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濟州道內 組織培養室의 實態 및 改善方案에 關한 硏究

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Survey and Improvement of Commercial Tissue Culture Laboratory in Cheju-do, Korea

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Summary

The results from the survey of commercial tissue culture labs distributed in Cheju-do, Korea, and their improvements on lab organization, facility, equipment, environmental control, hardening facility and produres, etc. are summarized as follows.

The lab organization varies, depending upon a situation that a lab operator faces when a lab is constructed. However, no matter what situations are faced, three distinct lab areas, media preparation, transfer, and culture growing rooms, must be established for long term efficiency. Three basic models are suggested for lab sizes: 48.6 ㎡ (15 pyung) for less than 2 technicians, 81.0 ㎡ (25 pyung) for 3 to 4 technicians and 129.6 ㎡ for more than 5 technicians.

To use more spaces of the culture growing room, the movable shelf frames with 4-5 stacked shelves are recommended to be used in a relatively large tissue culture lab. 60-75 cm in height and 90-120 cm in width as an adequate shelf size are also recommended to reduce overheat from lamps and ballasts, and to precisely control the temperature of the culture growing room. As a material of the shelf board, perforated plywood or wire mesh is better for air circulation.

Most labs in Cheju-do were equipped with relatively enough equipments for plant micropropagation except a water purification system. A water distiller or other distillatory apparatus is strongly suggested to be purchased for a successful tissue culture program. Tap, subterranean, or even demineralized water is not good quality for tissue culture media.

Light and temperature as environmental factors should be precisely controlled. Humidity and air movement might not be ignored. Most labs in Cheju-do do not provide optimal light intensities to the cultures, less than 1,000 lux being inadequate. Intensities between 1,000 and 3,000 lux are

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recommended to be supplied for the cultures with photoperiods of 12 to 16 hours. Fluorescent lamps are most widely used for plant tissue cultures because they provide a high output of photosynthetically active and photomorphogenetic radiations. Temperatures are relatively well controlled in many tissue culture labs in Cheju-do by using air conditions. However, lab operators do not realize temperature gradients from bottom to top shelves, and inside and outside of culture vessels, which sometimes cause a critical danger to the cultures Humidity and aeration have been almost ignored in Cheju-do. However, 70 % relative humidity is better to be maintained for the precise temperature control. A downward pattern of air movement is recommended preferable to reduce the temperature gradients of the culture growing room.

Crops propagated by tissue culture were very limited to mainly orchids, banana, and pineapple. For successful tissue culture programs in the future, plant species must be diversified or highly advanced culture techniques should be developed for plant improvement.

Greenhouse as a hardening facility is most widely used in Cheju-do and hardening procedures should be also improved to enhance a survival ratio when transplanting the cultures to potting media. A special hardening room similar to the culture growing room is recommended to be constructed in order that light, temperature, and constant humidity can be easily controlled.

Introduction

Plant tissue culture is an important new method of plant propagation now readily available to growers and widely used for the commercial application in the propagation of horticultural species. Commercial tissue culture techniques which are widely used have two distinct reasons: mass-propagation and establishment and/or maintenance of virus-free stocks. Due to these reasons, tissue culture methods have been extensively applied to the orchid industry for the last two decades (Hu and Wang 1983). Plant tissue cultures provide the growers with several advantages of less time, space, labor, and cost than the conventional propagation method (such as vegetative cuttings) to produce a significantly greater member of healthier plants (Kyte 1983).

There was only one commercial tissue culture lab until 1976, but the numbers of the labs have been rapidly increasing in Cheju-do, Korea during the last decade (Fig. 1).

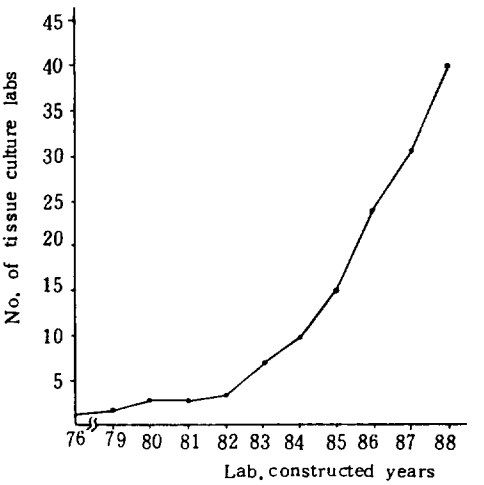

Figure 1. The trend of increase in tissue culture labs constructed from 1976 to 1988 in Cheju-do, Korea (as of July, 1988).

Now, totally more than 40 (commercial tissue culture) labs have already been or being built, as of July, 1988 in Cheju-do, Korea. Most of labs are located in Seoguipo city and south county, and only few labs in Cheju city and north county of Cheju-do (Fig. 2). Many growers are still inputing much money and planning to build the commercial labs. However, there are no guidelines to be consulted when constructing the labs. Many problems have being caused by already constructed labs. Therefore, this work is required to give the growers the guideline for the basic requirements of commercial tissue culture labs such as design, facilities, environmental conditions of the labs, etc., and to reduce the problems which will be caused in the labs to be constructed.

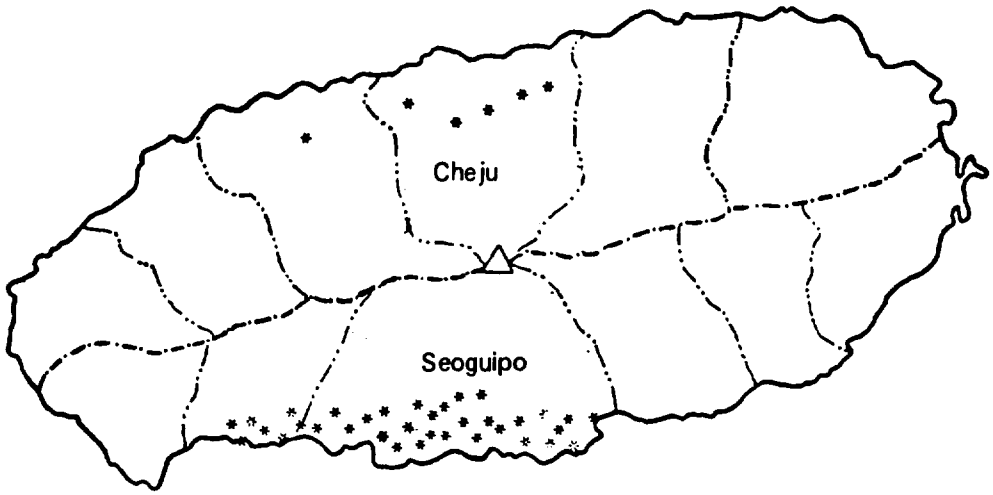

Figure 2. The locations of commercial tissue culture labs distributed in Cheju-do, Korea (as of July, 1988).

Survey

The survey of commercial tissue culture labs distributed in Cheju-do, Korea was made by K.C. Han, J.S. Lee, and S.H. Kim, who visited each lab to meet lab operator for the prepared questionaires in July, 1988. Total thirty commercial labs were surveyed to find the followings: total lab size and organization, design and structure of culture growing room, control of environmental factors, lab facilities, hardening facilities and procedures, major crops tissue-cultured in the labs, lab distribution in Cheju-do, lab constructed year, owner's personal informations. Some other labs which the locations were known were not surveyed because the surveyers could not meet lab operators when visited.

The informations callected from the survey (of questionaires) were analyzed and used to suggest the improvements of the labs constructed or to be constructed.

Results and Discussion

A) Commercial lab organization and facilities

The (mass) propagation of plants by plant tissue culture techniques does not per se require complex or expensive equipment. The extent to which more sophisticated apparatuses are necessary depends on the objectives of lab operators or the nature of culture techiques to be used (Biondi and Thorpe 1981).

No matter what objective of the operator and size of the lab are decided upon, three distinct lab areas must be established in the lab facilities: a media preparation room, a transfer room, and a culture growing room. In extreme or temporary circumstances, these three areas might occupy the same room, but long term operating efficiency and the need for cleanliness dictate each be in a separate room (Biondi and Thorpe 1981 and Kyte 1983).

From the planning stage on, careful provision for a clean environment is required if the tissue culture program is to be successful. Cleanliness is not only important in the sitting of a lab but the major consideration in its design. The cleanest prospective work flow and traffic pattern should govern the planning of room location, passage ways, work areas, doors, and pass-through windows. It may be desirable to plan an inexpensive, temporary setup to prove that the practice of tissue culture is appropriate, feasible, and applicable to the requirements of the nursery.

However, only a few labs were constructed for a new tissue culture lab in Cheju-do and most other labs were remodeled for the labs from family houses, warehouses, and citrus storage facilities. Therefore, the organization and facilities of the lab varied. The lab facilities in Cheju-do are characterized by 3 distinct types. The first type of tissue culture labs is composed of only one room playing combined roles of media preparation, transfer, and culture growing areas at the same time. The second type is consisted of two areas: one is for culture growing room and the other is for both media preparation and transfer room. The last type is made up of 3 distinct areas such as media preparation, transfer, and culture growing rooms in a separate room.

The average lab size in Cheju-do is 83.6 ㎡ (approx. 26 pyung) in total: the transfer room is 10.2 ㎡, the media preparation room 15.6 ㎡, and the culture growing room 39.8 ㎡ (Table 1). However, the labs, smaller than 50 ㎡ (approx. 15 pyung) are responsible for 46 percent, larger than 81 ㎡ (25 pyung) 30 percent, and inbetween 50 and 81 ㎡ 24 percent out of all tissue culture labs. The mean numbers of employees are 3 to 4 persons or technicians in each lab, as of July, 1988 (Table 1).

Basic models being suggested for the commercial tissue culture labs, the simplest design of small tissue culture lab for less than two technicians is shown in Fig. 3. The total lab size is 48.6 ㎡ (15 pyung). The media preparation room is 12.96 ㎡, the transfer room 6.48 ㎡, and the culture growing room 29.16 ㎡. The transfer hood can be widened to allow two technicians to transfer at the same time. Either entry can be a simple pathway or be developed further for storage, office, autoclave, water treatment, or restroom. In this design there are no upper cupboards because below bench access is available without overhanging cabinets. Windows, if desired, may be placed wherever

Table 1. Total lab sizes, mean sizes of 3 distinct lab areas (TR, MPR, and CGR), numbers of employees per lab (NEPL), and percentages of labs by size (PLBS) in Cheju-do, Korea (as of July, 1988).

Total size	TR[x] (㎡)	MPR[y] (㎡)	CGR[z] (㎡)	NEPL	PLBS (%)
A	6.8	9.7	24.3	2.6	46
B	8.1	17.2	34.7	3.0	24
C	17.2	25.3	69.2	4.8	30
D	10.2	15.6	39.8	3.3	

A: labs smaller than 48.6 ㎡
B: labs inbetween 48.6 and 81.0 ㎡
C: labs larger than 81.0 ㎡
[x]transfer room. [y]media preparation room.
[z]culture growing room.

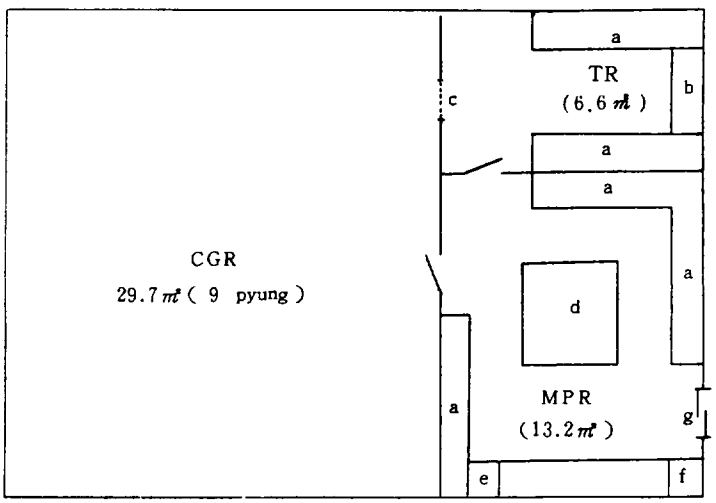

Figure 3. A basic model of commercial tissue culture lab for one or two tecnicians. CGR: culture growing room.
TR: transfer room. MPR: media preparation room.
a) counter. b) transfer hood. c) pass-through door.
d) work table. e) refrigerator. f) autoclave.
g) sliding glass door. h) sink.

convenient in the media preparation and the transfer rooms. There should be no windows to the outdoors in the culture growing room because outdoors light and temperatures reduce the ability to control the growing environment.

Fig. 4 shows a similar design to Fig. 3 but is for a somewhat larger operation for 3 to 4 technicians. The total size of the lab is 81.0 ㎡ (25 pyung). The media preparation room is 19.44 ㎡, the transfer room 9.72 ㎡, the culture growing room 45.36 ㎡, the storage room 3.24 ㎡, and the restroom 3.24 ㎡. The entry allows access to transfer and culture growing rooms with mimimal traffic through the media preparation room. Upper half glass doors provide access to and immediate visibility of the transfer and culture growing rooms.

Relatively large tissue culture lab is shown in Fig. 5. The lab can be operated by more than 5 technicians. The lab can be operated by more than 5 technicians. The total lab size is 129.6㎡ (40 pyung). It consists of 3 rooms--media preparation room (23.1 ㎡), transfer room (15.5㎡), and culture growing room (82.5 ㎡) and they are individually accessible from an outer hall or coridor. The media preparation room is supplemented with a media storage room (1.65 ㎡) and a general storage room (4.95 ㎡), and a restroom (3.3 ㎡). In the media preparation room, available counter space can be increased by a bench or work table in the middle of the room. The work table makes the technicians very convenient for preparing media and other works. All aisles and doorways should be wide

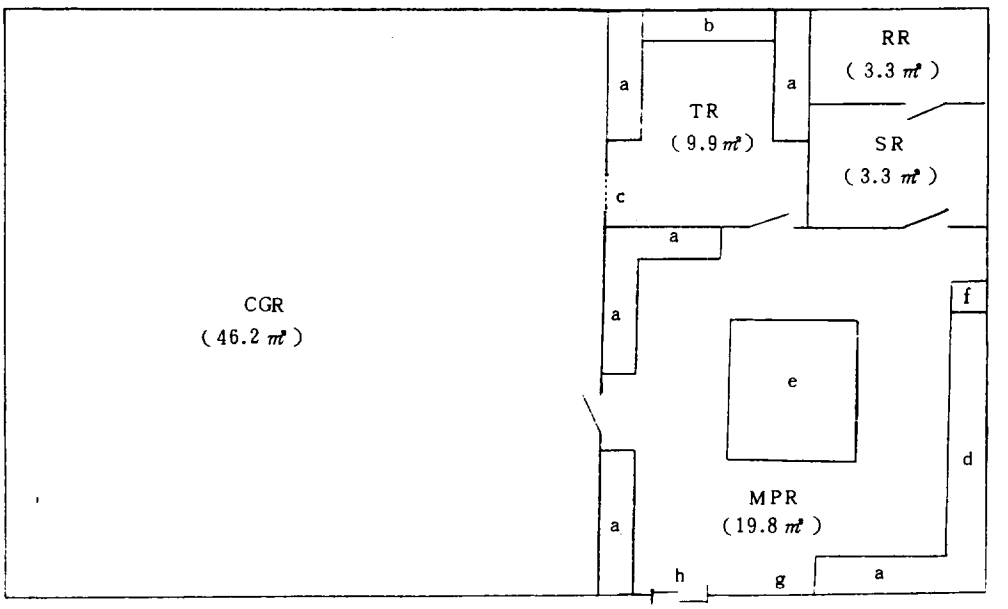

Figure 4. A basic model of commercial tissue culture lab for 3 to 4 technicians. CGR : culture growing room. TR : transfer room. MPR : media preparation room. SR : storage room. RR : restoom. a) counter. b) transfer hood. c) pass-through door. d) sink. e) work table. f) refrigerator. g) autoclave. h) sliding glass door.

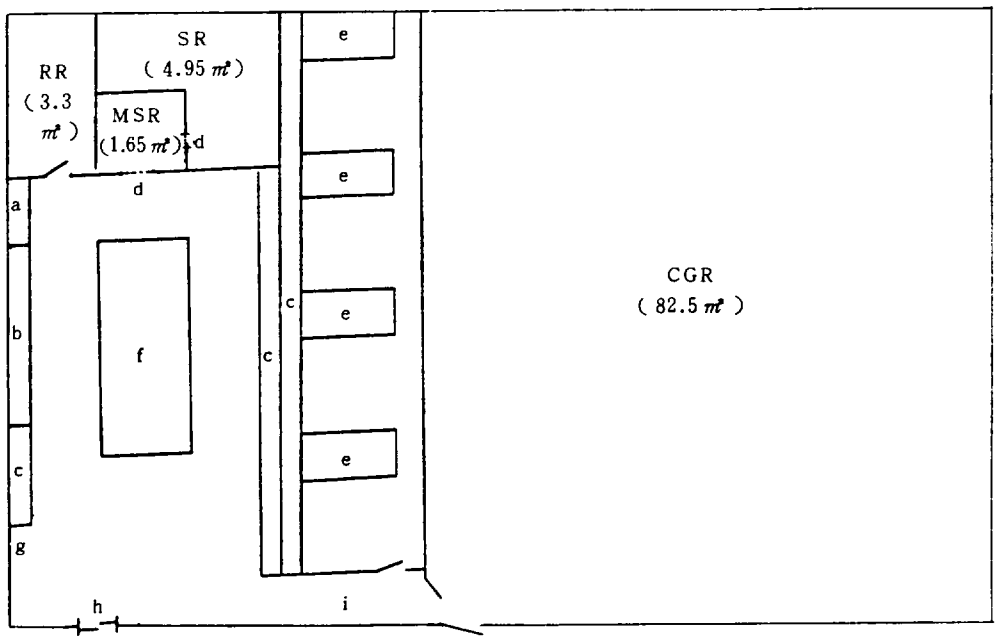

Figure 5. A basic model of commercial tissue culture lab for more than 5 technicians. MPR: media preparation room. MSR: media storage room. SR: storage room. RR: rest-room. TR: transfer room. CGR: culture growing room. a) refrigerator. b) sink. c) counter. d) pass-through door. e) transfer hood. f) work table. g) autoclave. h) sliding glass door. i) corridor.

enough to accommodate carts. The transfer room in Fig. 5 is designed for 4 technicians. The transfer hood is placed in a row to provide the technicians with enough individual work area as well as counter space. An alternative plan has two transfer hoods placed back-to-back in two locations in the room.

In all three model plans, pass-through windows between rooms are recommended. They save time by reducing traffic, they reduce contamination by airflow through opened doors, and they allow good visibility.

Fig. 6a shows the three dimentional views of a complete set of shelves (left) and a shelf frame (right) to hold culture vessels in the culture growing room. The shelf frame can be constructed by using slotted aluminum or steel plate as supports. When planning to construct nonmovable shelf frames, an adequate shelf size, 60~75 ㎝ in height and 90~120 ㎝ in width, is recommended. Depending upon the height of the culture growing room, 4~5 stacked shelves can be constructed in a frame. The length of the shelves depends upon the size of the culture growing room.

Perforated plywood or wire mesh is recommended to be used as shelf boards to allow better air circulation than solid materials. The shelf boards should be painted white to maximize available light. Two cool white fluorescent lamps can be istalled approximately 50~60 ㎝ apart in width just beneath the shelf oards and they will supply 1,000 to

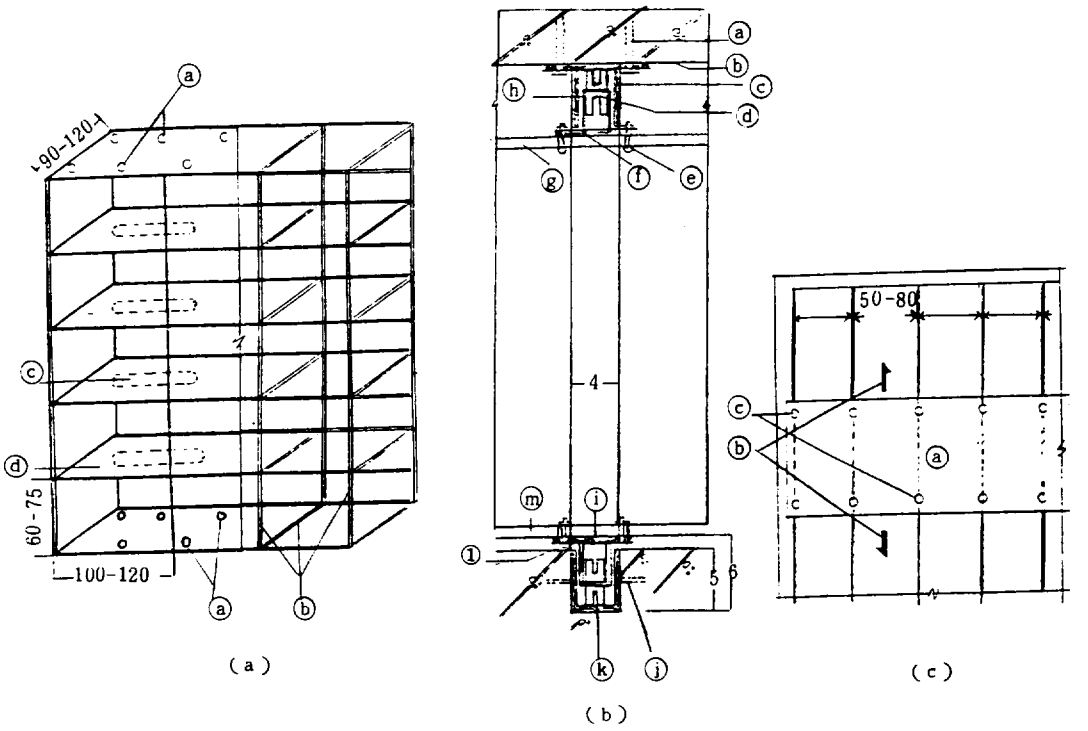

Figure 6. Three dimentional views of complete sets of shelves (a, left) and a shelf frame (a, right), cross-sectional views of the assemblies of a roller and a rail for the top shelf board and the ceiling (b, upper) and for the bottom shelf board and the concrete floor (b, bottom), and ground view of moving directions of the shelf frame in a culture growing room (c).

(a) a: rollers, b: aluminum or steel angle (4×4×0.2), c: fluorescent lamp, d: perforated plywood or wire mesh.

(b) a: nail, b: ceiling, c: 2㎜ thick steel plate, d: roller, e: bolt and nut, f: 2㎜ thick steel plate, g: 12㎜ thick plywood, h: roller holder, i: same as f, j: fixing iron bar of a rail no to move in the concrete floor, k: rail, l: floor, m: same as g.

(c) a: shelf frame, b: moving directions of the shelf frame, c: roller.

[z]Centimeter (㎝) as units of all numbers shown in the figure.

3,000 lux of light intensity to the cultures.

The movable shelf frame can be also constructed as an alternative way to use more spaces in the culture growing room. This frames might be better used in relatively large tissue culture labs than in small labs because they will save several aisles necessary for the growing room which non-movable shelf frames are constructed and one aisle is enough for passage ways in the culture growing room.

The cross-sectional views of a roller and a rail are shown in Fig. 6b to construct movable

shelf frames. The rollers can be attached or fixed 50~80 ㎝ apart in the several locations on the top and the bottom shelf boards. The upper design is for a roller and a rail to fix them together to the top shelf board and the ceiling, and the bottom design to fix them together to the bottom shelf board and the concrete floor in the culture growing room, respectively.

The size of the shelves for the movable frames should be smaller than that for the non-movable frames not to give much too heavy load to the rollers by culture vessels. The size of shelves, 70~75 ㎝ in height and 90 ㎝ in width, might be better. Due to the heat from the lamps when movable frames are used in the growing room, only one fluorescent lamp is recommended to be istalled in the middle of underneath shelf boards (Fig. 6a).

When constructing the rail way on the floor, rails should be placed and fixed in the concrete floor. Be sure to make the tops of rails be same levels as the floor. The rail ways must be same distances apart as the rollers. Then, the frames can be easily moved forward and backward directions by pulling and pushing (Fig. 6c). All electrical wires for the lamps are suggested to be connected from elctrical outlets in the ceiling to make the frames move easily when movable frames are constructed. If the fluorescent lights give off too much heat, then the ballasts must be removed and placed on a protected panel outside of the lab. Twenty-four hour timers will automatically control the lighting. Electrical requirements and safety precautions are important enough to deserve the best professional help available when lab is constructed because it is especially true when there are several unusual requirements in the tissue culture lab.

B) The equipments of commercial tissue culture lab

a) Laminar flow transfer hood (or clean bench)

The facilities for aseptic manipulations are most important and necessary to be equipped in the tissue culture labs. Aseptic conditions can be best achieved by using laminar flow transfer hoods or cabinets. They are manufactured by several companies (Chosun Sci. Co., Korea Manhattan Sci. Co., Daeju Engineering Co., Kongsin Sci. Co, etc.) in Korea and in many other countries (Japan, USA, England, etc.). They provide a sterile atmosphere in which to work with the cultures. In a clean bench or hood, the whole of the back is occupied by a sub-micron particle filter which strains out as small as 0.3 microns assuring a high degree of sterility. Air passing prefilter and entering the sub-micron flows at the rear of the hood toward the technician (Brown and Thorpe 1984 and Biondi and Thorpe 1981). The gentle air stream moves forward with uniform velocity (30 m/min.). Overhead ultraviolet lighting, although considered to be dangerous to both humans and plants, is a standard feature in the hood or clean bench. If the filters can be purchased it is possible and practical to construct rather than buy a satisfactory laminar flow hood.

b) Sterilizing equipment

The complete elimination or exclusion of contaminating micro-organisms such as virus, fungi, etc. is an essential feature of tissue culture. All nutrient media, culture vessels, and instruments used in handling the plant tissue must be sterile.

The most popular method of sterilizing both equipment and media is by autoclaving at 121℃ with the pressure of 15 psi for 15 minutes

(Brown and Thorpe 1984, Kyte 1983, Biondi and Thorpe 1981, and Hu and Wang 1983). However, depending upon the size of the lab and the objectives of lab directors, an autoclave well fitting to the lab can be purchased from Je-il Sci. Co., Chosun Sci. Co., and Dae Yang Engineering Co. A relatively small lab for one or two technicians can readily use a household pressure cooker for sterilizing the culture media. A relatively large lab should purchase an autoclave for all preparation works to be efficiently done.

c) Water purity

Water is the largest component of tissue culture media. So, the quality of water used is of critical importance in establishing a successful tissue culture lab. Tap water is usually unsuitable for tissue culture media because it may contain dissolved organic and inorganic materials, micro-organisms, particulate matters, and gases. These impure materials in tap water often lead to a failure of tissue culture lab operation (Brown and Thorpe 1984 and Street 1977). However, all tissue culture labs in Cheju-do have used tap water or subterranean water for preparing media and only a few labs have used distilled water or de-ionized water for making stock solutions. Distilled water is relatively good enough to be used for making stock solutions and preparing media, but de-ionized water is not pure enough to be used for and it is basically suitable for rinsing labwares.

As mentioned above, the impure chemicals, particularly inorganic materials in tap and subterranean water, cause to upset the precise balance of nutrients in media formulas and promote precipitates that make the nutrients unavailable to the plantlets. In addition, by introducing excessive amounts of unknown chemicals, a nutrient imbalance or a toxic medium may well result.

d) pH of tissue culture media

The pH adjustment is very important when preparing culture media because different plant species require different pH for optimal growth. The pH should be adjusted to be most suitable for plant growth just as it is in soils and potting media. The common pH range for tissue culture media is between pH 4.5 and pH 5.8.

While a pH meter is necessary for commercial production, a beginner can use pH indicator paper. Normal pH ranges for test paper are 2.9~5.2, 4.9~6.9, and 5.8~8.0 (kyte 1983). These ranges will measure pH adjustments of media to approximate pH requirements. Every commercial lab should invest in a pH meter. Purchasing a good quality meter is a wise investment. It can be purchased from several countries, USA, England, Canada, Japan, Spain, etc. It can be also purchased from You Sung Sci. Co., General Sci. inc., etc. in Korea.

e) Weighing equipment

Tissue culture requires a precise balance to accurately measure the small amounts of chemicals for the culture media. The expensive analytical or electronic balances are fast and precise, a worthwhile investment if affordable. However, the two-pan balances are satisfactory for weighing 10 milligram quantities or more, while slower and less precise than analytical or electronic balances. Dial-O-Gram is also satisfactory for the commercial tissue culture labs. Many labs in Cheju-do use this balance made in W. German, USA, or Korea.

f) Dissecting microscope

Microscope requirement depends upon the

type of tissue culture work to be done. A dissecting microscope is required for obtaining meristem explants. Even though a microscope is not essential for tissue culture lab when the explants are not microscopic, it is a helpful equipment to have available. Particularly, when culturing meristems cut to a length of shorter than 0.5 ㎜, it must be equipped in the lab. Most labs in Cheju-do purchase the microscopes, the brand of Nikon or Cannon from Japan.

g) Shaker or agitator

Some tissue culture works are conducted in liquid media for optimal performance of the cultures. Such cultures shoud be gently agitated by means of a shaker (80~100 rpm). Agitation aerates the medium thereby preventing the culture from 'drowning' (Kyte 1983). Agitation also disperses the waste products of the culture. The shaker can be purchased from several scientific companies such as Chosun, Je-il, You Sung, etc. in Korea.

h) Hot plate/stirrer

Although the hot plate/stirrer has been equipped in most commercial labs in Cheju-do, it has not been used at all. It is thought that the lab technicians do not realize the use of the equipment. A combined hot plate/automatic stirrer is among the most useful aids in preparing media. Agar media tend to stick and burn when heated unless they are constantly and effectively stirred by a rotating magnet until they boil.

Another important use for the magnetic stirrer is usually to provide adequate agitation in the cleaning of explants. The hot plate/automatic stirrer can be also purchased from Chosun, Je-il, or other scientific companies in Korea.

i) Glassware

Tissue culture work does not normally require more glassware or instruments than is found in a well stocked lab. The requirements are flexible and almost any glass container that can be easily cleaned and sterilized can be used for tissue culture, but it is preferable that lab glassware be of good-quality pyrex or borosilicate glass, as it is resistant to heat, breakage, and scratching, and can be cleaned with strong solvents and reprocessed repeatedly (Street 1977, Biondi and Thorpe 1981, and Brown and Thorpe 1984). Soda lime glass containers such as baby food jars have been widely used and are less expensive (than other glassware), but de Fossard(1976) suggested that they be discarded after 1 year's use or be treated with a dimethyl-dichloro-silane.

A plentiful supply of graduated measuring cylinders (10-2,000 ㎖), volumetric flasks (1-2,000㎖), and burettes (0.1-25㎖) is recommended. Wide-necked Erlenmyer flasks (50-400㎖), test tubes (25x150-250 ㎜), and glass petri dishes (60x15 and 100x200㎜) are particularly useful as tissue culture containers.

Thorough-washing and rinsing culture vessels are very important. All new culture vessels may release substances which affect the composition of the medium or is toxic to the tissue (Biondi and Thorpe 1981). Hence, they must be thoroughly washed before use and thoroughly rinsed after use. Rinsing usually discards the detergents which are often highly toxic to the tissue. Repeated rinsing in tap, de-ionized, and then distilled water is essential. For glassware coming into direct contact with living tissue, sterilization for 30 minutes in autoclave and rinses twice in double distilled water have been suggested (de Fossard 1976 and Brown and Thorpe 1984). Various glasswares can be purchased from Dong Sung and other scientific companies in Korea. The culture vessels most

widely used are 300-450$m\ell$ flasks in Cheju-do.

C) Environmental conditions of culture growing room

The culture environment includes all factors other than culture medium. Even though so many factors can potentially affect shoot-tip and meristem cultures, the most important factors are light, temperature, humidity, and aeration. However, the relative humidity of the culture facility is usually not important since the relative humidity of the microenvironment within a culture is about 100% (Murashige 1974).

a) Light

The light requirement of tissue cultures is not the same as these of autotropically developing plants since carbohydrate is adequately provided. However, several studies have shown that light plays very important roles to regulate certain morphogenetic and organogenetic processes (Hussey 1976 and Kato 1978a, b).

The three characteristics of light that affect plant tissue culture are: light intensity, photoperiod, and light quality. Morphogenetic and organogenetic requirements for light in tissue cultures may be satisfied by one or more of these factors.

i) Light intensity: Almost all of the tissue culture labs in Cheju-do have provided a light intensity of less than 1,000 lux to the cultures for shoot multiplication, *in vitro* seed germination, and other purposes. However, in the cultures of *Gerbera* and many other herbaceous genera, the optimal light intensity for shoot initiation is 1,000 lux, less than 1,000 lux being inadequate, and light intensity higher than 3,000 lux being strongly inhibitory (Murashige 1977).

In general, a wide range of light intensity is suitable for various cultures. Intensities below 1,000 lux are used only occasionally, while intensities between 1,000 and 3,000 lux are most common (Styer and Chin 1983). Light intensity has been shown to affect the type of growth in culture. The optimal light intensity sometimes appears to depend upon the daylength and plant species used (Hussey 1978). High light intensities between 6,000 and 10,000 lux are usually required before being transferred to soil to enhance their ability to survive in the greenhouse (Murashige 1974, Hasegawa et al. 1973, and Hughes 1982).

ii) Photoperiod: Like light intensity, the effective photoperiod varies with most plants growing *in vitro* Obviously, the key is the total radiant energy of specified quality to which the culture is exposed rather than the length itself that the culture is exposed to light (Murashige 1974). Moreover, the combined effect of both light intensity and photoperiod plays more important roles for manipulating certain morphogenetic or organogenetic processes. It is reasonable to expect varying optima in the length of the daily exposure to light for a given species, depending upon the light intensity used. Most common photoperiod is 16 hours of light and 8 hours of darkness. Murashige et al. (1974) mentioned that photoperiods of 12 to 16 hours provided the most favorable length of daily exposure for shoot division and shoot quality in clonal multiplication on *Gerbera*. However, photoperiod should be differed, depending on tissue culture purposes and plant species.

iii) Light quality: Spectral quality of light can be very important in regulating various

morphogenetic effects. The quality of light, particularly spectrum or wavelength, can be manipulated by artificial illumination, For use with tissue culture, the lamp should contain adequate doses of blue and red light, since both root initiation and shoot formation are promoted by these wavelength (Murashige 1977). Other spectral regions for plant tissue culture should not be ignored in selecting the lamps. Fluorescent lamps have been commonly used for plant tissue cultures because they provide a high output of photosynthetically active radiation (PAR) between 400 and 700 nm (Heuser 1983, Stoltz 1984, and Ettinger and Preece 1985) and photomorphogenetic radiation between 700 and 850 nm (McFarlane 1978 and Blazich and Novitzky 1984). The spectrum from the lamps generally matches the requirements of plants.

b) Temperature

Few studies have been carried out to find the optimal incubation temperatures. The temperatures vary with plant species and culture purposes being considered. Most researchers studying meristem and shoot-tip cultures have selected a constant temperature between 20 and 28℃ (Hu and Wang 1983).

A diurnal fluctuation in temperature might be desirable for maximum shoot formation, and shoot initiation might not be desirable with the decrease in night temperature. A constant 26℃ is probably the optimal temperature for the multiplication of shoots and roots and for the development of transplantable plants. The diurnal temperature fluctuation may be desired for some plants, especially for those adapted to emperate and desert climates (Murashige 1974 and 1977).

Practically speaking, constant incubation temperatures between 24 and 26℃ are common for shoot-tip and meristem cultures for most families although diurnal temperature fluctuations have been employed for some species (Styer and Chin 1983). Recently, most researchers have employed a constant temperature of 24-26℃ to maintain the cultures of most plant species (Kerbauy 1984, Tamura et al. 1984, and Stephens et al. 1985).

The precise control of temperature in the culture growing room is actually difficult to be manipulated because of shelf to shelf and bottom to top variations of temperatures due to the heat from light lamps, poor air circulation, and high humidity levels in the growing room. The temperature gradients from bottom to top show 2 to 4℃ differences from the set temperature. Other temperature gradients also occur from inside to outside of culture vessels. Many cases are that an inside temperature is approximately 2℃ higher than an outside temperature of culture vessels.

Normal symptoms by temperature gradients are yellowing, browning, and sometimes dying of the cultures in a stepwise. The severity of the symptoms by the gradients sometimes causes to kill the cultures or strongly inhibit the culture growth. Therefore, the precise control of temperature is essential for successful tissue culture program.

c) Humidity and aeration

Humidity control is important in regulating plant growth in the culture growing room. Precise humidity levels are difficult to control and also difficult to maintain over long periods of time. Thus, almost all of the culture growing rooms are without adequate humidity control for tissue culture.

The humidity level in the growing room is characterized by large variations. If no control

is provided, the variation between light and dark period and over each heating and cooling cycle will be quite large. Between 15 and 30 ℃ most culture growing rooms will be at about 50 percent relative humidity during the light period unless humidification is provided. During dark period the culture growing room may be more than 80-90 percent relative humidity if no significant amount of cooling is required. The excessive dryness if the culture growing room will result in the dryness of culture medium during night period, while the excessive moisture will cause the contaminants such as bacteria, fungi, molds etc. to grow rapidly and result in increasing contaminations of the cultures. Thus, it is suggested that 70 percent relative humidity is maintained for the culture growing room in the tissue culture lab (Street 1977).

Aeration or air movement has been recognized an important but complicated determinant of the culture growing room in the tissue culture lab. There are three possible patterns of air flow in the growing room-upward, downward, and horizontal. The importance of the directions of air flow depends to a large extent upon the size and the type of the growing room (Krizek 1978).

For the large growing room, a horizontal pattern of air flow is generally undesirable, since a permanent gradient in temperature will exist between the intake and the exhaust side of the growing room. A downward pattern of air movement is considered preferable for the growing room. It provides a means of carrying the excess heat away from the lamps and avoids large temperature gradients (Morse 1963). For good air circulation and no temperature gradients in the growing room, perforated shelf boards should be used to support culture vessels (Thorpe 1982), and a low speed and large blade fan is well suited for these purposes (Biondi and Thorpe 1981 and Street 1977). An air velocity of lower than 300 cm per miunte is generally considered optimum for the culture growing room.

D) Major crops tissue-cultured in Cheju-do

The crops or plants that can be propagated by tissue culture techniques vary, depending upon the objectives of the lab directors. Theoretically, most plants can be successfully propagated by tissue culture methods. However, the crops or plants cultured in vitro actually have been very limited. Most commercial labs in Cheju-do mainly propagate very limited plant species such as banana, pineapple, and orchids (table 2). Recently, the labs culturing *Gerbera* tend to increase and only one or two labs start

Table 2. List of all plant species and percentage of tissue culture labs culturing these species in vitro in Cheju-do, Korea (as of July, 1988).

(%)[z]

Orchid	Banana	Pineapple	Gerbera	Lily	Strawberry	Grape
97	94	64	18	9	3	3

[z]percentage calculated by total lab numbers culturing these plant species divided by total numbers of labs surveyed x 100.

to propagate lilies, grapes, and strawberries by tissue culture.

To operate the commercial lab successfully, all lab directors must try to widen the plant species to be cultured in Cheju-do or try to develop the highly advanced tissue culture techniques to propagate plants with the limited species in the future. Another way to be successful is to develop the techniques for plant improvement by in vitro breeding methods.

E) Hardening facility and procedure

The plantlets produced by tissue culture are often difficult to establish in natural or even greenhouse conditions. They behave as tender seedlings and need to be hardened off with the special care. Various facilities such as hardening room and greenhouse can be used for hardening off, depending upon the locality, the particular cultivar, and the quantity of the cultures.

Most labs use greenhouse as a hardening facility in Cheju-do. They are all hardening the cultures with similar procedures in the greenhouses. Culture vessels are taken out from the culture growing room and placed in the hardening greenhouse. Then, the tops of culture vessels are taken off, the cultures in the vessels are left for 2 to 5 days, and then transplanted into 3 to 4 inch plastic pots containing soil mixes. After the seedlings root well, they are again transplanted into larger pots containing potting media.

However, a special hardening room similar to the culture growing room is a good option in which to establish tissue culture plantlets in the potting media. The hardening room being used, light and heat can be easily controlled and constant humidity can be also maintained. The humidity is gradually lowed and light is gradually increased to accustom the plantlets to normal greenhouse conditions. When the seedlings are covered with plastic (0.4 ㎜ in thickness), humidity can be lowed by making a hole in the plastic cover and enlarging it each day, or by removing the cover for longer periods each day (Kyte 1983).

To prevent mold growth, it is desirable to wash plantlets coming out of cultures to remove any culture medium adhering to the base or roots. Agar sticks to some roots more than it does to others. Therefore, it is strongly recommended to completely remove agars from the cultures or plantlets before transplanting them into the potting media for hardening off (Biondi and Thorpe 1981 and Kyte 1983).

摘　　要

濟州道內에 施設된 組織培養室의 現況과 實態를 調査하고 이들 培養室의 構造와 設備, 環境制御, 幼苗 馴化 施設 및 方法의 改善 方向에 關한 結果를 要約하면 다음과 같다.

組織培養室의 構造는 運營者의 實情에 따라 매우 多樣하였으나 效率性을 考慮할 때 必須的으로 準備室과 無菌室 및 培養室의 3部分으로 區分되어 있어야 한다. 이에 따른 組織培養室의 基本 形態를 크기별로 區分해 볼 때 2人用인 境遇 48.6 ㎡ (15坪型), 3～4人用에 81.0㎡ (25坪型), 그리고 5人 以上인 境遇에는 129.6㎡ (40坪型)이 適合한 것으로 思料된다.

培養室의 空間을 效果的으로 活用하기 爲하여 比較的 넓은 培養室의 境遇 4～5段式 移動 旋盤臺가 바람직하며 螢光燈에서 發生하는 熱을 줄이기 爲하여 旋盤의 높이는 60～75㎝ 그리고 넓이는 90～120㎝로 하는 것이 바람직하다. 旋盤의 材料로는 有空합판이나 철망을 使用하는 것이 培養室의 空氣循環을 圓滑하게 하는 데 效果的이다.

道內 大部分의 組織培養室은 比較的 充分한 培養 機資材들을 갖추고 있었으나 蒸溜를 하지 않은 水道물이나 地下水는 培地製調用으로 適合치 못하기 때문에 蒸溜水를 使用하는 것이 바람직한 것으로 思料된다.

培養室內의 光線條件과 溫度는 매우 重要한 環境要因 等이기 때문에 正確하게 調節되어야 한다. 그러나 大部分의 培養室들은 1,000 *lux* 以下의 낮은 光度條件으로서 充分치 못하므로 螢光燈을 利用하여 1,000 以上~3,000 *lux* 以下의 光度를 12~16時間의 光週期로 照射해 주는 것이 좋다. 溫度는 에어컨을 利用하여 比較的 잘 調節이 되고 있으나 旋盤의 下段部와 上段部 그리고 培養用器 內外의 溫度差는 전혀 考慮되고 있지 않다. 培養室의 濕度와 空氣의 循環은 거의 無視되고 있으며 正確한 溫度調節을 하기 爲해서는 70% 程度의 濕度를 維持해 주어야 하고 培養室內 溫度差를 줄이기 爲해서는 下向式 空氣循環 方法을 摸索하는 것이 바람직하다.

組織培養에 依하여 增殖되는 作物의 種類는 주로 東,西洋 蘭類와 그리고 파인애플로 制限되어 있어서 앞으로 作物의 種類를 多樣化하고 品種의 改善을 爲한 高度의 培養技術이 開發되어야 한다.

培養된 植物體의 馴化施設로는 비닐하우스가 一般的으로 利用되고 있으나 그 方法이 適合치 못하므로 生存率을 높이기 爲해서는 光과 溫度 및 濕度를 容易하게 調節할 수 있도록 하기 爲하여 培養室의 環境調節裝置와 類似한 馴化施設을 갖추는 것이 바람직하다.

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