한란의 영양생리에 관한 연구

홍경애 · 강순선 · 유장걸

The Nutritional Physiology of *Cymbidium kanran*

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要 約

本 實驗은 寒蘭의 生育에 가장 중요한 要因 중의 하나로 생각되고 있는 相對濕度가 燐酸의 吸收 및 移行과 urea permeability에 끼치는 影響, 그리고 寒蘭의 無機成分 組成과 V.A. mycorrhizae 有無를 研究 檢討하여 寒蘭의 營養 生理 特性에 관한 基礎 資料를 提供하고져 遂行되었다.

1. 寒蘭의 AFS 값은 0.44~0.50㎖/g.fresh weight로 다른 식물 平均値(0.10~0.15㎖/g. fresh weight)와 비교하여 큰 것이 特徵이었다.
2. 시간 經過에 따른 뿌리에서 잎으로의 養分移動은 매우 느려서 P-32 燐酸을 吸收시킨 뒤 14시간(840분) 이내에는 잎에서의 P-32 檢出量이 아주 적었다.
3. 뿌리 연령에 따른 寒蘭의 養分移行을 보면 뿌리에서 뿌리(촉 포함)로의 養分移行이 뿌리에서 잎으로의 養分移行보다 活發하였고 젊은 뿌리일수록 燐酸의 移行 能力은 컸다.
4. 寒蘭을 잎, 촉, 뿌리로 나누어 部位別 無機成分 分析을 해 본 결과 K는 잎중에, Mg, Cu, Al은 뿌리에, 그리고 P, Ca, Fe, Mn, Mo, Zn, B, Si, Na는 촉에 많이 含有되어 있었다.

   Fe, B, Mo 含量은 다른 植物의 平均値에 비해서 그 含量이 매우 높았고 K 含量은 낮은 것으로 나타났다.
5. 35%, 50%, 65%, 90%의 相對濕度下에서 자란 寒蘭이 urea permeability를 測定한 결과 相對濕度가 낮을수록 浸透速度(Ks)가 컸으며 相對濕度가 클수록 Ks 값은 낮았다.
6. 寒蘭의 燐酸 吸收能은 相對濕度가 낮은 곳에서 자란 寒蘭일수록 吸收能이 낮았으며 相對濕度가 높은 곳에서 자란 寒蘭일수록 吸收能이 컸다.
7. 野生 寒蘭에서는 V.A.mycorrhizae가 거의 觀察되었으나 組織培養된 寒蘭과 野生 春蘭에서는 V.A.mycorrhizae가 觀察되지 않았다.

Introduction

Orchids are the most varied and beautiful of all flowers. There are about 660-800 genera and 25000-35000 species and new ones are being discovered and artificially bred every year. Among orchids, *Cymbidium* genera are around 70 and some of them are called "oriental orchids"; *Cym. forreslii, Cym. ensifolium, Cym. gyokuchin, Cym, pumilum,* etc.. (North, 1983) which have been elegantly loved by many famous scholars and noblemen in Korea, China, and Japan from the old time.

On Halla Mt. many kinds of oriental orchids are natively growing; *Cym. kanran, Cym. virescene, Cym. nipponicum, Cym. koran, Cym. lancifolium* (Lee, 1984) and so on. Although *Cym. kanran* has been kept as the most valuable horticultural flower due to its delicate colors, eligant shapes and incredible fragrances, any experimental research on the nutritional physiology have never been tried yet because of remarkably high price and shortage of homogeneous plant materials.

This paper deals with mainly the foundamental aspect of inorganic element contents, phosphate ion transport, and phosphate absorption by roots and root cell membrane permeability constant under the different conditions of relative humidity.

Since it has been said that V.A. mycorrhizae (V.A.M) were closely associated with *Cym. kanran* the lactophenol staining method was introduced to observe V.A.M. infection in *Cym. kanran* roots.

Considering that *Cym. kanran* is one of the shadow plants having specific physiological characteristics, the present work is only begining step of *Cym. kanran* study but believed to provide some basic data needed to understand the nutritional physiology of *Cym. kanran.*

Materials and Methods

1. The plants used

1) *Cym. kanran*: The sample plants were purchased from the Galsan Farm in Seogwipo, where the germinated seedling of *Cym. kanran*(Kyungsa) were propagated by the tissue culture technique, hardened out of the culture bottles, and grown in the pots for two to three years.

Therefore the used *Cym. kanran* had two or three bulbs (Fig.1)

On the other hand, the root segments for V.A.mycorrhizae observation were collected randomly from 25 different wild *Cym. kanrans* which were not artificially propagated.

2) *Cym. virescenes*: The samples offered for V.A.mycorrhizae observation were

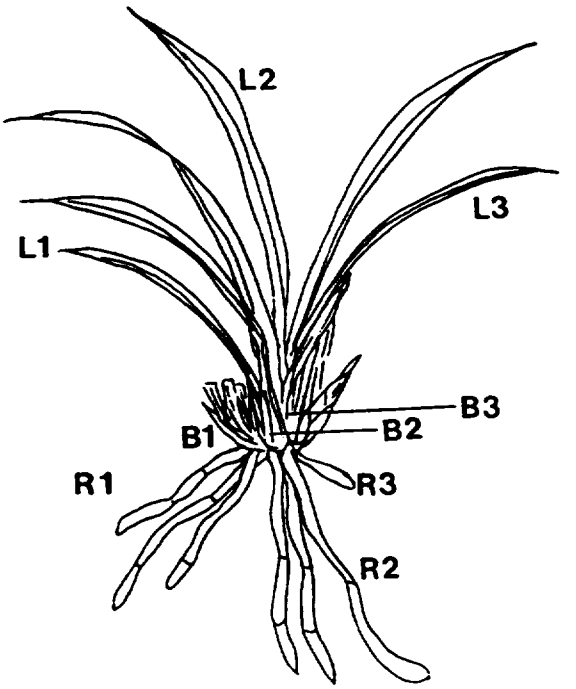

Figure. 1. The picture of *Cym. kanran* with three bulbs.

collected from the forest area around Cheju National University or purchased from the five-day market.

2. Determination of the apparent free space(AFS) of *Cym. kanran* roots.

To measure AFS of *Cym. kanran* root (Arisz, 1956; Briggs et al., 1958), the plant roots were dipped in the P-32 labelled Hoagland solution(1/10 strength) having 16 μci/200$m\ell$ of specific activity for 16 minutes. After the extra surface water on the roots was removed with tissue papers, the roots were transfered into 100$m\ell$ of distilled water and left with little shaking for 20 minutes for the P-32 labelled solution existed in AFS to be released into the water. 10$m\ell$ of the solution was taken into the glass vial and its P-32 activity was measured by the Cerenkov counting method using the liquid scintillation counter(Berthold Model 8000).

AFS was calculated by the following

equation (Epstein, 1972).

$$AFS = \frac{\text{the total activity released from the unit weight of fresh roots (cpm/g.fresh.wt)}}{\text{the specific activity ot the P-32 labelled Hoagland solution (cpm/ml)}}$$

3. Translocation rate of phosphate ions from roots to leaves using the P-32 autoradiography technique and the Cerenkov counting method

1) P-32 absorption by plant roots with time

Seven plant samples were put into 200 ml of Hoagland solution labelled with 16 μci P-32 and left for 16 minutes altogether. After 16 minutes, the roots of one plant among the seven were rinsed in the tap water to take out the P-32 solution in AFS.

The plant was divided into leaves and roots which were fixed on the hard board and placed in a freez-dryer at -25 ℃ for seven days.

Rest of the *Cym. kanran* were replanted in the pots individually after the surface water had been removed by tissue papers. The replanted six *Cym. kanran* were cultivated in the tissue culture room (1500lux, 22℃) without watering and taken out from the pots after different periods of time : 90 min., 160 min., 840min., 2400min., 7200min., 16000 min. Those plants were treated just same as the first plant to dry out completely without shape damage.

2) Preparation of autoradiography

The dried samples in the freez-dryer were taken out, placed on the X-ray film (Agfa Curix 100 NIF, 10×12 inch), covered with the black papers and put into the dark chamber for two days in case of roots and for five days in case of leaves.

The X-ray films exposed to the β-ray emitting samples were developed in X-Dol for 15 minutes, fixed in X-Fix for 15 minutes and washed with tap water. These procedures were carried out at about 20℃ under the safety light condition every time.

3) Radioactivity counting

After autoradiography, all the plant samples were cut into small pieces (about 5㎜ length) with the scissors, placed in the 50㎖ Kjeldhal flask, and digested with 3㎖ of conc. H_2SO_4 and 10 ㎖ of H_2O_2 to fill up to 15㎖ with H_2O. The digested and diluted solutions were transferred into the glass vial and their radioactivities were measured with the liquid scintillation counting system.

4. P-32 translocation patterns depending on the different ages of *Cym. kanran* roots

Two kinds of plants (two bulb and

three bulb) were used.

1) P-32 absorption by roots

The P-32 translocation patterns were observed after the different ages of roots (from the first bulb, the second, and the third) were dipped into the P-32 labelled culture solution (16μci/800$m\ell$) separately (Fig. 2).

2) Autoradiography and radioactivity counting

Measurements were conducted by the same methods used in the experiment 3-2).

5. Composition of inorganic elements

The three-plant composite sample was prepared to determine the inorganic element contents of the *Cym. kanran* sample. After the plants were divided into three parts (roots, leaves, and bulbs), dried, weighed, and digested with conc. H_2SO_4 and H_2O_2.

The spectrophotometer was used for phosphorous analysis and the atomic absorption spectrometry was introduced for the determination of K, Ca, Mg, Fe, Mn, Zn, Cu, B, Mo, Si, Na, and Al (Shouichi et al., 1971).

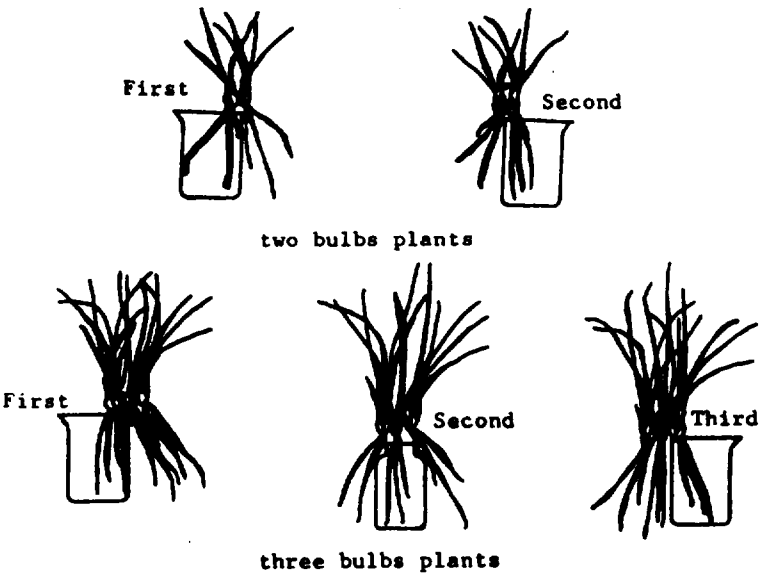

Figure. 2. Treating the different ages of *Cym. kanran* roots with the P-32 labelled Hoagland solution.

6. Measurement of root cell membrane permeability of *Cym. kanran* grown under different relative humidities.

1) Treatment of different relative humidities

Duplicate *Cym. kanrans* were cultivated for 7 days in a glass box independently adjusted at 35%, 50%, 65%, and 90%, relative humidities (Fig. 3)

The conditions of the cultivation room were 20~22℃ of temperature, and 2000 Lux of light intensity with 11 hours of light time and 13 hours of dark time. The size of glass box was 33㎝×23㎝×40㎝ and the air in the boxes was replaced continuously by the convection current and the electrical pump.

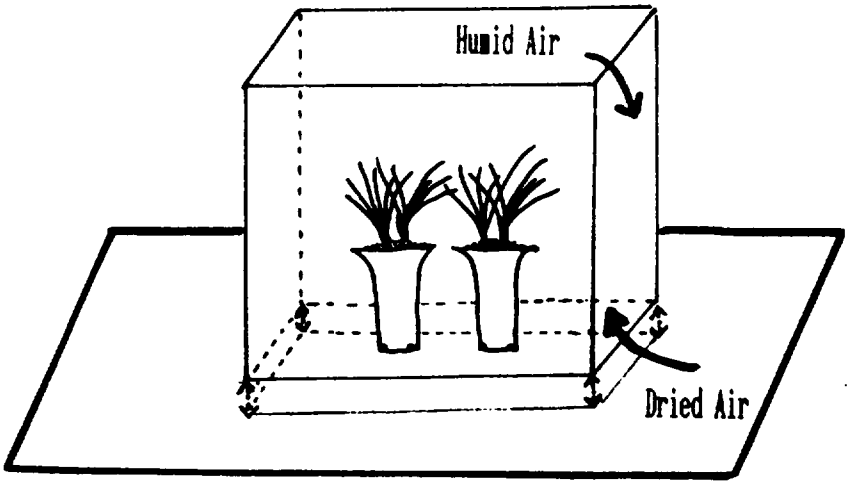

Figure. 3. Control of relative humidity by the convection current in the glass box and by the exchange of humid air inside and dried air outside.

2) Measurement of urea permeability

To observe membrane permeability, the roots were cut into 100㎛ thickness by the vibratome (Series 1000, American Scientific Products) and this contained 2 to 3 cell layers. To remove air in the tissue vacuum infiltration was applied in the ion-balanced medium (mixture of $CaCl_2$+KCl) to protect cell membrane damage.

For plasmolysis, the roots were dipped in the sucrose solution ranging from 0.3 M to 1.2 M step by step.

The plasmolyzed cells, to observe permeability, were placed in the perfusion chamber (Fig. 4). The plasmolized cells placed in groove were tightly closed with a cover glass.

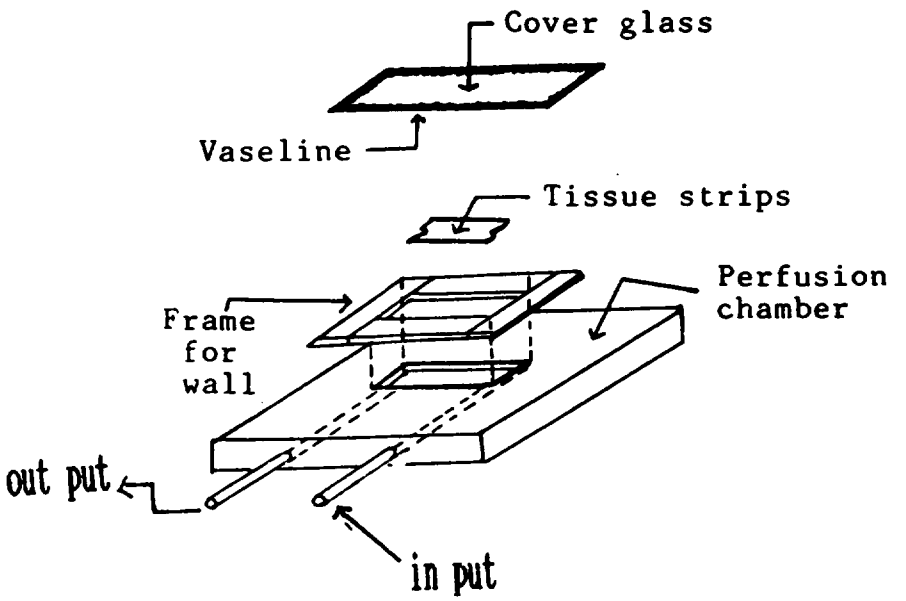

Figure. 4. The schematic diagram of the perfusion chamber used for urea permeability mesurement.

The length of the intact protoplasts was measured with micrometer under the microscope until the protoplast volume reached a final value at equilibrium with the concentration of the plasmolysing solution, that is deplasmolysis.

After the protoplast size versus time was plotted, the Ks values were calculated by the following equation (Stadlemann et al., 1989) at $t_1=20$ min. and $t_2=40$ min.

$$Ks=\frac{1}{8}\frac{d_2^4-d_1^4}{d_0^3(t_2-t_1)}\ (cm/sec)$$

Ks=urea permeability constant in cm/sec

d_0=diameter of spherical protoplast just before urea soln. introduction

d_1=diameter of spherical protoplate at t_1

d_2=diameter of spherical protoplast at t_2

t_1=time of the measurement.

t_2=time of the measurement.

7. P-32 absorption by *Cym. kanran roots* under the different conditions of relative humidity

After 7 days, the treated plants were placed in the P-32 labelled Hoagland solution (16 μCi/800㎖, 1/10 strength) for 20 minutes.

Radioactivity counting was carried out in the same way of the experiment 3-3).

8. Observation of V.A.M. associated with *Cym. kanran* roots

The root segments were placed in the vials, treated with 10% KOH and autoclaved at 1.03×10^5N/㎡ for 10 minutes. After autoclaving, roots were rinsed with at least three complete changes of regular tap water, bleached with alkaline H_2O_2 (alkaline H_2O_2 solution was made by adding 3㎖ of NH_4OH to 30㎖ of 10% H_2O_2 and 567㎖ of water) and rinsed thoroughly with tap water three times. Roots in vial were soaked with 1% HCl for 3 minutes. Roots were stained with 0.01% acid fuchsin (lactophenol staining solution) and autoclaved for 10 minutes at 1.03×10^5N/㎡. The lactophenol solution consisted of 300g of phenol, 250㎖ of lactic acid, 250 ㎖ of glycerin and 300㎖ of water (Paul et al., 1979).

After roots were destained with lactic acid solution, V.A.M. was observed with microscope.

Results and Discussion

1. Determination of the apparent free space (AFS) of *Cym. kanran* roots.

Since the cell walls have a system of microcapillaries which offers little resistance to water and solute movement, a portion of the root volumne can be freely penetrated and is directly accessible to the outside solution. This proprotion is known as the apparent free space which is defined as that volumne into which a solute can move without the restraint of membrane barriers.

The determined AFS of *Cym. kanran* was 0.44-0.5 milliliter per gram fresh weight. This volume is rather higher than those of the most higher plant roots (0.10-0.15㎖/g fresh weight) (Epstein, 1972).

It is well known to the *Cym. kanran* fanciers that too much watering is harmful but the plants should not be allowed to dry out. The *Cym. kanran*, however, don't need so much watering as the another plants, although the amount of watering depends on the environmental conditions. The reason why the *Cym. kanran* can last for several days without watering is though to be that the roots have large water holding capacity brought by the high AFS.

2. Translocation of phosphate ions from roots to leaves

1) Autoradiography observation

According to the autoradiograph, the image of leaves (on the X-ray film) could be observed from the plants which were allowed for the absorbed P-32 ions to move up to leaves for 840 minutes. No image was found in 16, 90 and 160

minutes. There were no severe differences of X-ray film darkening after 840 minutes though the darkening degree was increased with time as shown in Photo 1.

This fact means that the translocation of phosphate ions in *Cym. kanran* is very slow comparing to another higher plants.

2) Result from Cerenkov counting

The percent of P-32 amount translocated to leaves was calculated by dividing the P-32 activity in leaves by the total P-32 activity in the plants (roots +shoots).

Figure 5 shows that the percent of P-32 activity in leaves was negiligibly small until 840 minutes but increased up to about 5% after 16000 minutes.

The relations between the amounts of P-32 translocated to leaves and time were analyzed by "Minitab" statistical package (Fig.6). A typical linear regression was observed from 16 minutes to 2400 minutes. The translocation rate was reduced after 7200 minutes because the plants began to suffer from water stress.

Considering that solute translocation is a passive process in the xylem stream, it is expected that the rate of transport would be governed by the rate of transpiration. Therefore the reduced transpiration 5 days (7200 min.) after watering could decrease the translocation of P-32 at the later time.

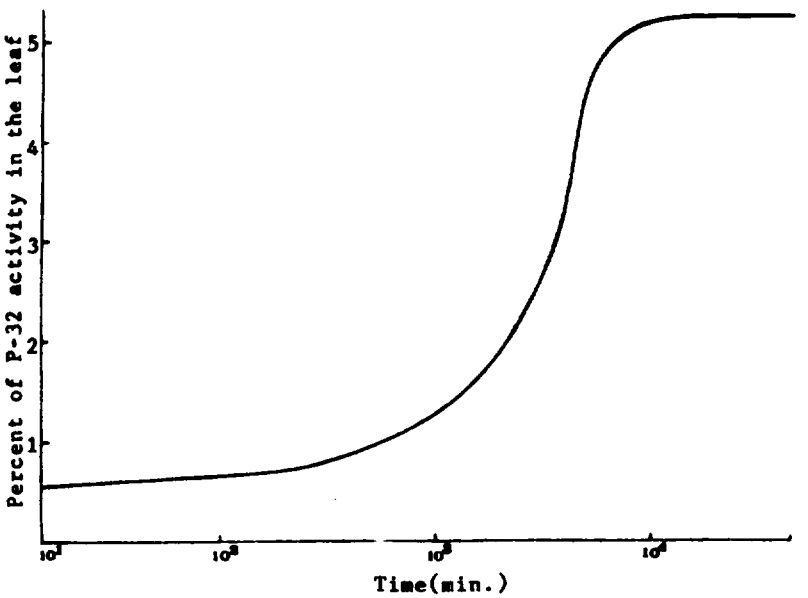

Figure. 5. Increase of P-32 activity percent in the leaf with time.

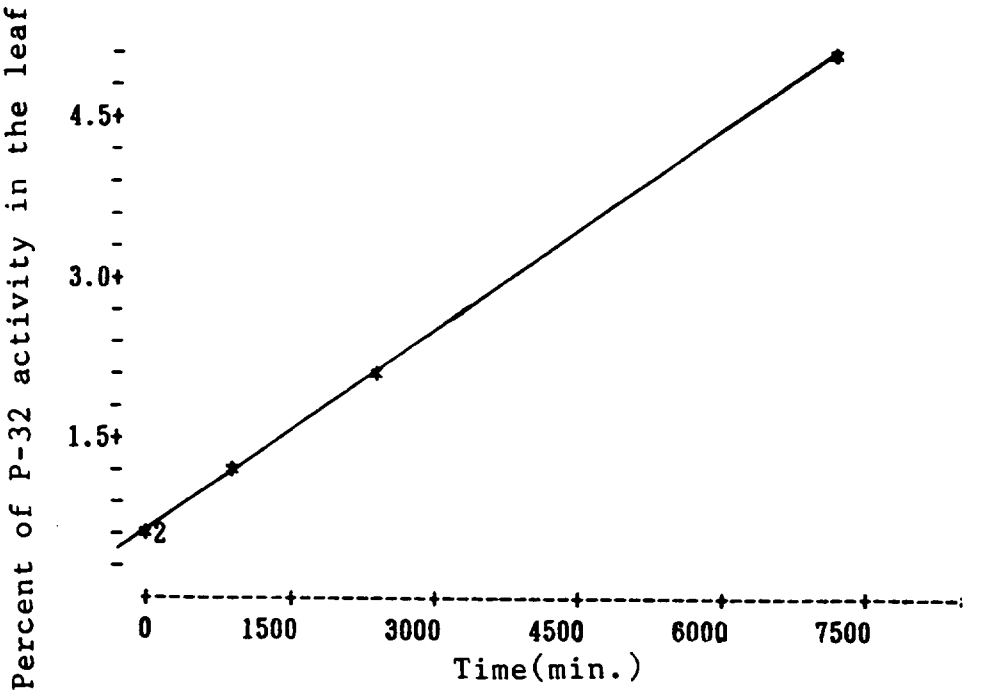

Figure. 6. Increase of P-32 activity percent in the leaf with time.

Also this result indicates that the proper watering period would be 5 days under the experimental conditions taken.

3. The P-32 transport patterns depending on the different ages of *Cym. kanran* roots

The younger roots could transport much more phosphate ions to the bulbs and the leaves in the two bulb plant as well as the three bulb plant as shown in Table 1.

In the two bulb plant, 7% of the total phosphate was moved to the bulb and the leaves when the first root(old) was treated with P-32 solution, while 13% was transported when the second root (younger) was treated. Also in the three bulb plant, only few percent was translocated to another parts from the root when the first(oldest) root was treated with P-32. But about 12% and 25 % of the total phosphate were transported to the bulbs and the leaves when the second root and the third (youngest) root were treated respectively. In the two bulb plant, the transport from roots to roots was more active than that from roots to another parts.

In the three bulb plant, however, the transport patterns seemed to be some-

Table 1. The distribution patterns of phosphate absorbed by the different ages of roots.

	Plant Parts	Distribution of phosphate absorbed (%)
Two bulb plant	*First root	90
	Second root	3
	First leaf	1
	Second leaf	0.6
	First root	6.4
	*Second root	87
	First leaf	5.1
	Second leaf	1.1
Three bulb plant	*First root	96.7
	Second root	1.2
	Third root	0.4
	First leaf	0.5
	Second leaf	0.9
	Third leaf	0.08
	First root	2.4
	*Second root	87.9
	First leaf	0.
	Second leaf	3.9
	Third leaf	4.9
	First root	0.4
	Second root	15.9
	*Third root	75.2
	First leaf	0.3
	Second leaf	1.4
	Third leaf	6.5

* sites labelled with P-32

what complicate. Though the transport from roots to roots were still dominant but quite high amounts of phosphate were transported to the younger leaves, especially to the third leaves came from the third buld which did not have its root yet.

4. Composition of inorganic elements

Table 2 shows the mineral contents of *Cym. kanran* used for this experiment. There are many factors influencing the mineral composition of the plants such as species, ages, organs, and the

Table 2. Mineral contents (%) of *Cym. kanran*

Elements	Plant parts		
	Root	Leaf	Bulb
P (%)	0.21	0.20	0.37
K	0.08	0.16	0.10
Ca	0.37	0.51	0.80
Mg	0.35	0.19	0.22
Si	0.92	0.71	1.20
Mn (ppm)	32	206	209
Fe	1131	340	1217
Zn	86	42	150
Cu	10	5.2	9.5
B	-	38	136
Mo	9.2	5.5	28
Na	405	192	876
Al	500	0	181

– not measured

environmental conditions. Especially the media where the plants grow and the nutritional characteristic of the plants will be the most important factors affecting the mineral composition.

As shown in Table 2 most of elements except K, Cu, Al and Mg were contained in bulb more than in roots or leaf.

The higher content (0.16%) of K was found in leaf, even though the value seemed to be rather lower than the average K content (1.66-2.75%) of plant leaves (EPSF, 1976).

Root and bulb gave higher Cu content (10ppm) than leaf (5.2ppm). These values are very common in the wide range of plant species (Shaklette, 1980).

Root contained more Mg (0.35%) than leaf or bulb. The lower K content of root could bring about this result because Mg accumulation in root occurs in the soil of K deficient.

Very high amount of Al (500 ppm) which is not essential for higher plant, was found in the root but the leaf did contain no Al (Montford et al., 1980; Burton, 1979; Liu et al., 1974). This might be due to high amount of aluminum oxide in the cultivation media derived from volcanic ash debris. Average Al content of plants is ranged from 0.5 ppm to 100 ppm (EPSF, 1976).

P contents of root (0.21%), leaf (0.20 %) and bulb (0.37%) were quite ordinary, average P content of plants being about 0.2% (EPSF, 1976) and grains as well as herbage 0.3-0.5% (Mengel, 1975).

Ca and Mn contents as like P showed the ordinary values, average Ca content of monocotyledon being 0.23% and Mn contents ranging from 10 ppm to 300 ppm (Loneragan, 1975).

Fe content seemed to be higher than those of grass (80 ppm), clover (115 ppm) and green plant tissue (100 ppm) (Ebens et al., 1982; Takahashi et al., 1977).

Zn content of *Cym. kanran* was not peculiar, being more or less 100 ppm (Mengel et al., 1987).

B and Mo contents, however, were comparatively higher. B content of plants ranged from 2 to 95 ppm (Wells et al., 1977) and most of plant bulbs contained 10-30 ppm (EPSF, 1976). It means that *Cym. kanran* belongs to the category of high B requiring plants. Mean value of Mo content in plants is below 5 ppm (Thornton, 1977; EPSF, 1976).

The contents of Na and Si were not so specific. These elements are generally known not to be essential for the higher plants and the contents are very much various depending on the circumstances and the plant species.

5. Root cell membrane permeability of *Cym. kanran* grown under the different relative humidities

Figure 7 shows the increase of intact protoplast diameter with time when the plasmolyzed cells were placed in the urea solutions.

The intact protoplast of the *Cym. kanran* roots grown under 35% relative humidity gave the highest urea permeability constant. This fact means that water stress could alter the membrane structure for urea solution to make quicker passive permeation.

The higher relative humidity, the lower urea permeability constants were observed.

6. P-32 absorption by *Cym. kanran* grown under the different relative humidities

As Table 3 shows, the lower relative humidity brought about decreases of P-32 uptake by roots. Considering that phosphate absorption by plant roots depends on mainly metabolic active transport rather than passive process, water stress induced by low relative humidity could inhibit the P-32 uptake.

The transport from root to bulb gave almost same tendancy as the P-32 absorption by roots.

But no clear explanation can be given for the phosphate transport to leaves; 50 % and 65% relative humidities giving higher values of P-32 transport than 35% and 90%.

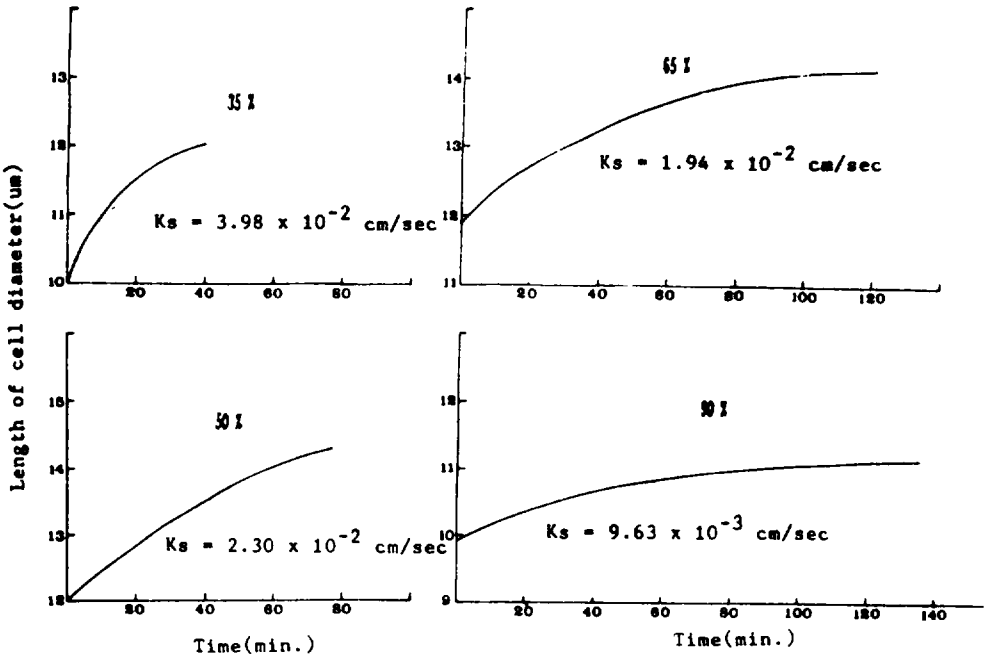

Figure. 7. Change of root membrane permeability in the *Cym. kanran* grown under the different relative humidities.

Table 3. P-32 uptake by the *Cym. kanran* grown under the different relative humidities

R.H. Treatments	Plant parts	Net cpm/dry wt.
35 %	Root	15,651
	Leaf	275
	Bulb	3,326
50 %	Root	16,436
	Leaf	502
	Bulb	4,026
65 %	Root	17,145
	Leaf	458
	Bulb	3,760
90 %	Root	20,019
	Leaf	143
	Bulb	5,110

The inversed relationship between urea permeability and P-32 absorption was found. Therefore the measurement of urea permeability can be used as a tool to predict P-32 uptake capability by the roots.

It should be, however, investigated more in detail if urea permeability has a negative or positive relation with each essential element absorption.

7. Observation of V.A.mycorrhizae (V.A.M.)

V.A.M. was observed from most of wild *Cym. kanrans* and not from cultured *Cym. kanrans* and wild *Cym. virescenes*. V.A.M. associated with wild *Cym. kanrans* seemed to be infected from soils while the tissue cultured *kanrans* did not have chance to be infected.

It is not explicable, however, why there was no V.A.M. in wild *Cym. virescene* roots. To make confirm, much more trials should be done.

Summary

The study is concerned with the apparent free space of *Cym. Kanran* root, characteristics of P-32 absorption by roots, and translocation from roots to upper parts and urea permeability under different relative humudities (35%, 50%, 65%, 90%). The composition of inorganic elements in *Cym. kanran* was determined and the infection of V.A.mycorrhizae was examined.

1. AFS of *Cym. kanran* was 0.44-0.5ml/g.fresh weight, being rather higher than those of most higher plant roots.

2. The phosphate translocation rate of *Cym. kanran* root to leaf was very slow comparing to another higher plants.

3. The phosphate transport from roots to roots was more active than that from roots to another parts, regardless of old and young roots.

4. Bulbs contained higher amounts of P, Ca, Mn, Fe, Mo, Zn, B, Si, and Na than another parts but the concentrations of Cu, Al, and Mg were higher in roots and more K was in leaf. Fe, B, and Mo contents seemed to be higher than another plants while K content was lower.

5. The lower relative humidity, the higher urea permeability.
The quicker permeation of urea might be brought about by increase of cell sap concentration and membrane structure alteration induced by water loss.

6. The lower relative humidity, the lower P-32 uptake by roots. This fact indicated that active absorption of phosphate was inhibited by water deficit.

7. V.A.mycorrhizae was observed from wild *Cym. kanrans*, but not from cultured *Cym. kanrans* and wild *Cym. virescenes*.

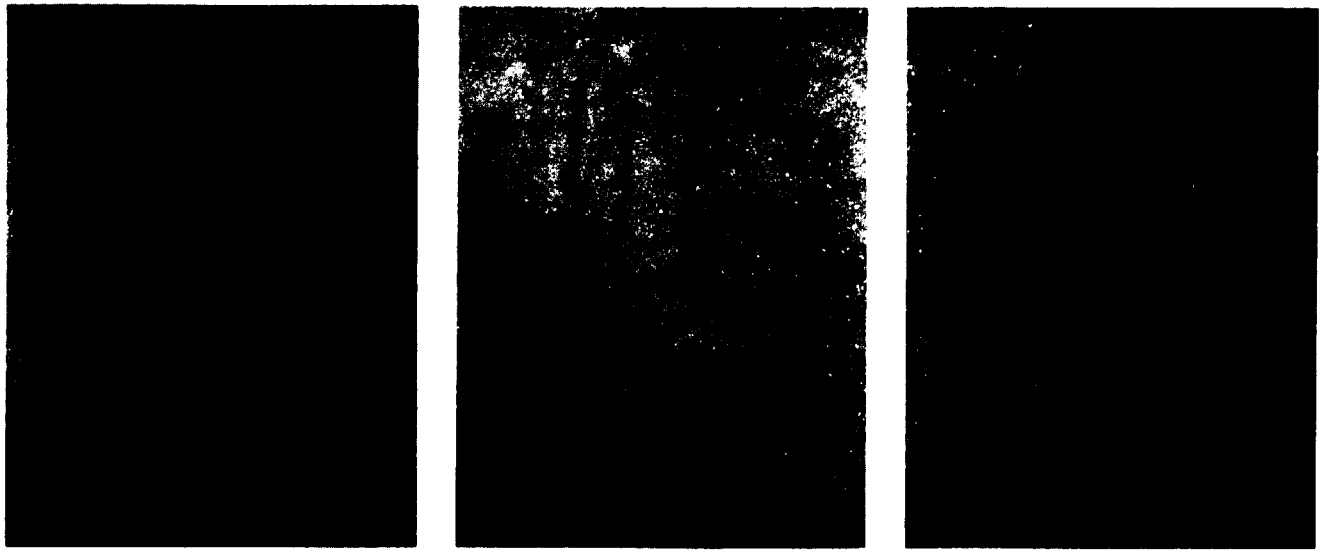

Plate 1(2400min.) Plate 2(7200min.) Plate 3(16000min.)

Plate 1-3. The autoradiograph of *Cym. Kanran* leaves, 2400 min., 7200 min., and 16000 min. after the plant roots were treated with the P-32 labelled culture solution.

7200 min.,
th the P-32

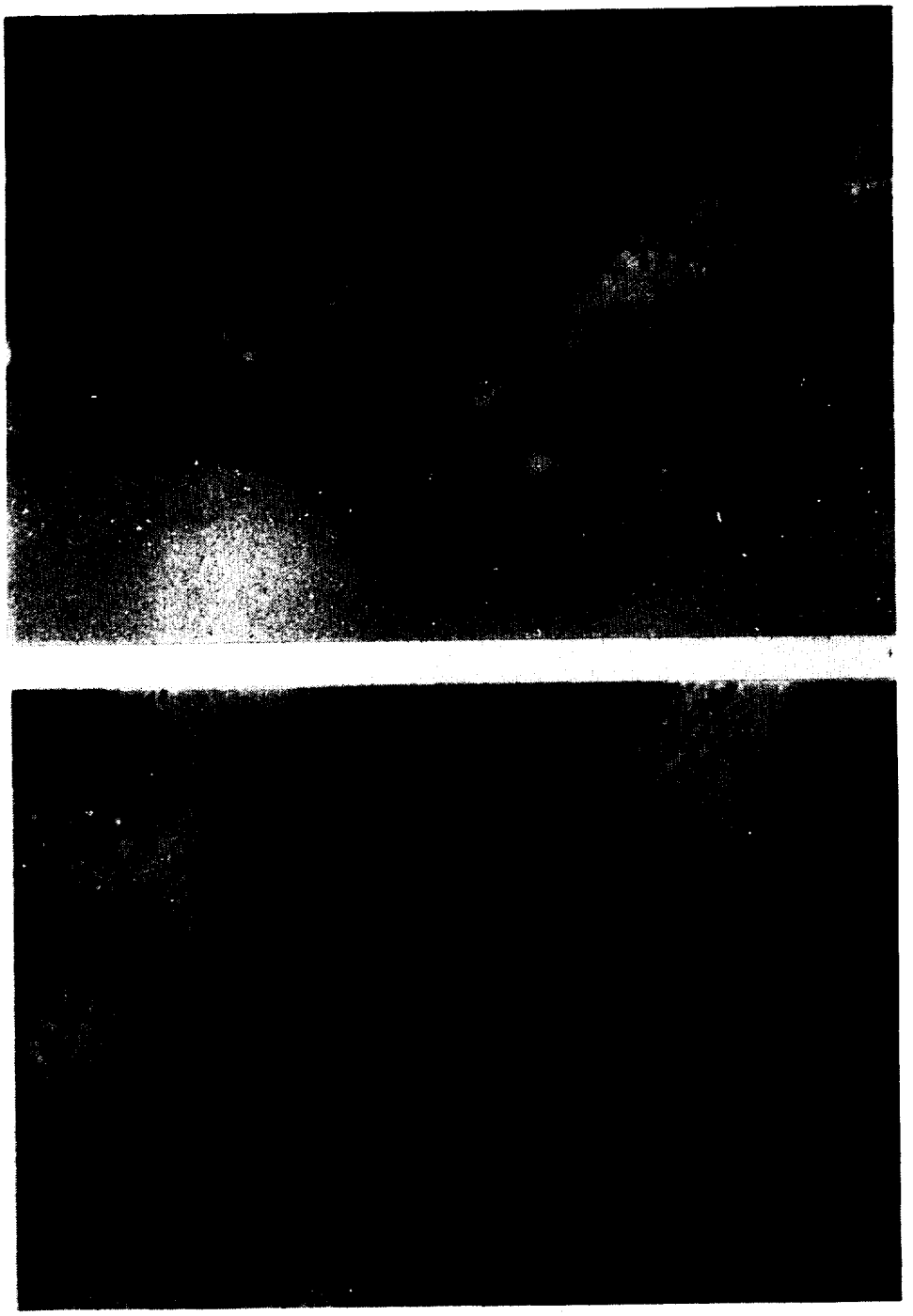

Plate 4. Arbusculars of V. A. mycorrhizae found in the wild *Cym. kanran* roots.

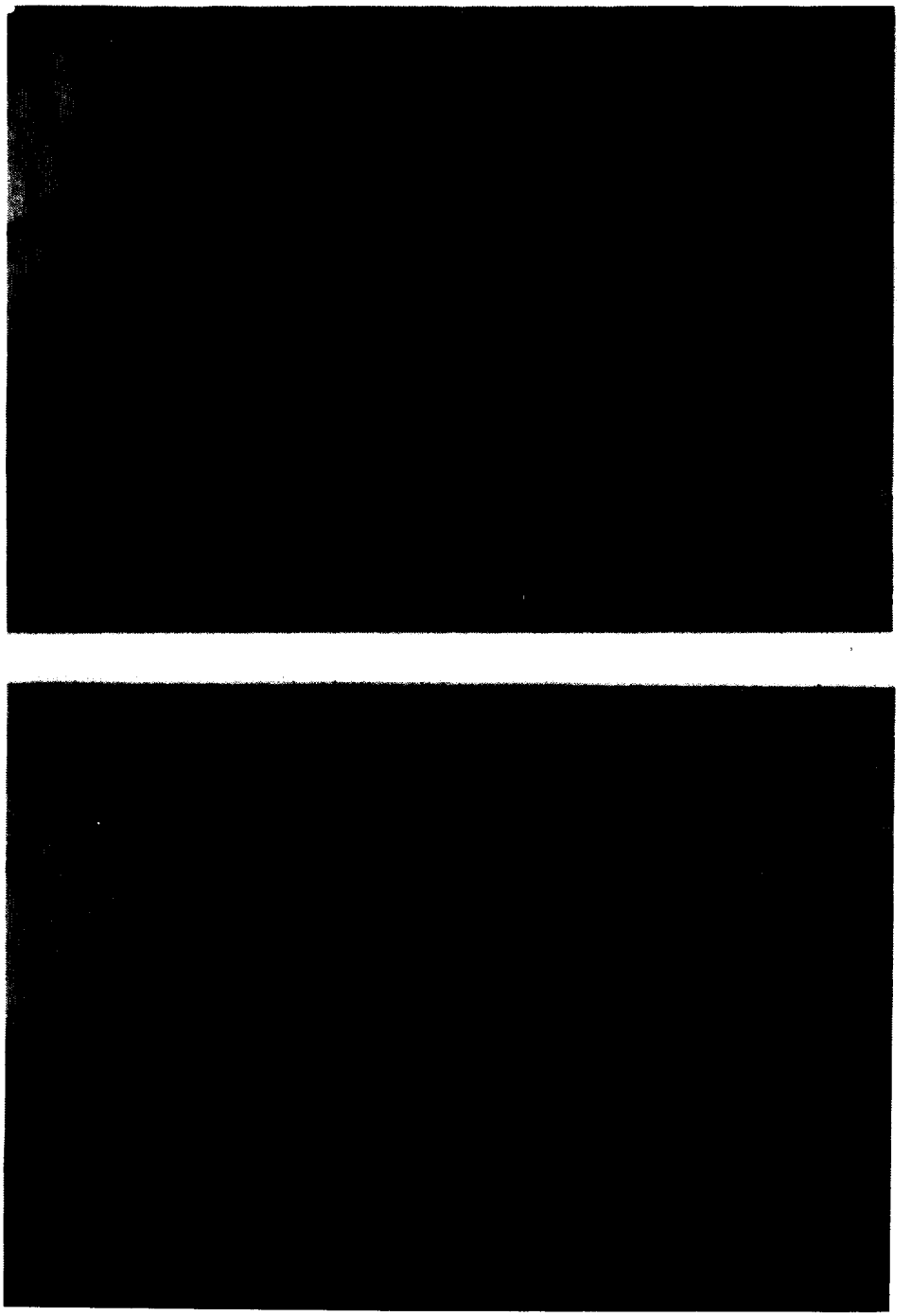

Plate 5. Hyphae of V. A, mycorrhizae found in the wild *Cym. kanran* roots.

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