growth and development. In addition, the mineral concentrations in the old plant parts, i.e. root and leaf obviously decreased during the new organs were developing, e.g. shoot, bud, flower and root development. The starch concentration obviously changed during sprouting of new shoot after a dormancy period.

Very few studies have been carried out on the nitrogenous substances in orchids. According to the changing in the level of nitrogen reported by Arditti (1992), the perianth and gynostemium of *Cattleya labiata* contain 7.5 mg and 2 mg per flower, respectively. The nitrogen content in the perianth dropped about 63 % of the initial level within 167 hours after pollination, while the level in the gynostemium increased about 161 % of the original content. In *Cymbidium tracyanum*, nitrogen level at 16 days after pollination was 160 % of the initial content in gynostemia, 155 % in ovaries, 43 – 100 % in the perianth, and 113 % in the whole flower. Among the amino acids, arginine and ornithine could serve as satisfactory nitrogenous sources for *Cattleya* seedlings cultured *in vitro*. Proline and γ-aminobutyric acid were moderately good sources of nitrogen. Phenylalanine, citrulline, tyrosine, aspartic acid, glutamic acid, glutamine and asparagine could not serve as nitrogen sources. While glycine, α-aminobutyric acid, leucine, phenylglycine, hydroxyproline, canavanine, and threonine were inhibitors. The contents of amino acids in the leaves of several orchid species were analyzed. It showed that asparagine was the dominant free amino acid in *Haemaria discolor*, *Cirrhopetalum ornatissimum* and *Cymbidium aloifolium*, while arginine was a dominant free amino acid in *Paphiopedilum insigne* and *Phalaenopsis*

amabilis, and both asparagine and serine were dominant in *Dendrobium kingianum* leaves.

In another bulbous plant, arginine and glutamic acid were the dominant free amino acid in the rhizome and storage root of *Curcuma alismatifolia*, respectively (Ruamrungsri *et al.*, 2001). In general, plants used asparagine as a nitrogen storage and transport compound. Most evidence suggests that ammonia is first incorporated into the amide position of glutamine and is then transferred to aspartate to yield asparagine. The synthesis of aspartate is probably via oxaloacetate from the tricarboxylic acid cycle. In addition, arginine is the major amino acid synthesized from glutamate, it is also able to regulate the rate of its own synthesis. The other source of carbon and nitrogen for arginine synthesis is carbamoyl phosphate which is synthesized from glutamine and also utilized for pyrimidine synthesis (Barrett, 1985).

The study on the changing of plant growth regulators in terrestrial orchids is known to be scarce. It has been reported in another bulbous plant, i.e. *Curcuma alismatifolia*; the level of free abscisic acid (ABA) in its stubbed rhizomes increased from the beginning of dormancy stage to the middle of dormancy stage and then decreased when entered the last stage of dormancy, and thence shoot bud sprouting occurred. Nevertheless, the level of ABA in the storage roots continuously decreased from the beginning to the end of its dormancy stage (Ruamrungsri *et al.*, 2001).

For plant dormancy, some terrestrial orchids can survive, overriding the unsuitable season by translocating the photosynthetic nutrient to the sink organ, i.e. tuber and then further convert to the nutrient reservoirs before the plant parts above ground dry out and enter into its dormancy stage completely (Bechtel *et al.*, 1986). The tuber contains salep, a polysaccharide which consists mainly of mucilage and starch. The mucilage is served for both water retention and reduces the freezing point of tuber tissue that results the tuber fairly drought and frost resistance, especially in the terrestrial orchids in the temperate zone (Rasmussen, 1995). The tropical terrestrial orchids especially in the deciduous group will be dormant when the dry season has come. However, they have a few differences, in timing of dormancy period depending on plant species, environment and culture conditions. Chanaken (2007) reported that *Habenaria malintana* was dormant during mid-December to mid-April and *H. rhodocheila* was dormant during November to March; while Chaithanu (1998) reported that *H. rhodocheila* was dormant between December to April, and *H. carnea* was dormant during mid-November to mid March and *Pecteilis sagarikii* was dormant for 4 months during December to March.