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# Orchid breeding: Recent advances in biotechnology and considerations

**So-Young Park, Ph.D**

**Department of Horticultural Science, Chungbuk National University**

**Republic of Korea**





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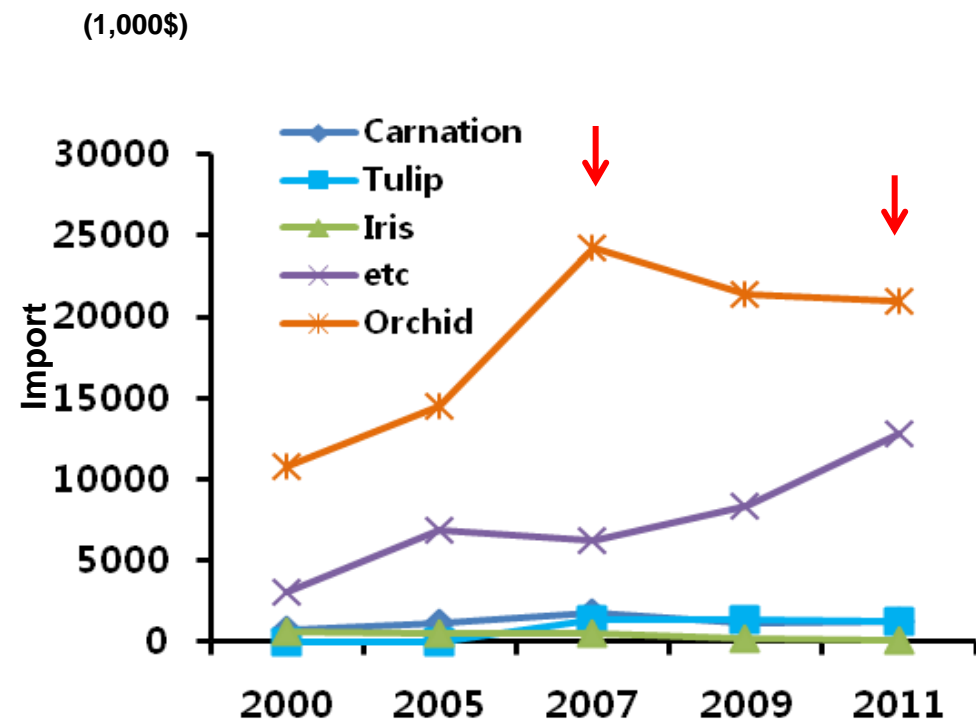
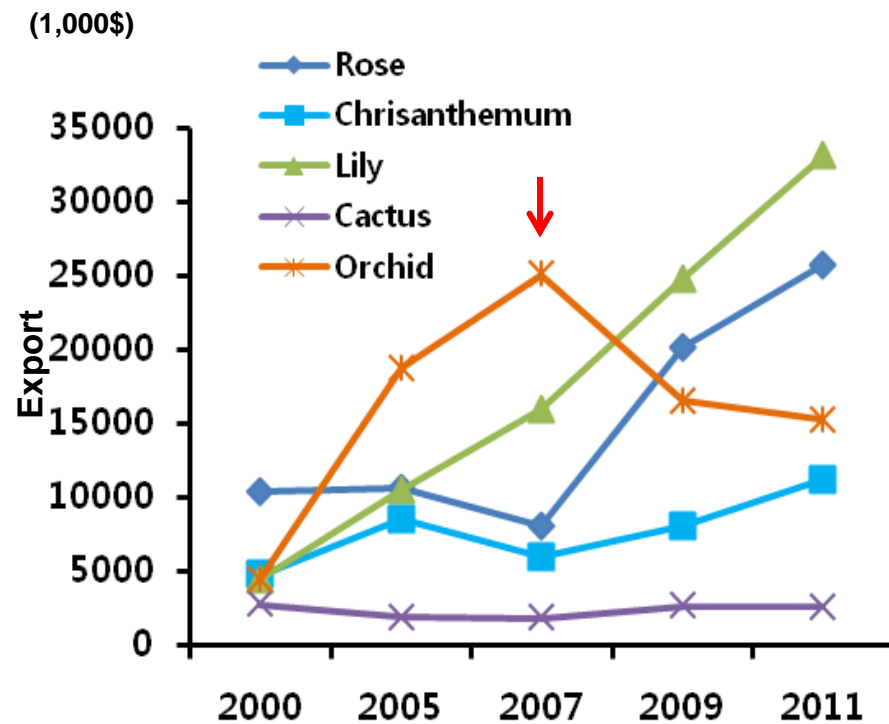
# Orchid Industry in Korea

- Total area utilized for floriculture was around 6,640 ha and the economic value reached US\$ 720 million. Among them 29% of the total pot flower cultivation area is allocated for orchid production.
- The value of orchid production reached up 36.8% of the total pot flower production value in Korea.
- The number of orchid growers is around 804 (7.5%) out of 10,690 flower growers.
- Orchid production is one of the most representative bio-agricultural industries in Korea.



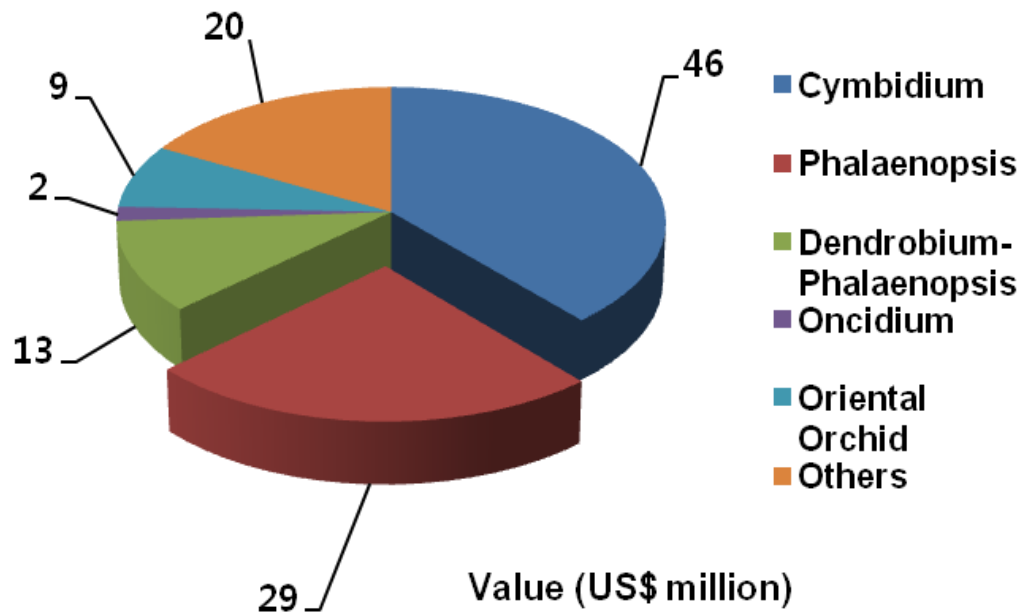


# Orchid Industry in Korea





# Orchid Industry in Korea



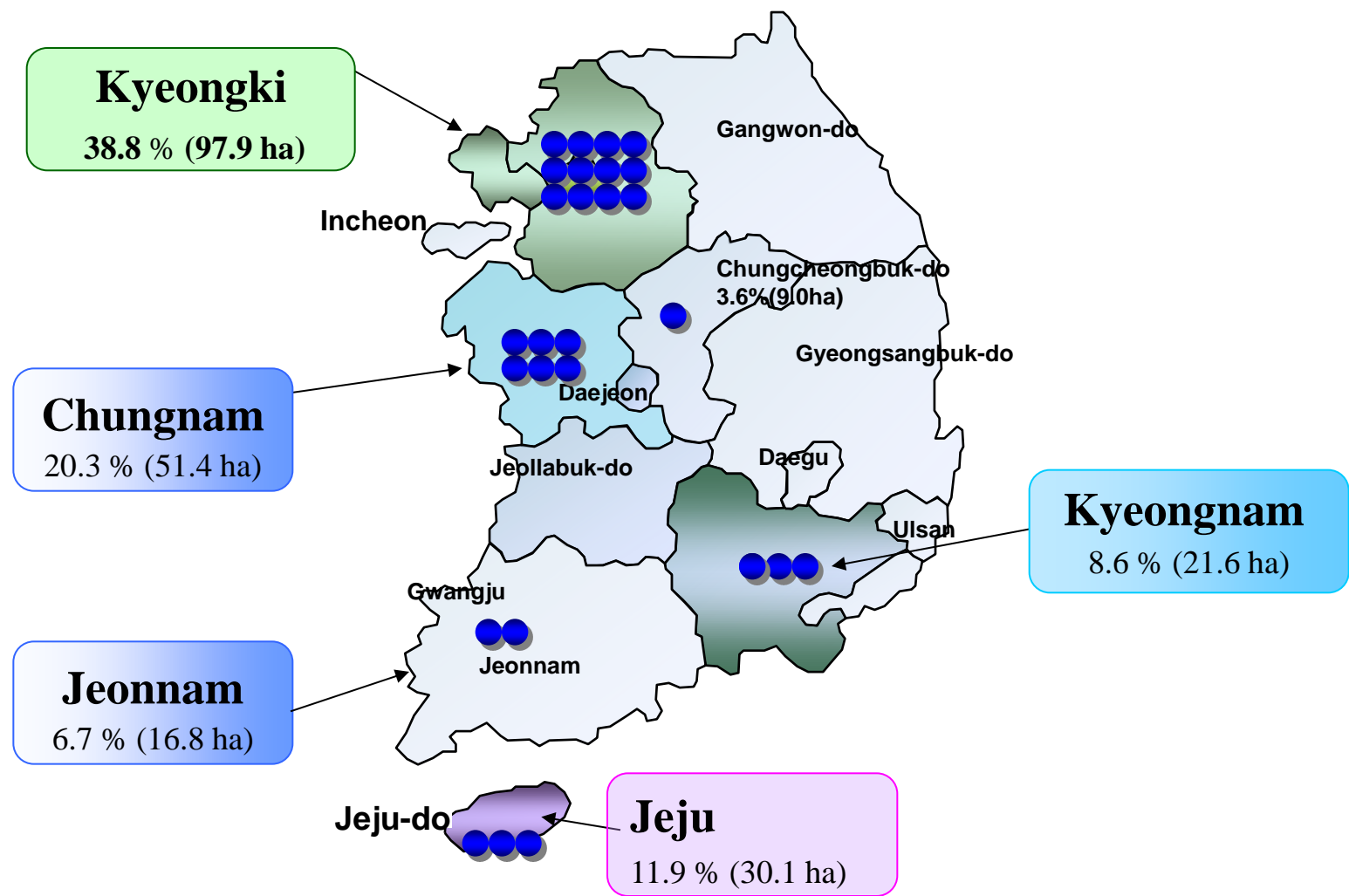
Orchid production (RDA, 2009)







# Orchid Cultivation in Korea



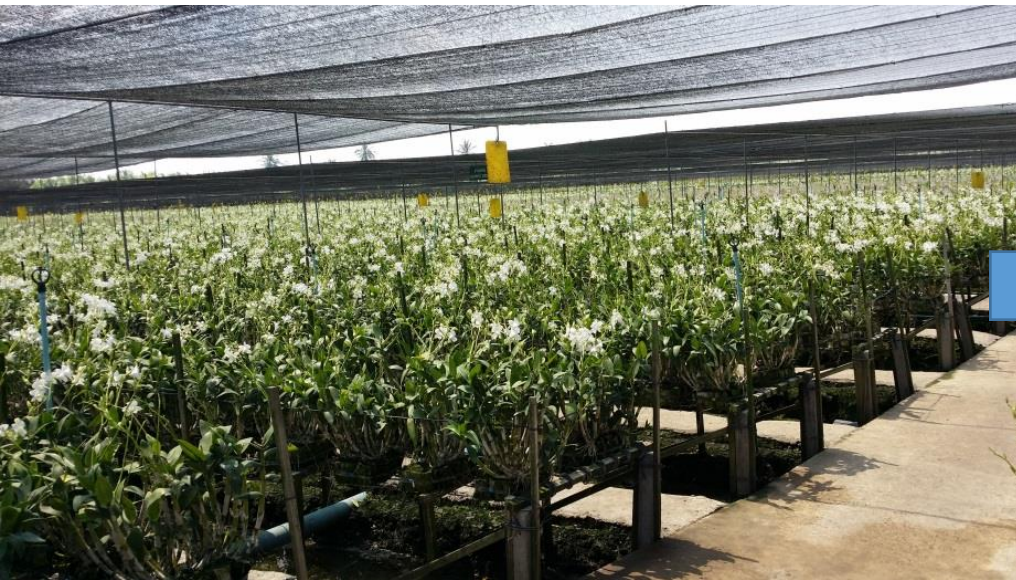


# Micropropagation

- Regeneration and Development -



# Conventional propagation by cutting and division



Mother plants for  
flowers and pseudo  
bulbs for propagation

Dendrobium-Phalaenopsis  
propagation (TOC group in  
Thailand)





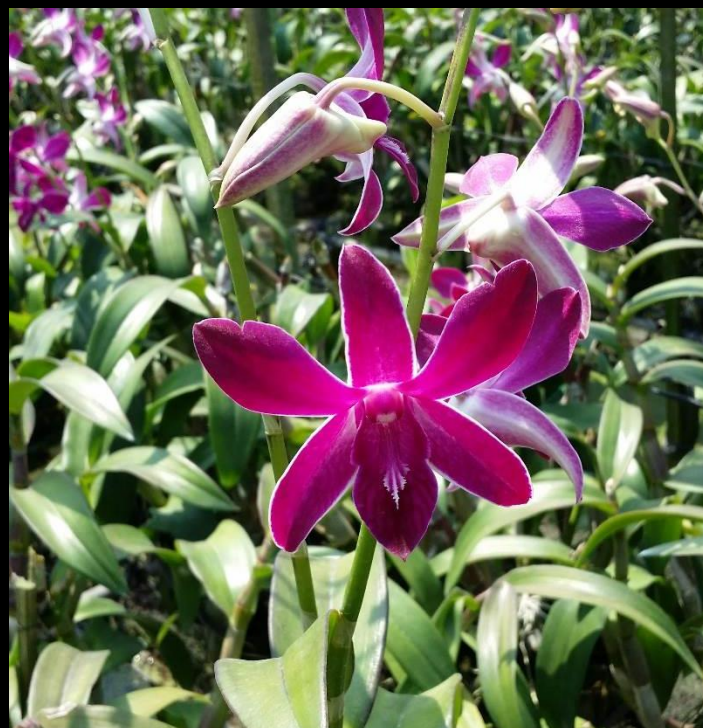
**Dendrobium-Phalaenopsis propagation (TOC group in Thailand)**



**New shoot production from old pseudobulb**



# Dendrobium-Phalaenopsis propagation (TOC group in Thailand)





# Dendrobium-Phalaenopsis propagation (TOC group in Thailand)

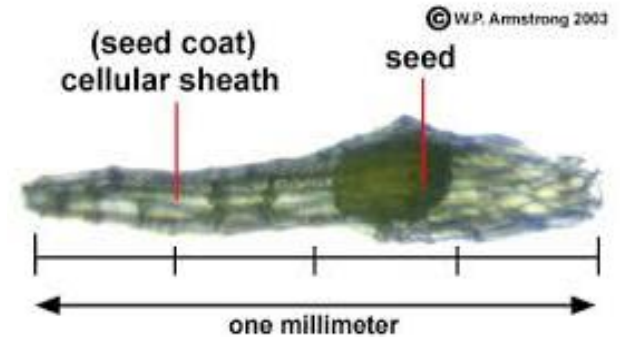




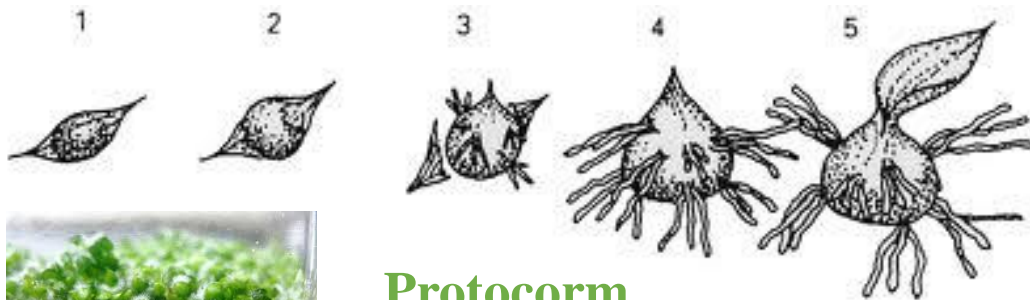
# Dendrobium-Phalaenopsis propagation (TOC group in Thailand)



# Micropropagation by seed germination



Orchid capsule and seeds



Protocorm



In vitro seed germination



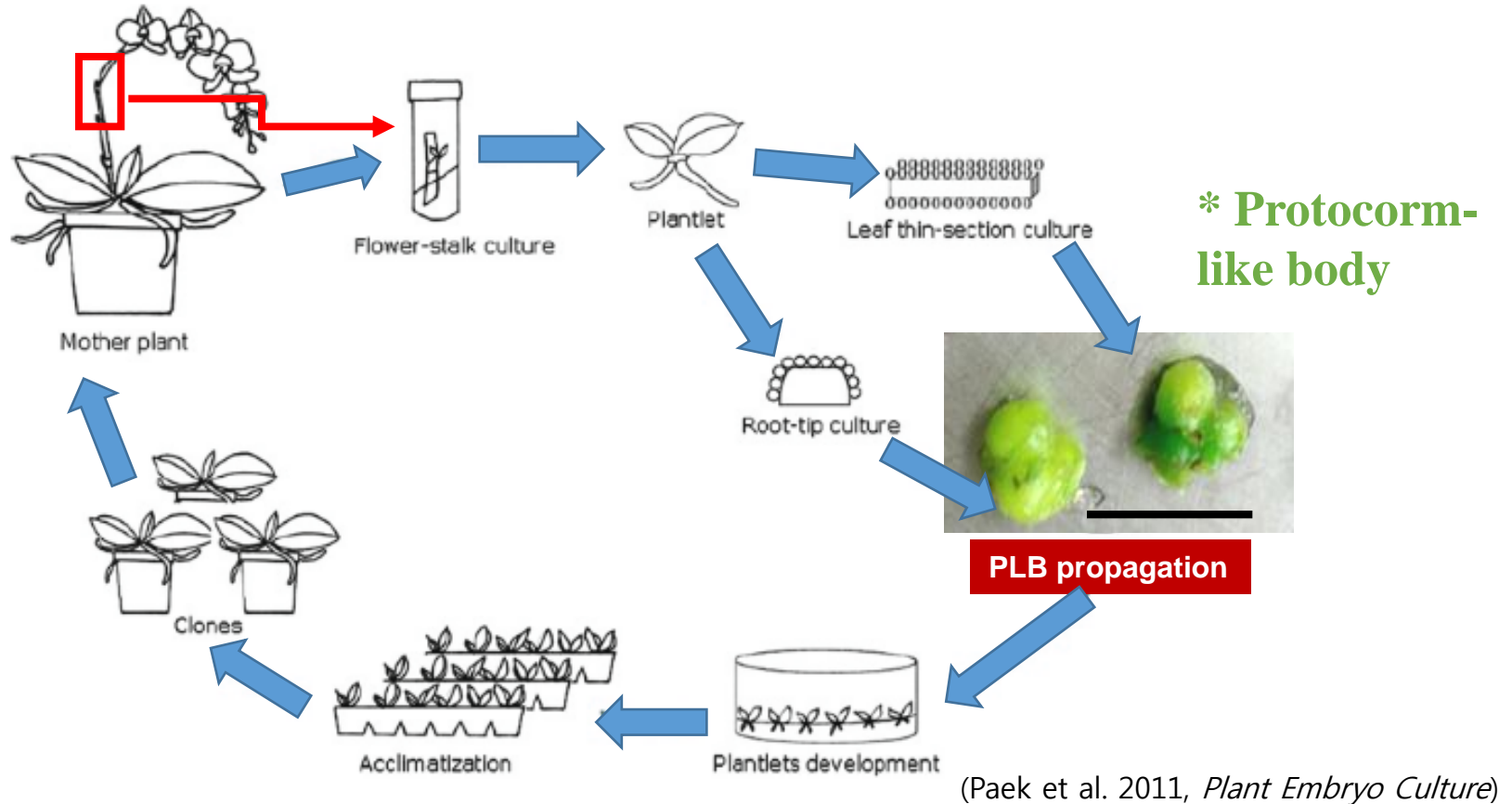


# Dendrobium-Phalaenopsis propagation (TOC group in Thailand)



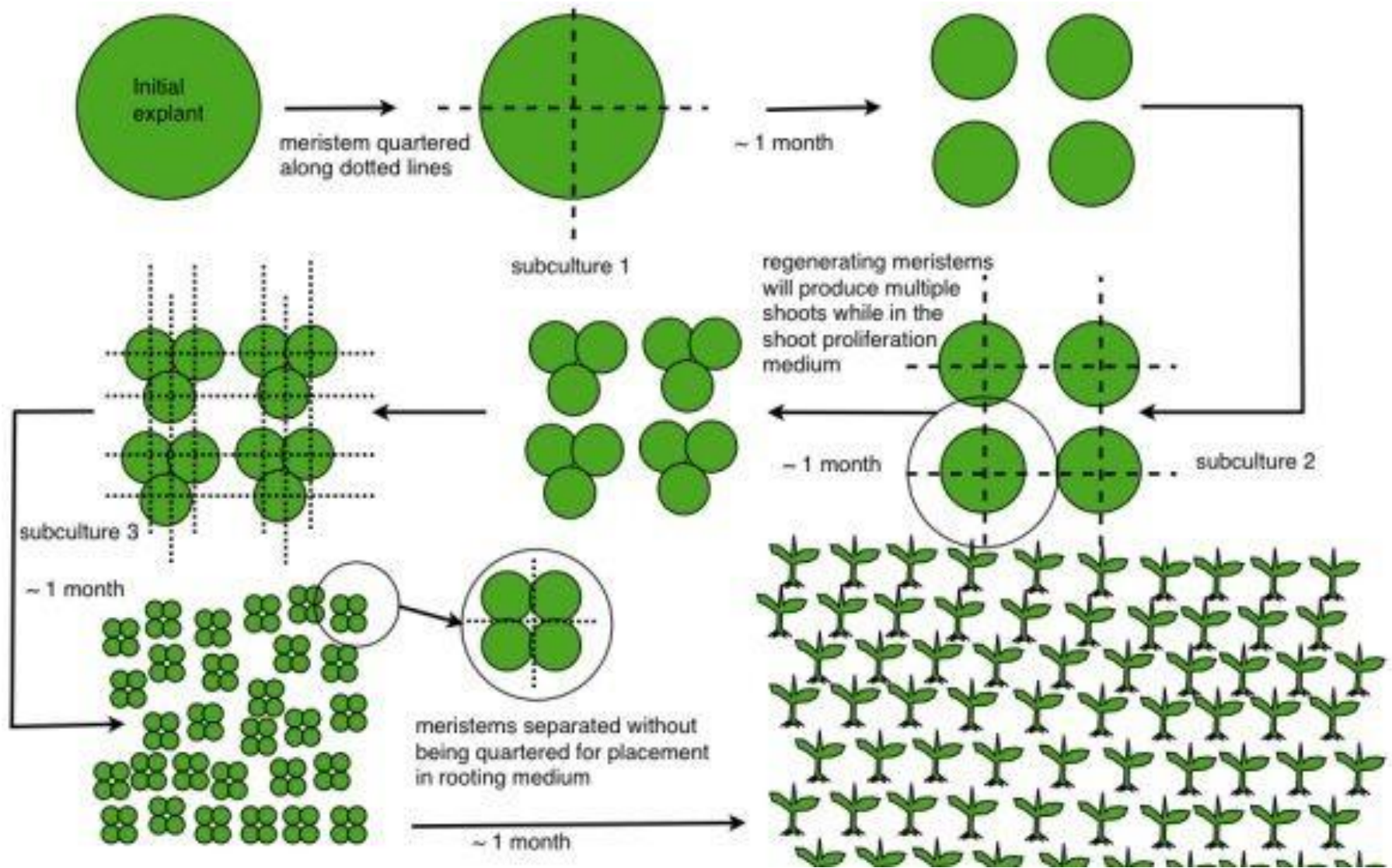


# Micropropagation by clonal propagation



**Fig. Vegetative propagation of elite orchid via PLB multiplication.**

# Concept of mass production system via micropropagation





# Breeding by in vitro biotechnology

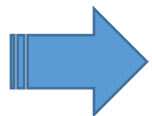
- 1. Micropropagation**
- 2. Polyploidy breeding**
- 3. Mutation breeding**
- 4. Genetic transformation**



# Micropropagation



- **Mass propagation of elite genotype by selection from seedling populations**
  - Flower color, disease resistance, flowering time..
- **Regeneration ability of plant**
- **Essential of plant cell and tissue culture technique**



**Rapid establishment of new variety**

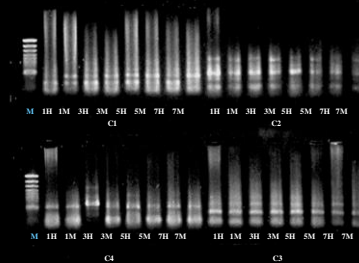
# Orchid MICROPROPAGATION (Phalaenopsis)



Proliferation via PLB/multiple-shoots



Mass production via solid/liquid culture



Variant detection



Acclimatization and  
greenhouse cultivation



PPF ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )



50 100 150 200 300



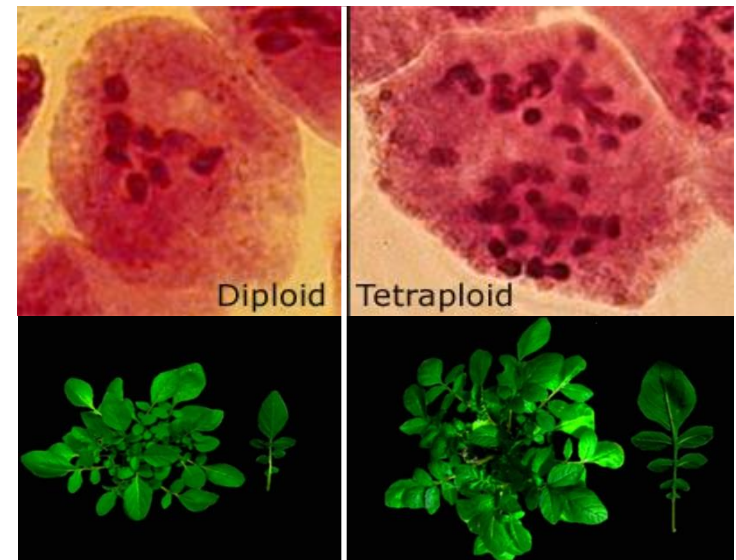
# Ploidy breeding



- Polyploidy is plants or cells containing more than two paired sets of chromosomes.
- Polyploidy is responsible for increases in cell size, a characteristic that leads to larger vegetative and reproductive organs (Kim et al, 2004).



*Phalaenopsis equestris* var *cyanochilus*



*Solanum commersonii* (Riccardo et al, 2012)

# Polyploidy orchid

## Chemicals for chromosome doubling

### 1. Colchicine( $C_{22}H_{25}NO_6$ )

- Narcotic alkaloid extraction from *Colchicum autumnale*
- Inhibition of microtubules during mitosis of plant cell

### 2. Oryzalin( $C_{12}H_{18}N_4O_6S$ )

- A kind of herbicide
- The disruption of microtubules during mitosis

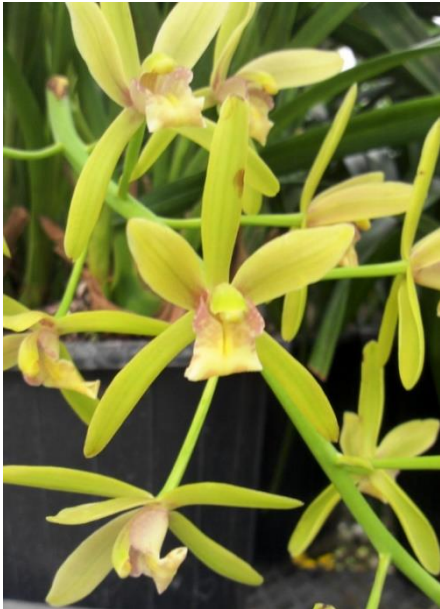


*Cymbidium Show Girl 'Silky'*



## Breeding in National Institute of Horticultural & Herbal Science

- Species  
: *Cymbidium* Elma 'Orient Toyo', C. Golden Elf 'Sundust', C. Show girl 'silky'
- Diploid and tetraploid plants by treatments of Colchicine 100-500 mg/L for 1-2 week



*Cymbidium* Elma 'Orient Toyo'



*Cymbidium* Golden Elf  
'Sundust'

# Analysis of DNA content for ploidy determination

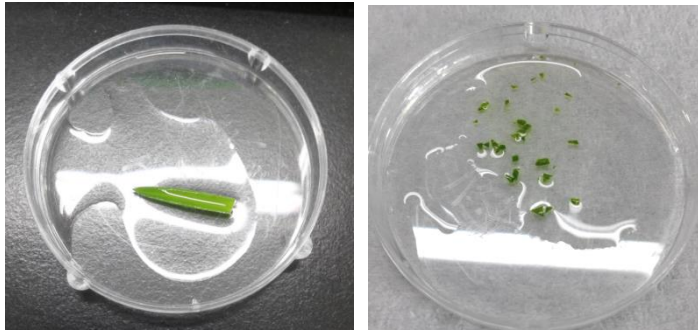


Treatment of colchicine and oryzalin

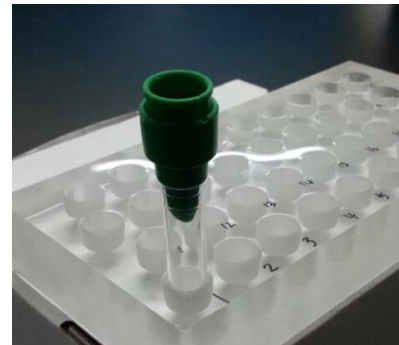


Flow cytometry

Extraction



Chopping and extraction of nuclei



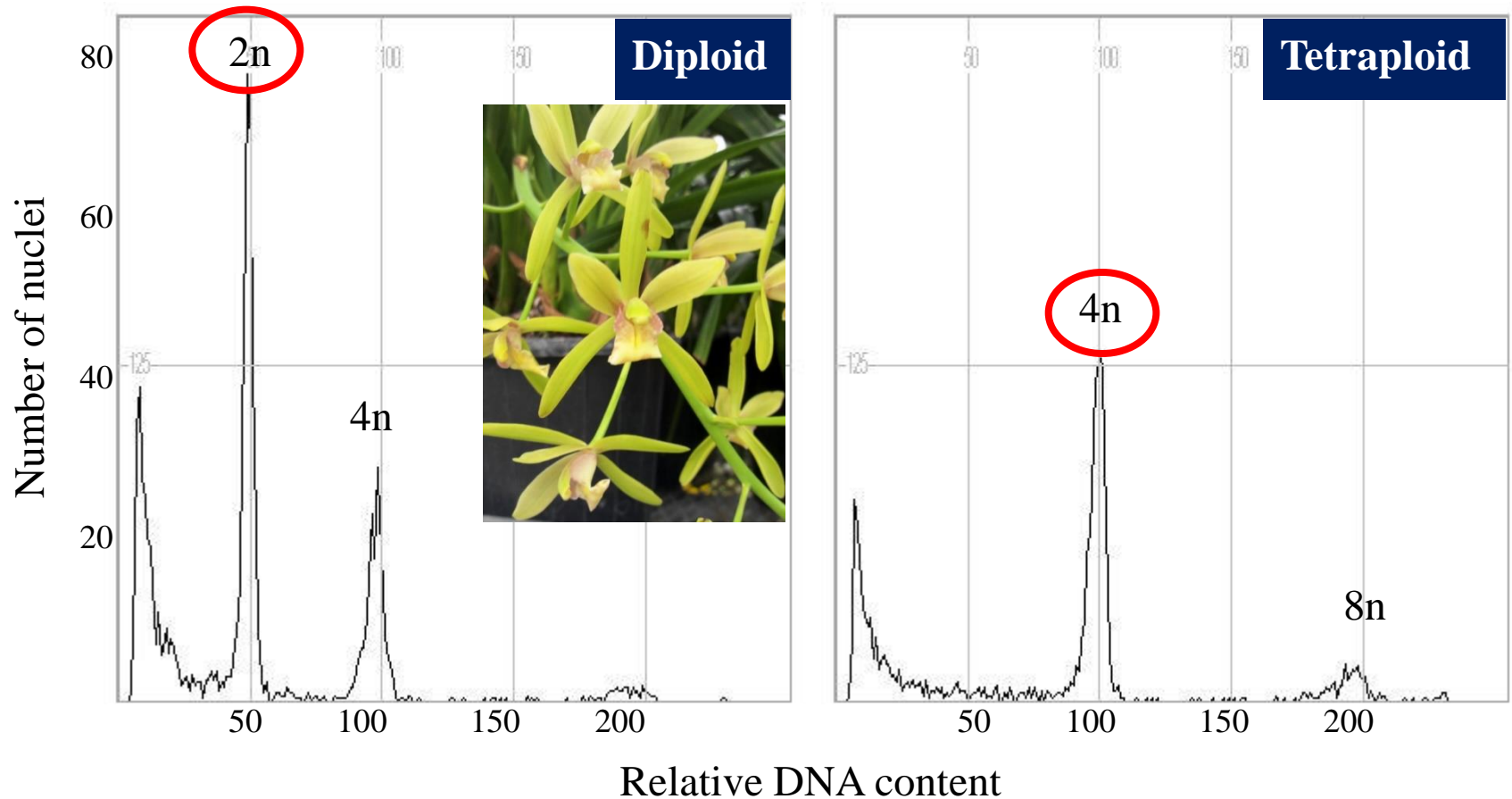
Filtering



Nuclei staining

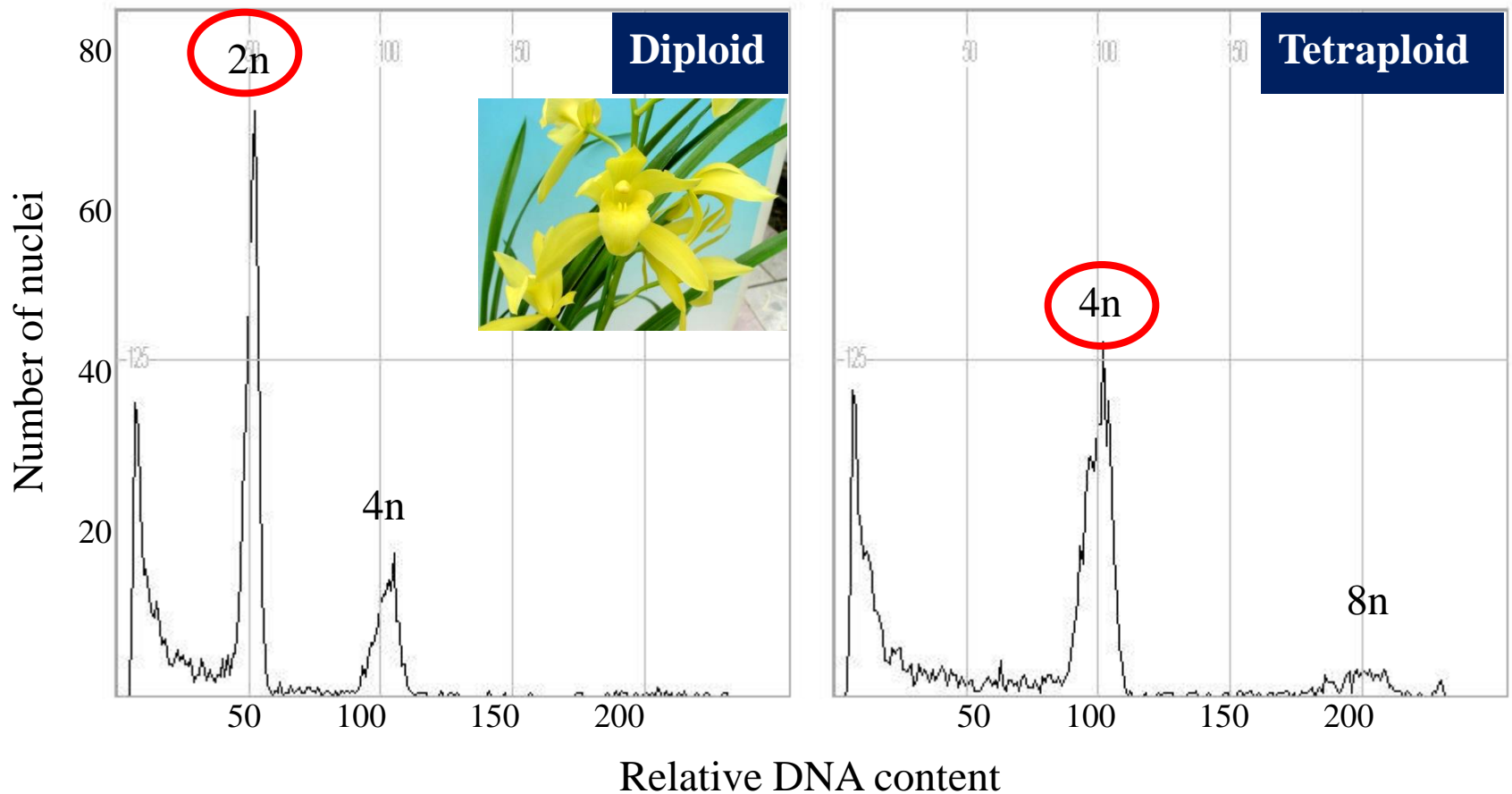


## *Cymbidium* Elma 'Orient Toyo'



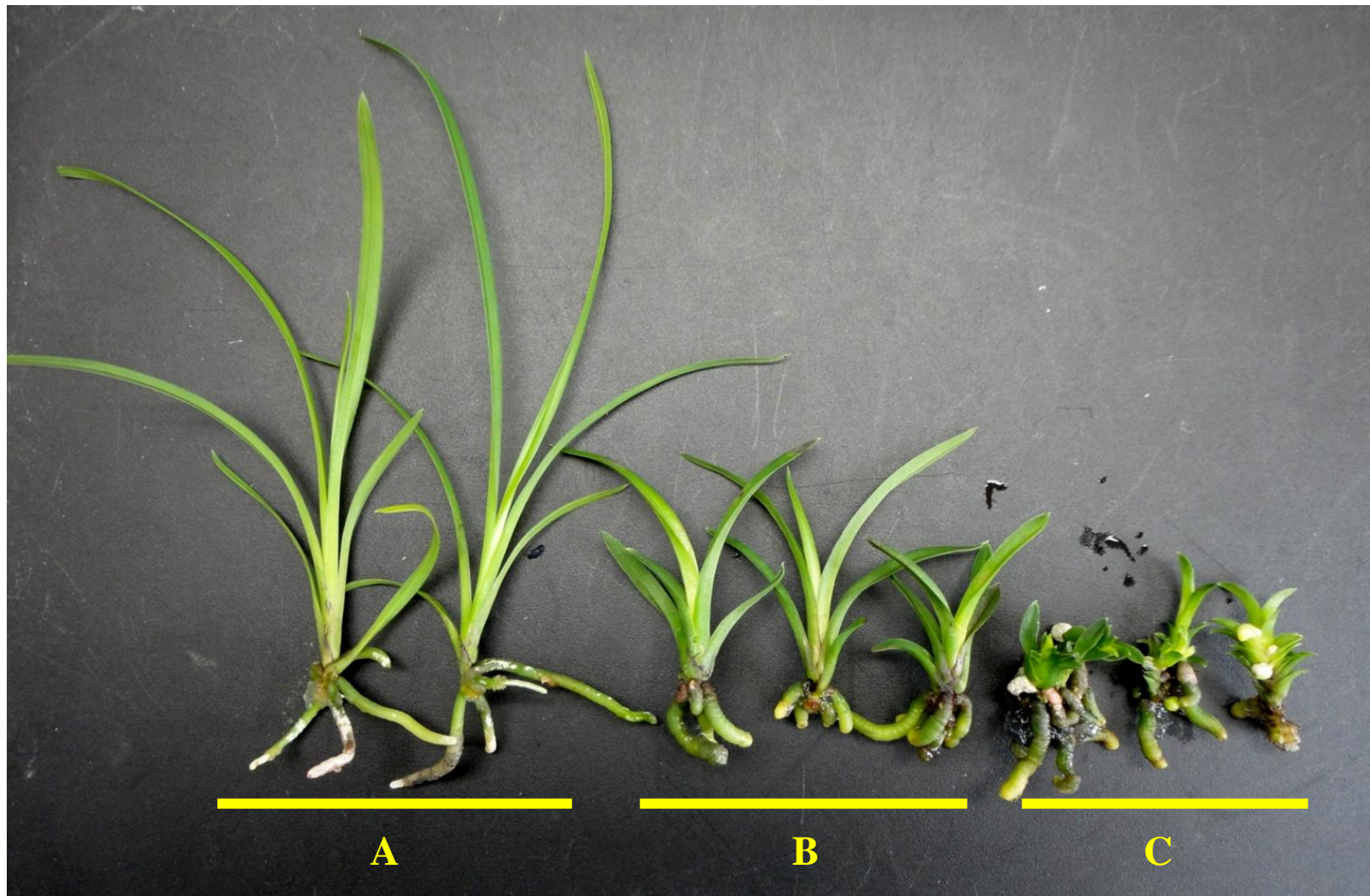
**Fig. Flow cytometric DNA histograms of diploid and tetraploid of *Cymbidium* Elma 'Orient Toyo'.**

## *Cymbidium* Golden Elf 'Sundust'



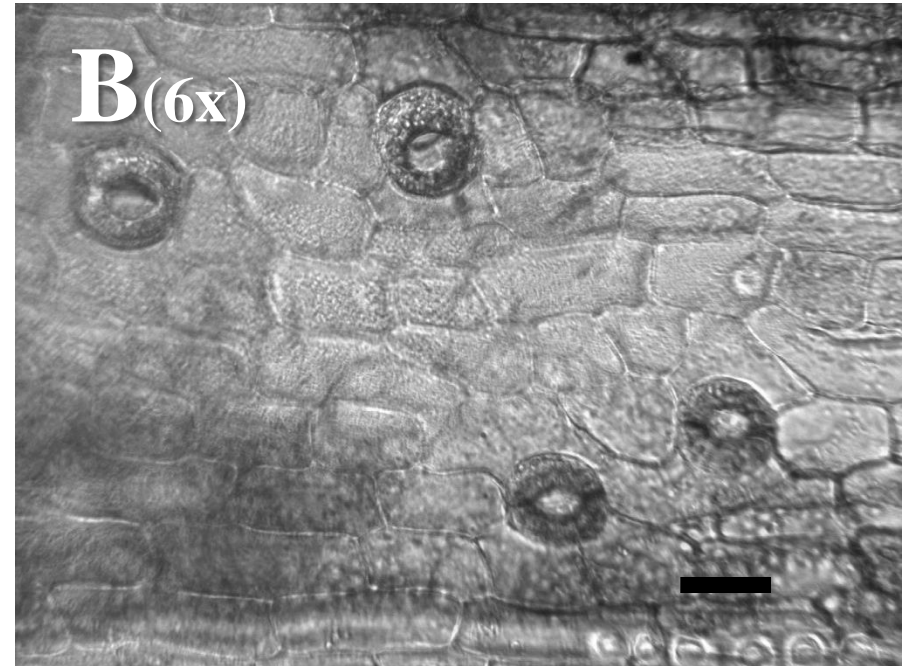
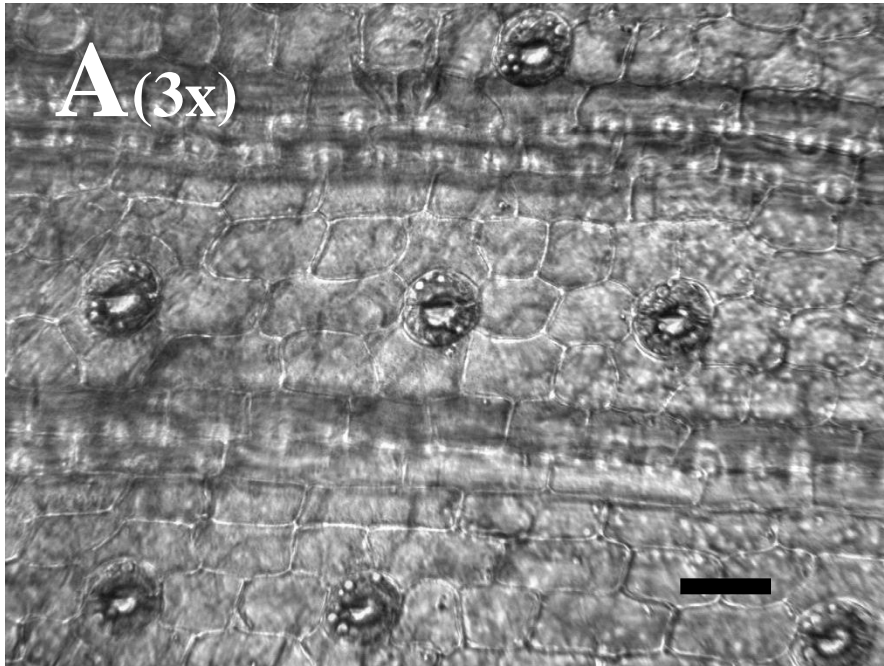
**Fig. Flow cytometric DNA histograms of diploid and tetraploid of *Cymbidium* Golden Elf 'Sundust'.**





**Fig. Control plants (A), chromosome doubled plants (B), ploidy chimera plants (C) of *Cymbidium* Show Girl 'Silky'.**

## *Cymbidium* Show Girl 'Silky'



**Fig. Guard cells of control and chromosome doubled plant of *Cymbidium* Show Girl 'Silky'. 400× magnification. Bars = 40μm.**



## Flower morphology by ploidy level

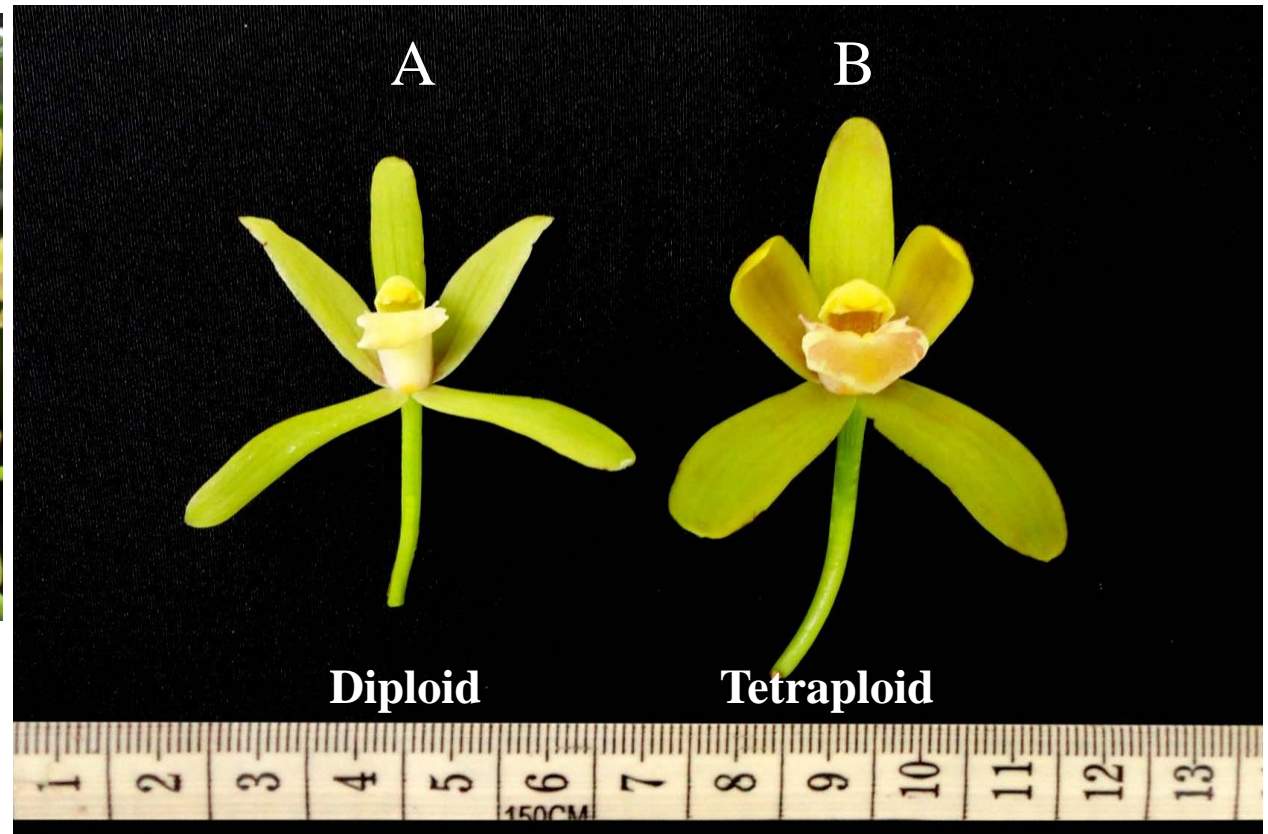
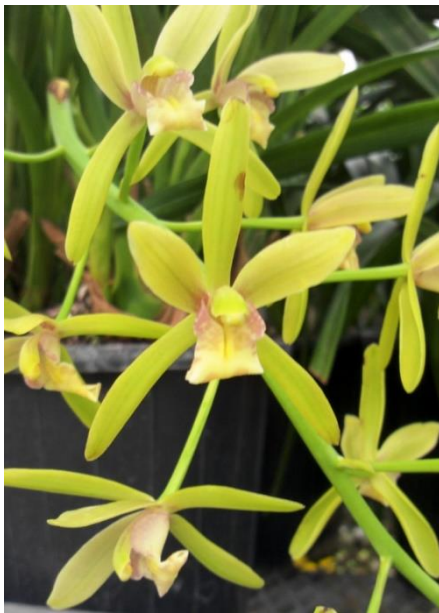


Fig. Diploid C and tetraploid D flowers of *Cymbidium* Elma 'Orient Toyo'. (Hwang et al. unpublished data)

## Flower morphology by ploidy level

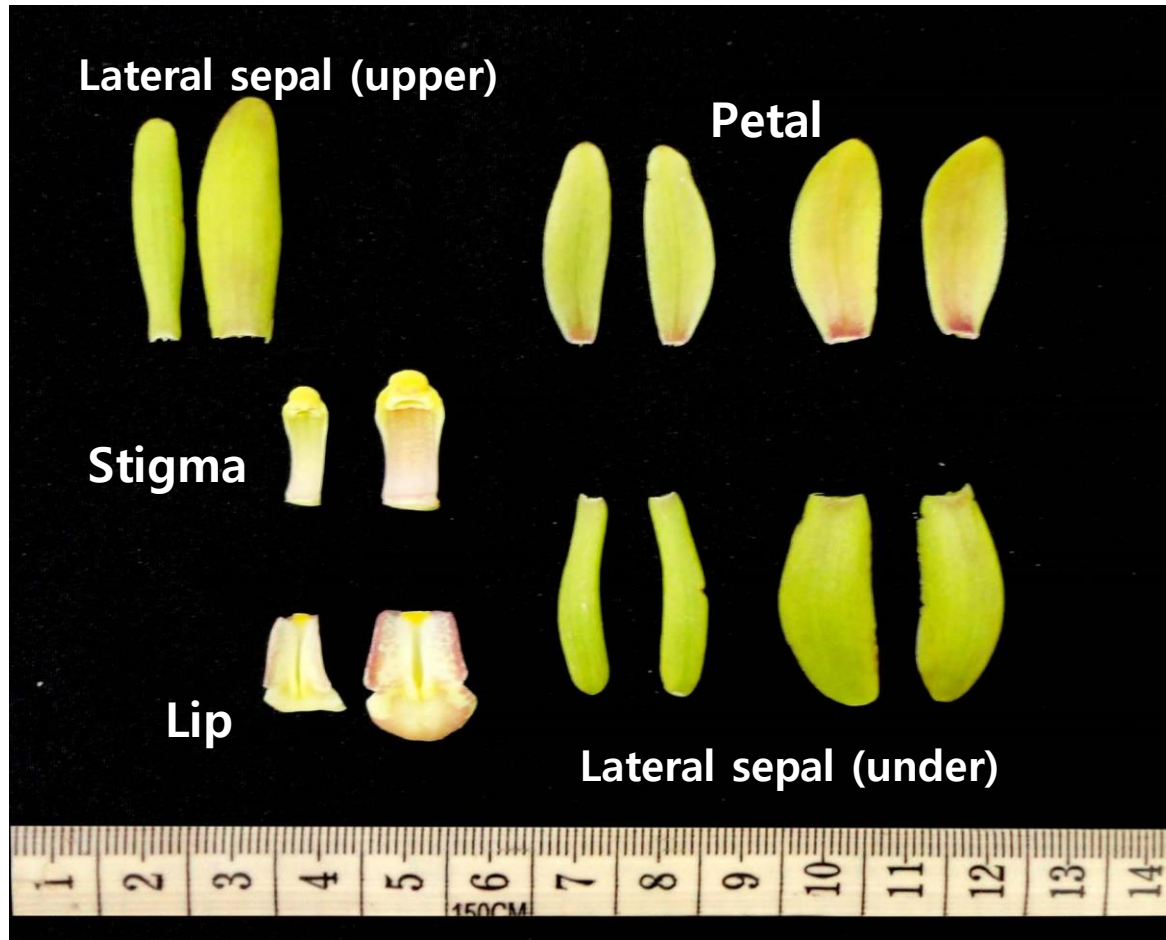


Fig. Diploid (Left) and tetraploid (Right) floral parts of *Cymbidium Elma* 'Orient Toyo'.  
(Hwang et al. unpublished data)



## Flower morphology by ploidy level

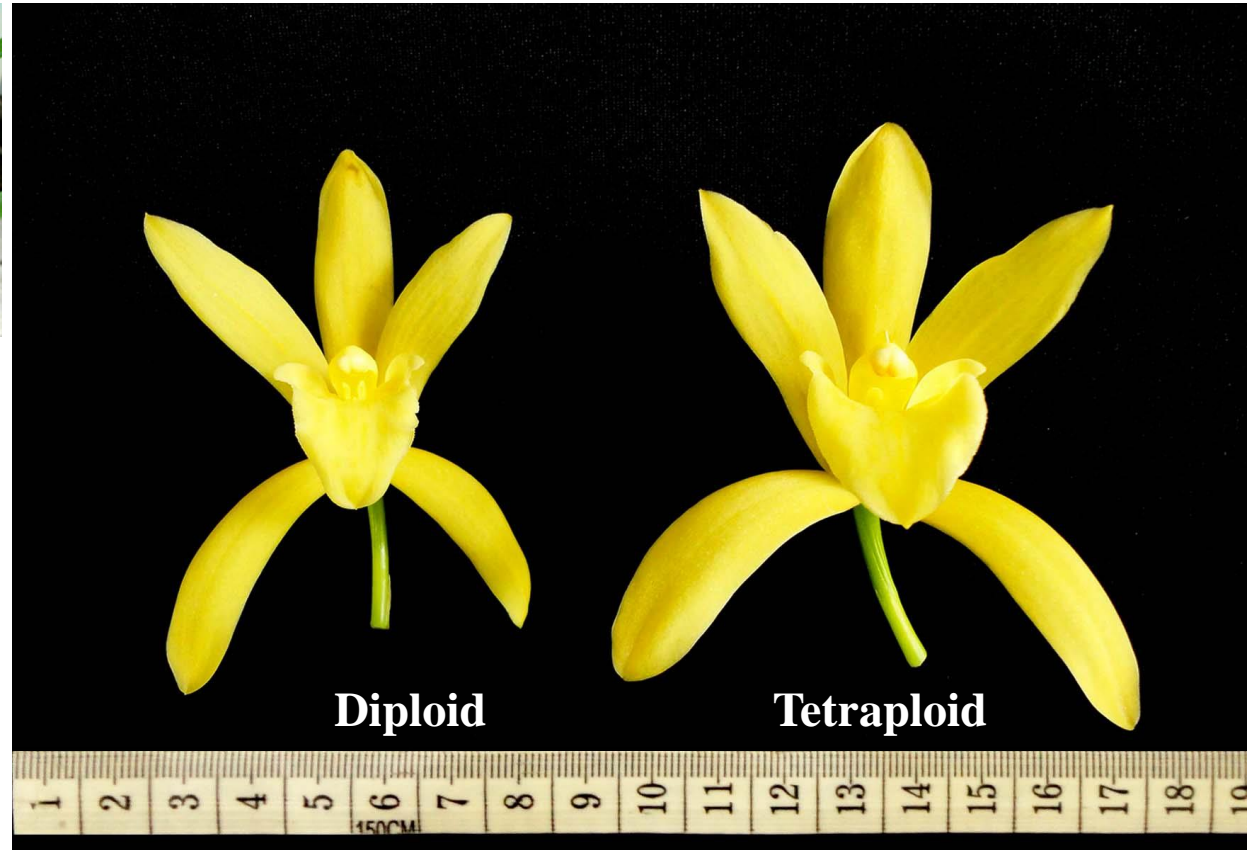


Fig. Diploid A and tetraploid B flowers *Cymbidium* Golden Elf 'Sundust'. (Hwang et al. unpublished data)

## Flower morphology by ploidy level

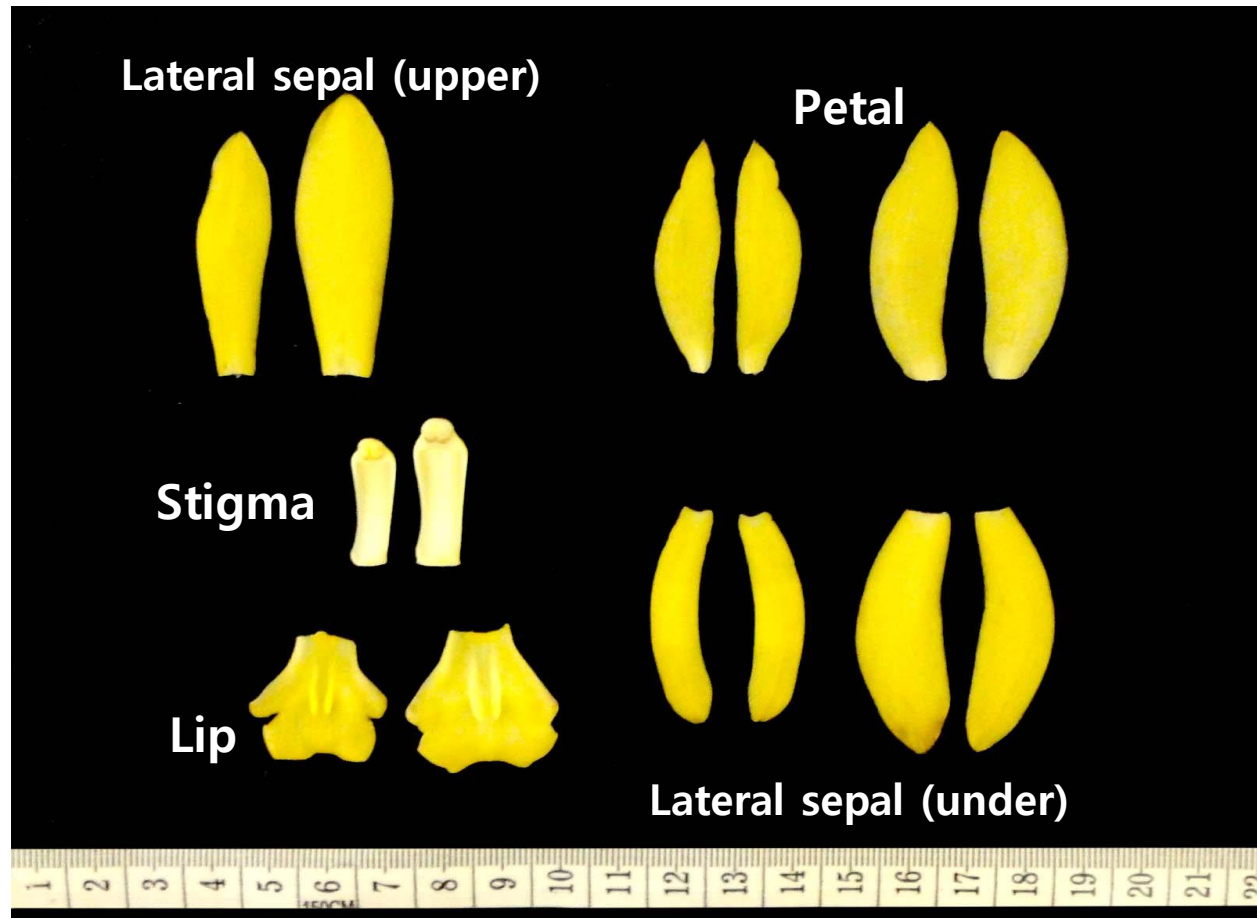
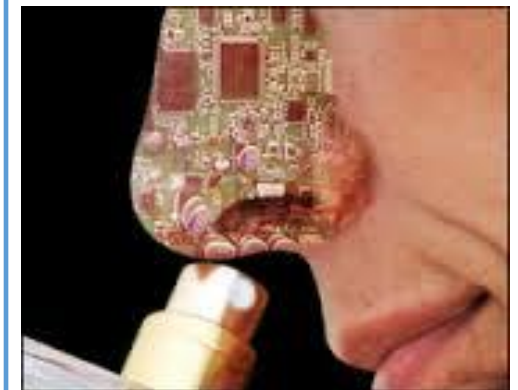
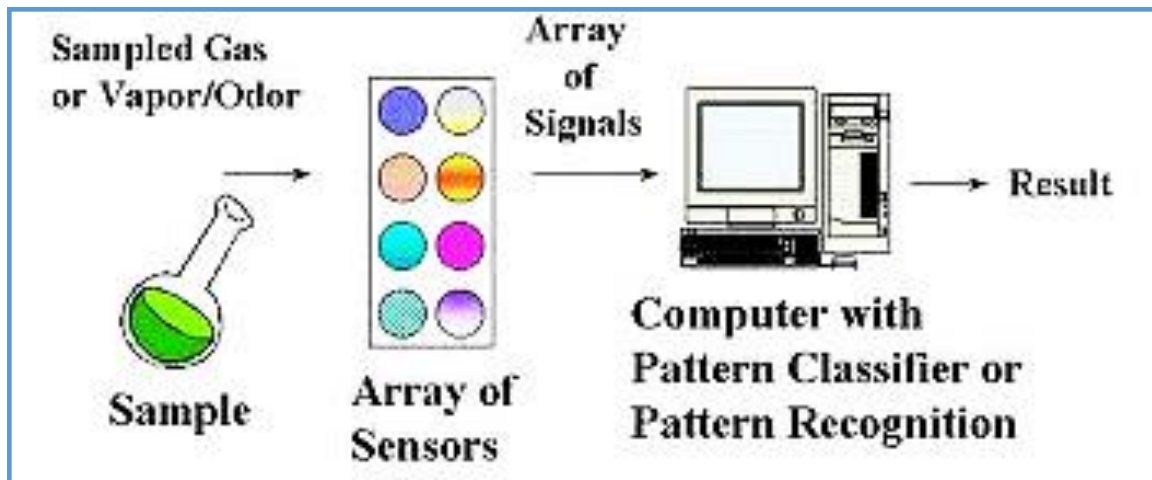


Fig. Diploid (Left) and tetraploid (Right) floral parts of *Cymbidium Golden Elf 'Sundust'*. (Hwang et al. unpublished data)

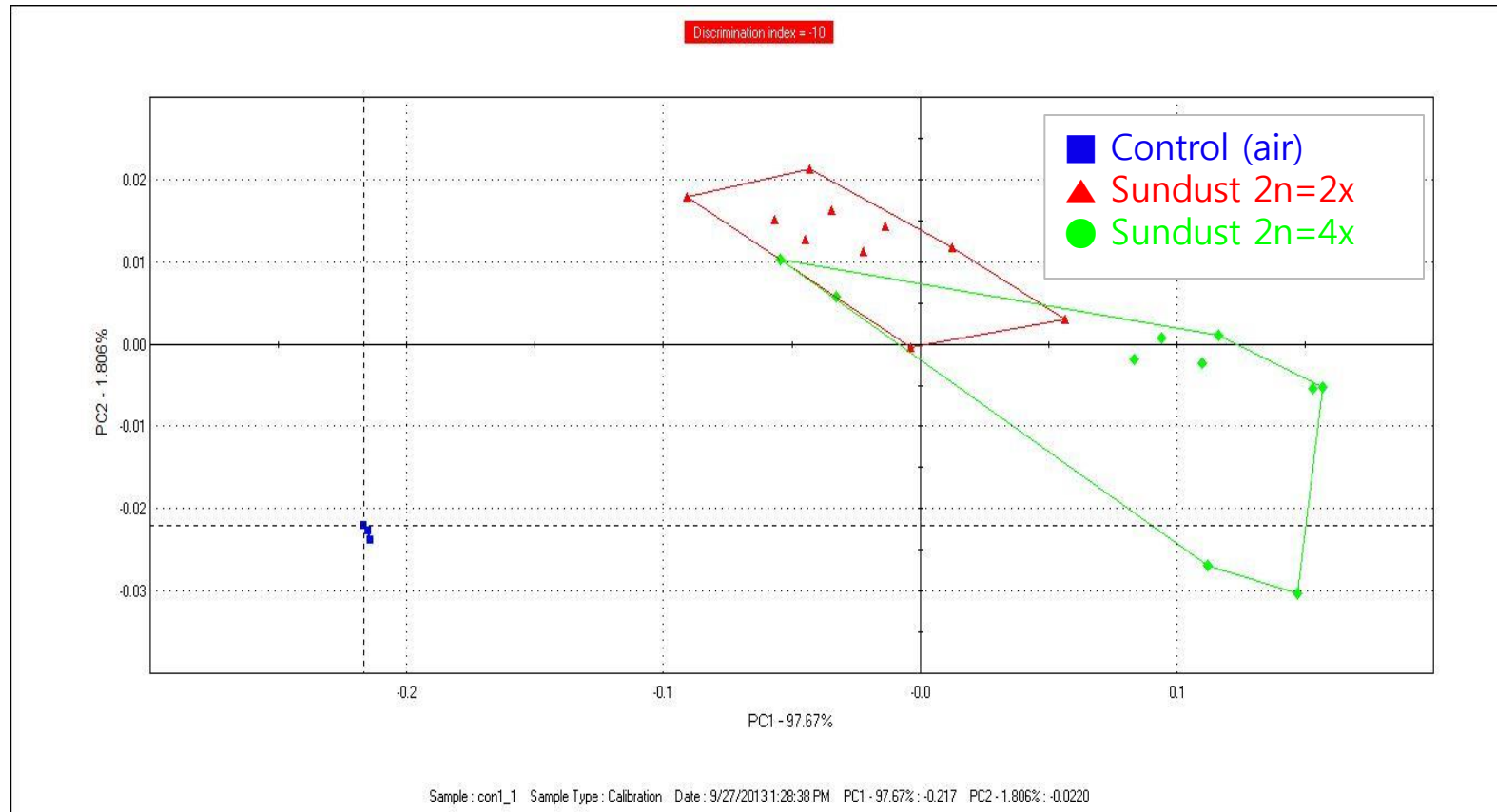


# Fragrance pattern in ploidy plant

- **Analysis of fragrance pattern using electronic nose**
  - MOS : High similarity with human olfactory sense
  - Use : Fox2000 with Sensor Array System (Alpha M.O.S)
- **Materials** : *Cymbidium* Golden Elf 'Sundust' di-, tetraploid
- Alpha soft model : **PCA** (Principle component analysis),  
**DFA** (Discriminant factor analysis)



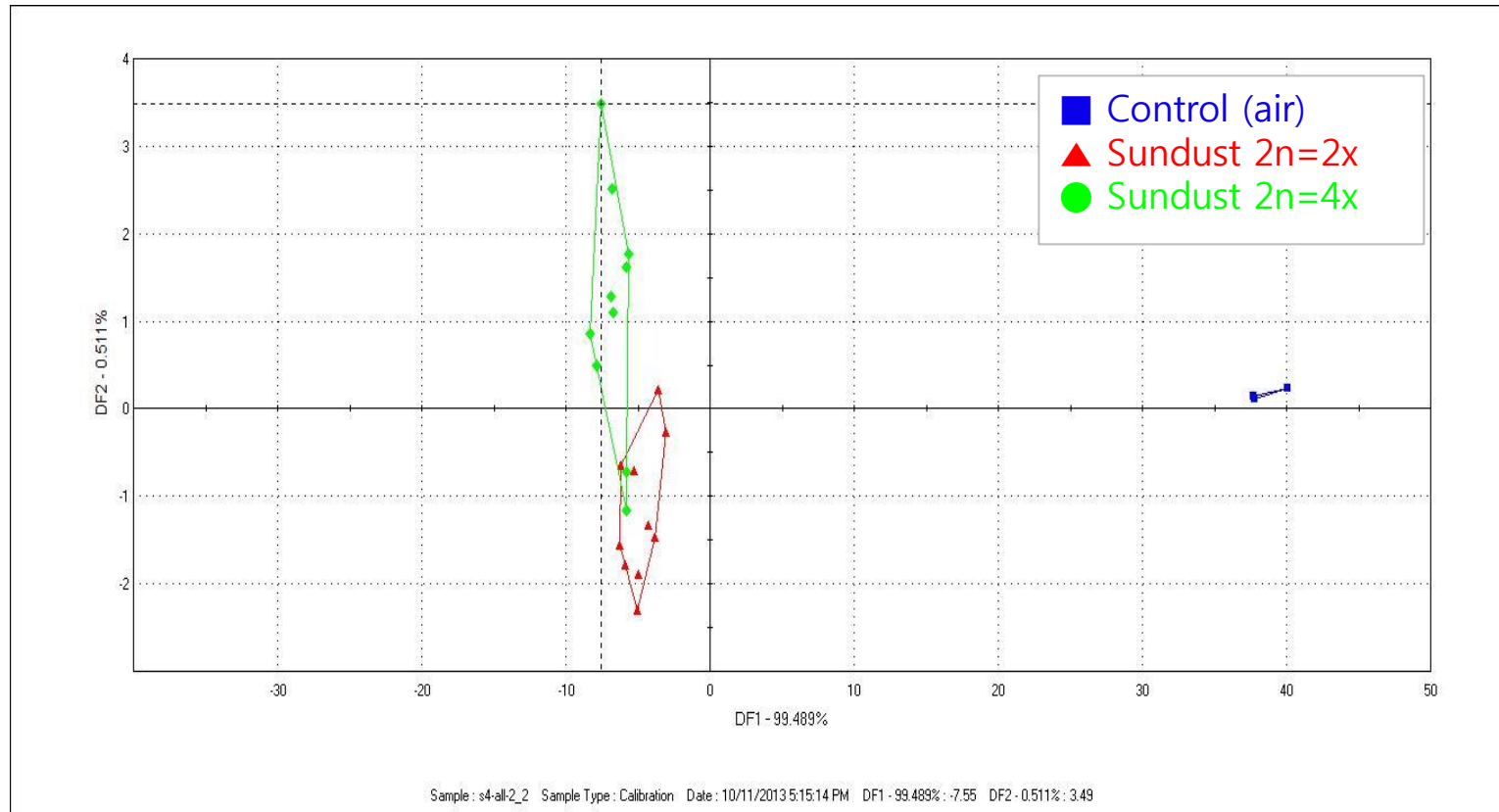
# Scent pattern of di- and tetraploid flower (PCA)



**Fig. Principle component analysis (PCA) plot of the scent from the 2n and 4n flowers in C. Golden Elf 'Sundust' using the electronic nose. (Hwang et al. unpublished data)**



## Scent pattern of di- and tetraploid flower (DFA)



**Fig. Discriminant factor analysis (DFA) plot of the scent from the 2n and 4n flowers in *C. Golden Elf* 'Sundust' using the electronic nose. (Hwang et al. unpublished data)**

# Mutation breeding



- **X-ray, gamma ray irradiation, and chemical treatments**
- Powerful tools for plant breeding as well as for physiological and molecular studies
- **Gamma ( $\gamma$ ) radiation**
  - a type of ionizing radiation
  - produce free radicals in cells which damage or modify DNA of plant cells and affect differently the morphology



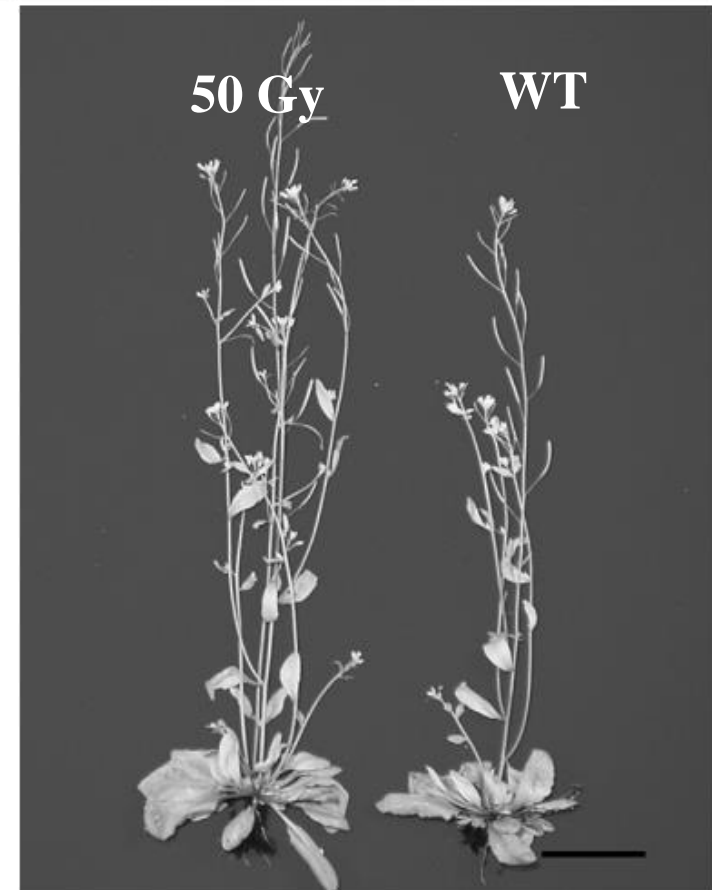
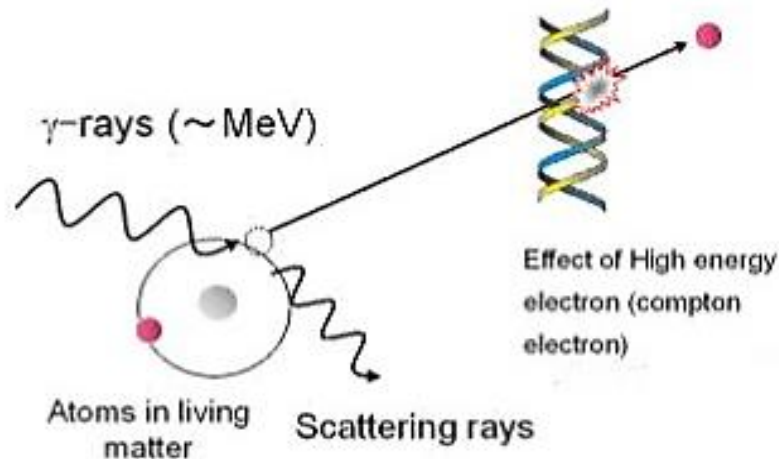
# Mutation breeding



## Gamma rays

- Convenient operation
- Short cycle
- High mutation quantity

## Gamma ray effect on damaging DNA



(Wi *et al.*, 2007)



**한국원자력연구원**  
Korea Atomic Energy Research Institute

Mother plants

Mutants





# Mutation breeding



(Taheri et al. 2014)

***Curcuma alismatifolia* varieties by gamma ray**

# Genetic mutation



Some genetic variants are popular and useful



Variants of *Aerides japonicum* obtained from asymbiotic seed cultures



Very popular in special grower with high price



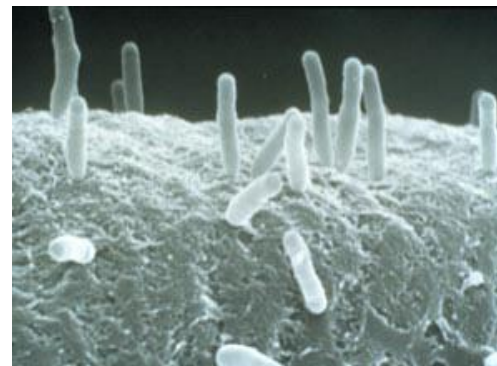
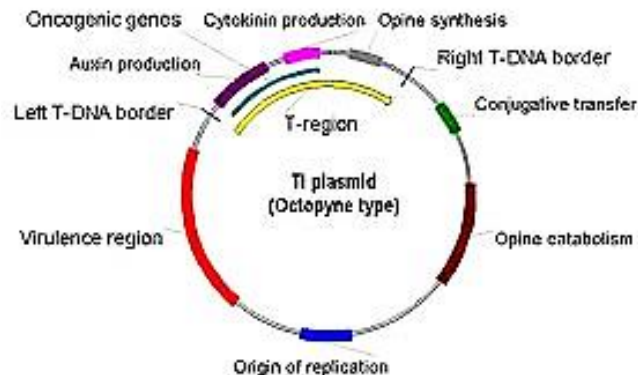


# Genetic transformation

# Genetic transformation

## Delivering the gene to the plant

- Transformation cassettes are developed in the lab
- They are then introduced into a plant
- Two major delivery methods
  - *Agrobacterium*
  - Gene Gun (Particle bombardment)



*Agrobacterium*

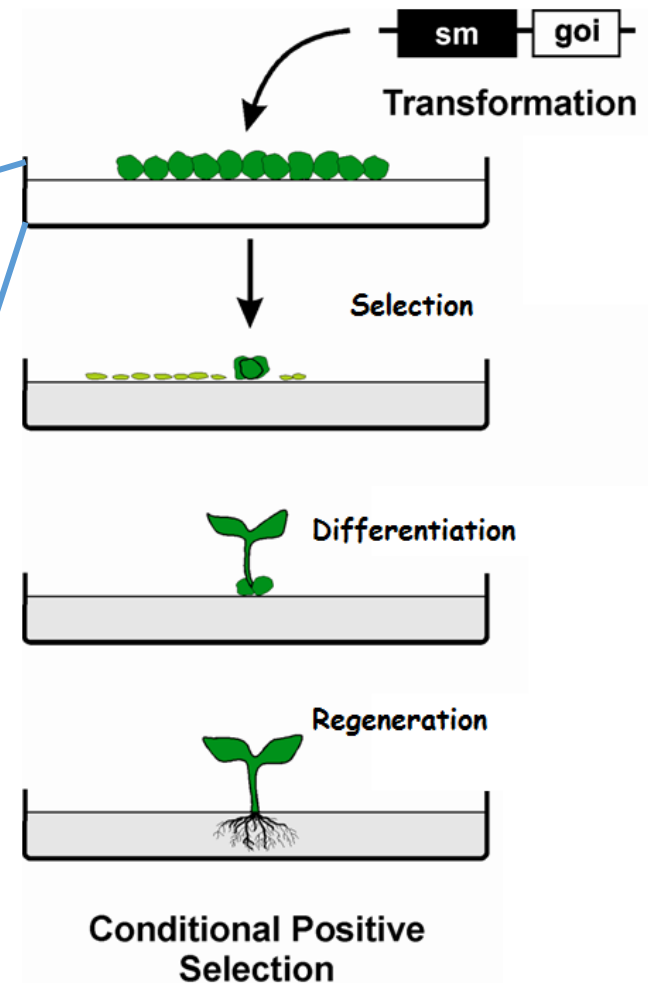
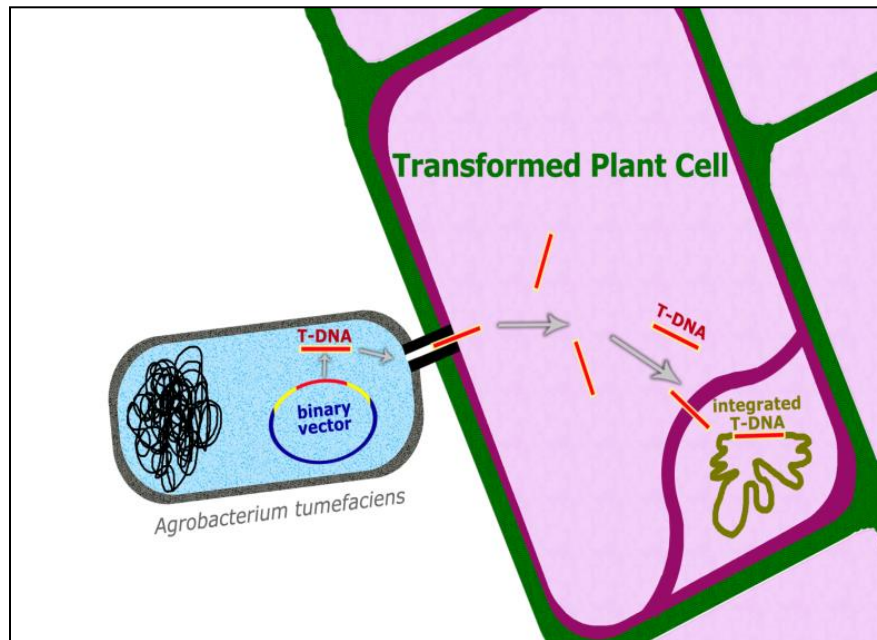


Gene gun



# Genetic transformation

by *Agrobacterium*



# World Orchid Exhibition 2012. Okinawa in Japan

Genetic transformation of orchid





# World Orchid Exhibition 2012. Okinawa in Japan

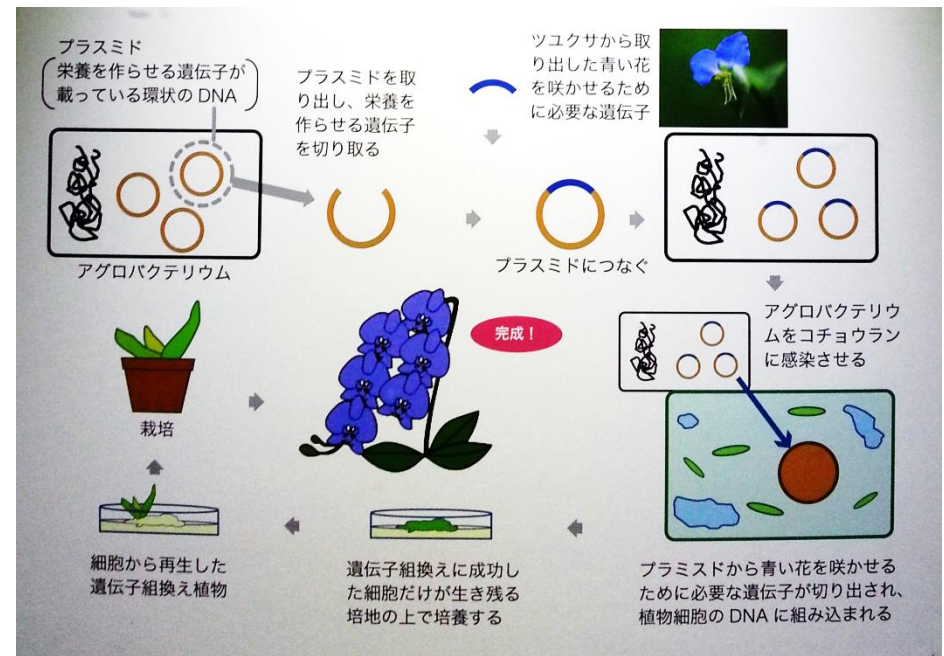
Genetic transformation of orchid



P1(P)  
MILWAUKEE 1992  
MILWAUKEE 1992  
MILWAUKEE 1992

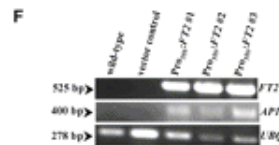
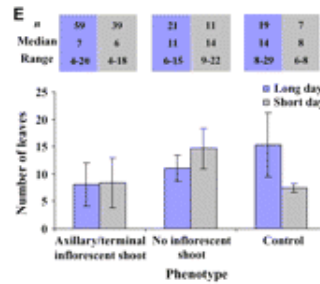
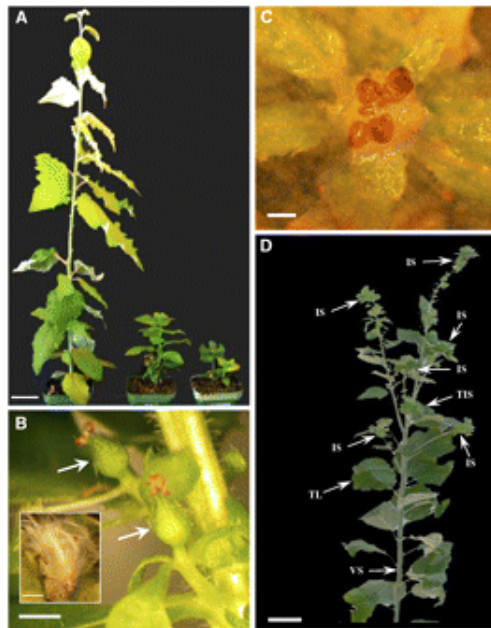
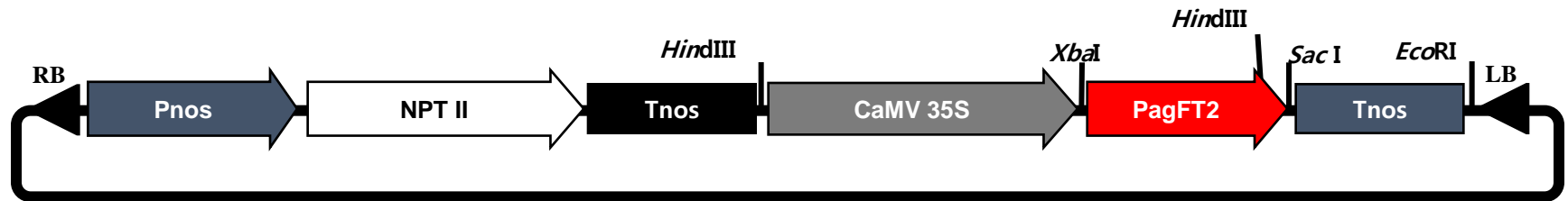




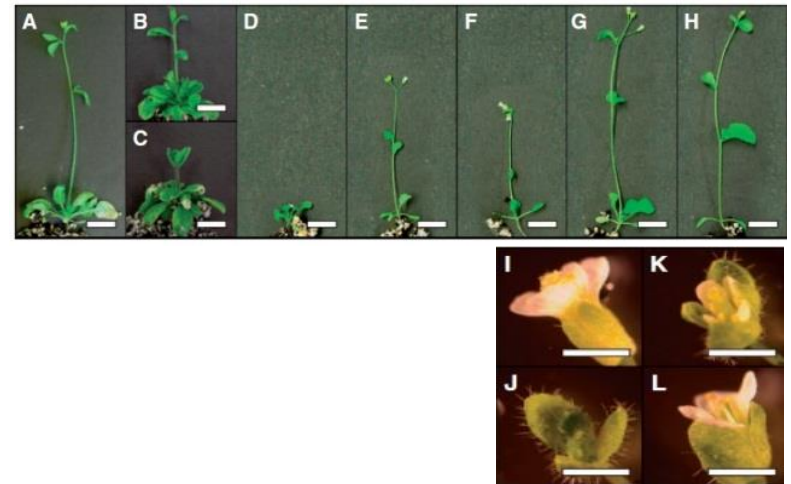


# Control of flowering time by transformation

## *FT2* Shortens the Juvenile Phase and Promotes Seasonal Flowering



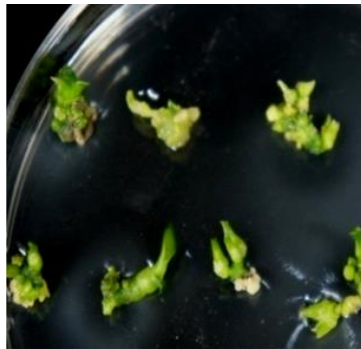
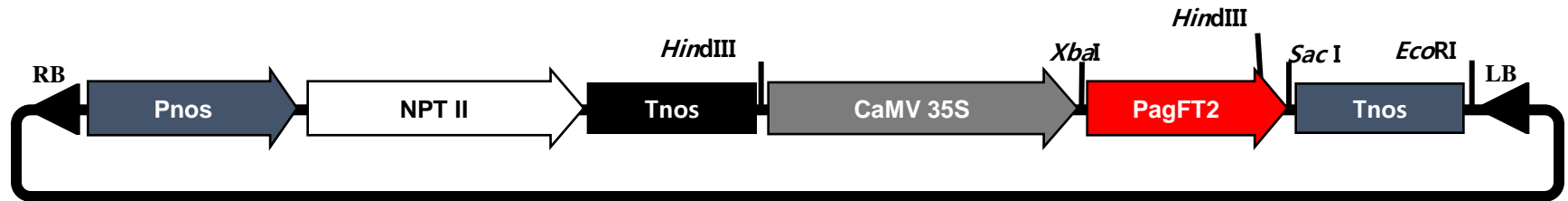
(Hsu et al., 2008, Plant Cell)



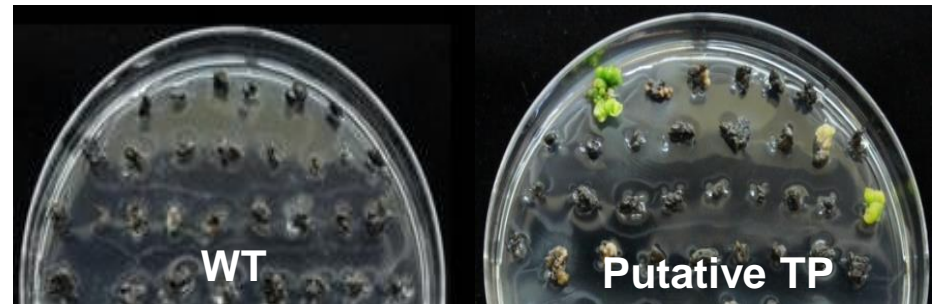
(Igasaki et al., 2008, Plant Cell Physiol)

# Control of flowering time by transformation

## *FT2* Shortens the Juvenile Phase and Promotes Seasonal Flowering



- *Phalaenopsis* cv 'Spring dancer'
- Destination vector pBI121
- 35S::*FT2*



Selection of transgenics on 50  $\mu$ M Kanamycin

(Park et al, unpublished data)

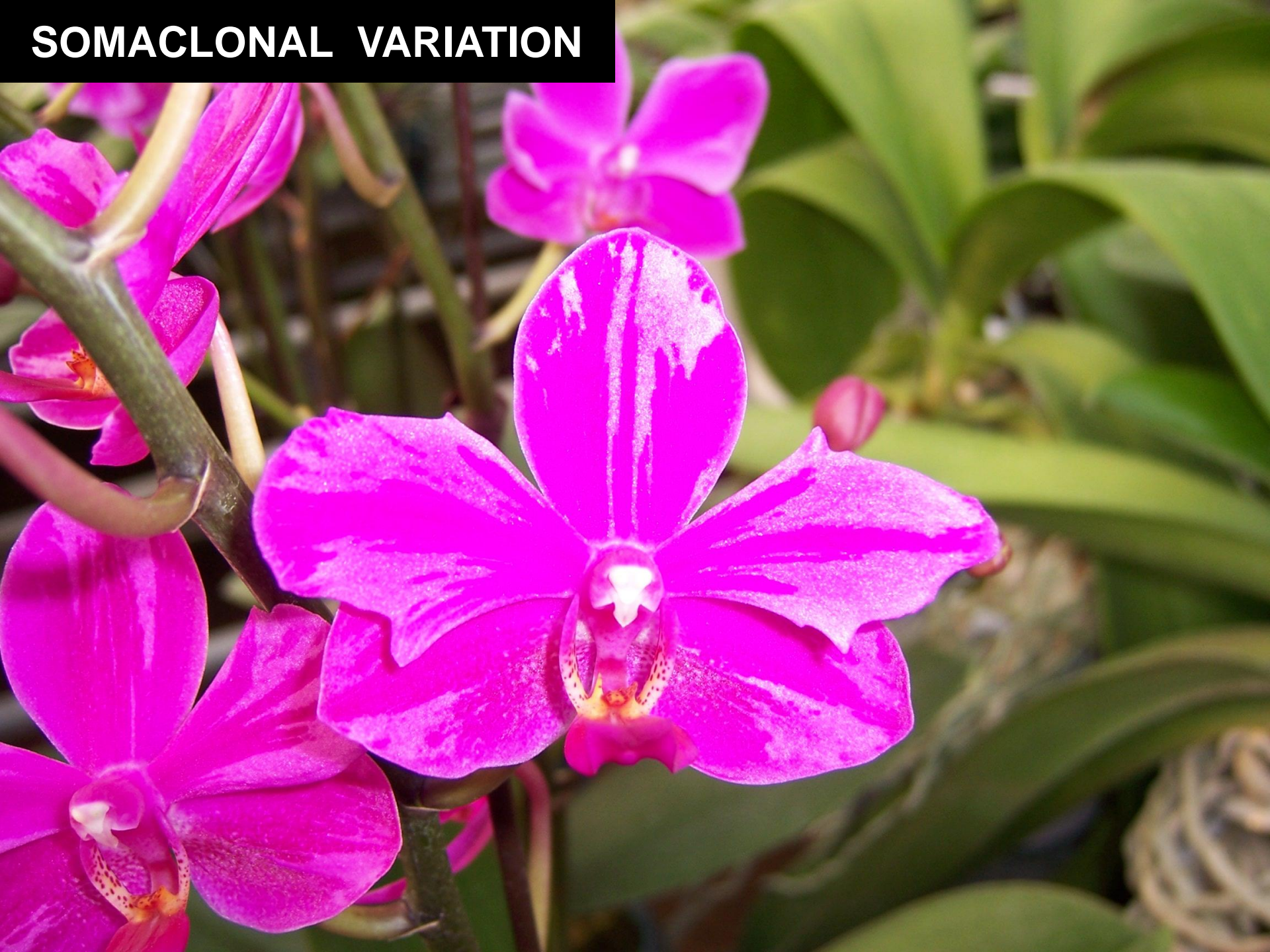




# Somaclonal Variation

- Things to be consider -

# SOMACLONAL VARIATION





# Somaclonal variation

## ❖ What's somaclonal variation?

- The variation seen in plants that have been produced by long-term micropropagation
- Somaclonal variation
  - Genetic variation : caused by DNA changes
  - Epigenetic variation : caused by temporary phenotypic changes





# Problems of somaclonal variation

- Morphological changes during floral development in *in vitro* clonally propagated Orchid
- Decrease of plant quality in uniformity

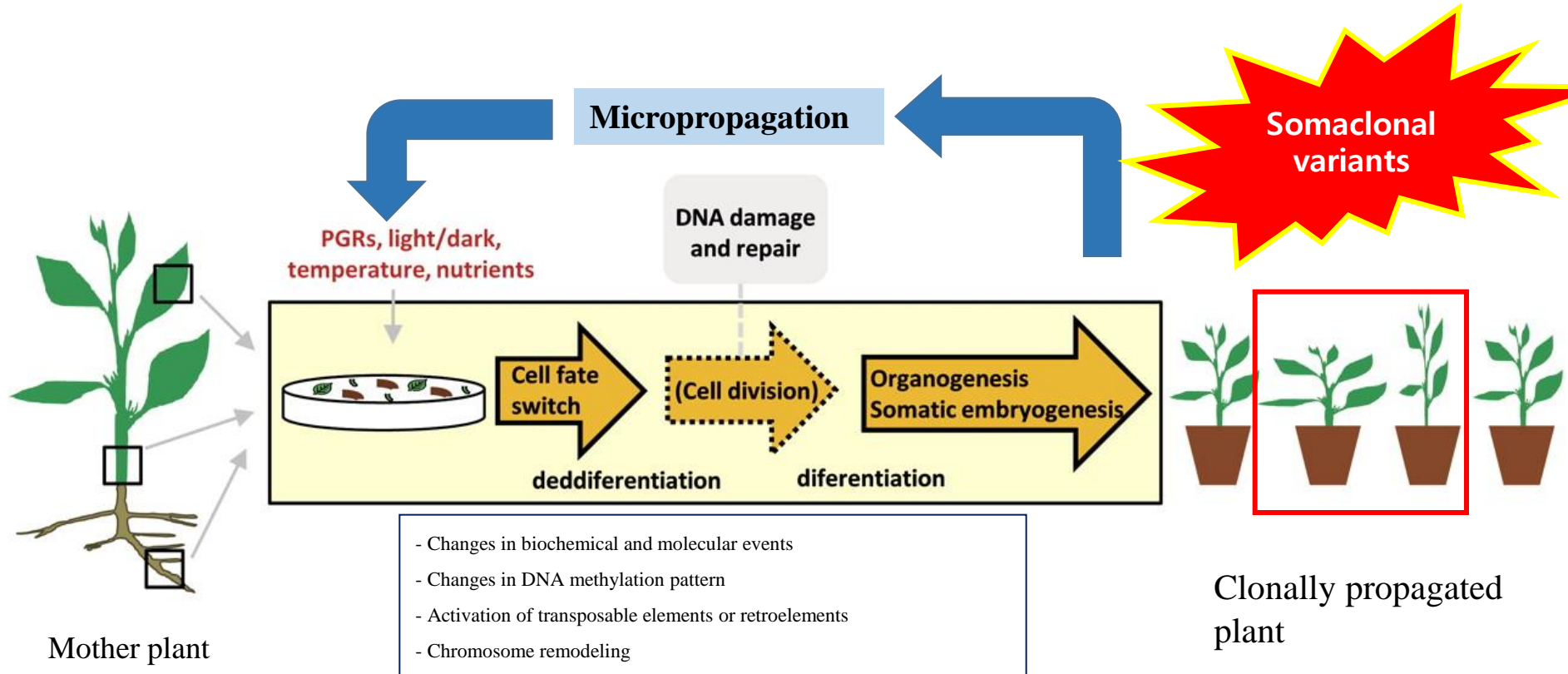


Normal plant

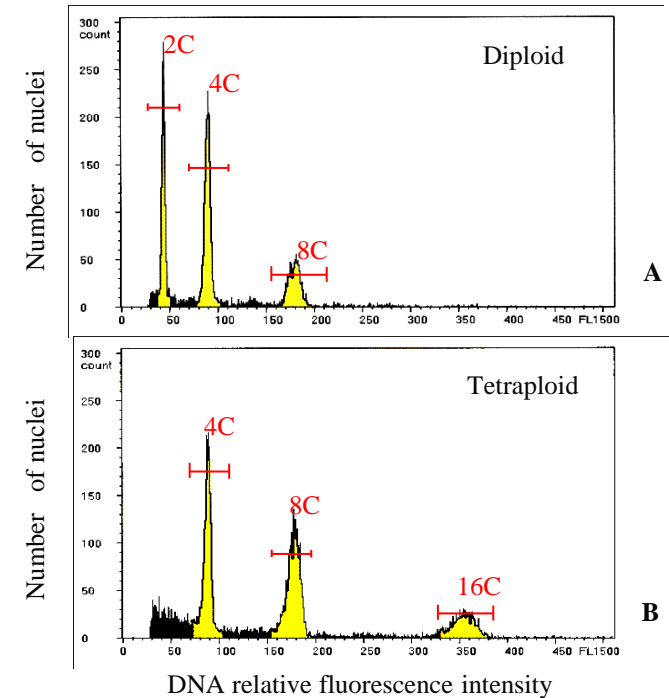
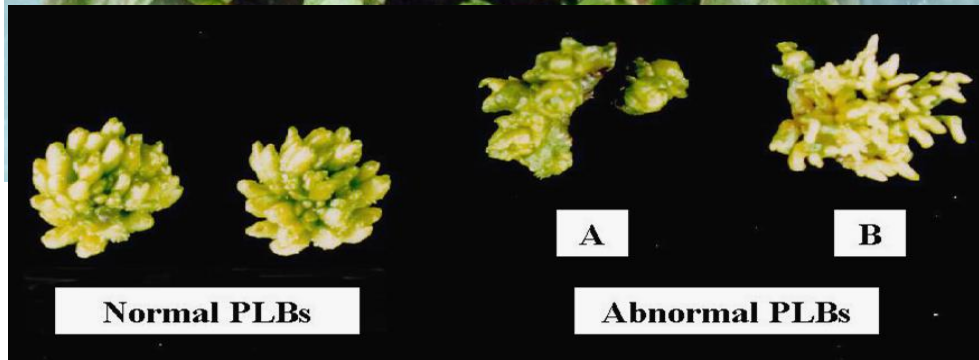


Variations in flower morphology

# What cause somaclonal variation?



# Somaclonal variation – **polyploid**



➡ Easily detected during  
in vitro culture process





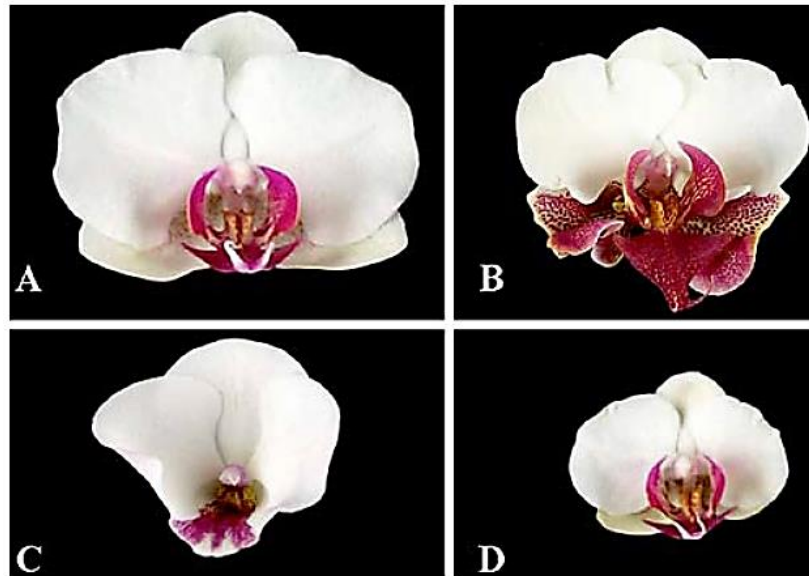
# Various mutants in flowers (*Phalaenopsis*)



*P. Baldans Kalaidescop*

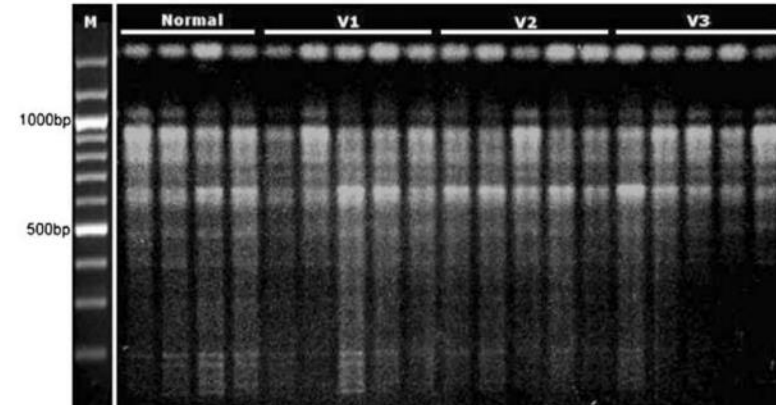


# Detection of epigenetic variation in tissue-culture-derived plants of *Doritaenopsis*

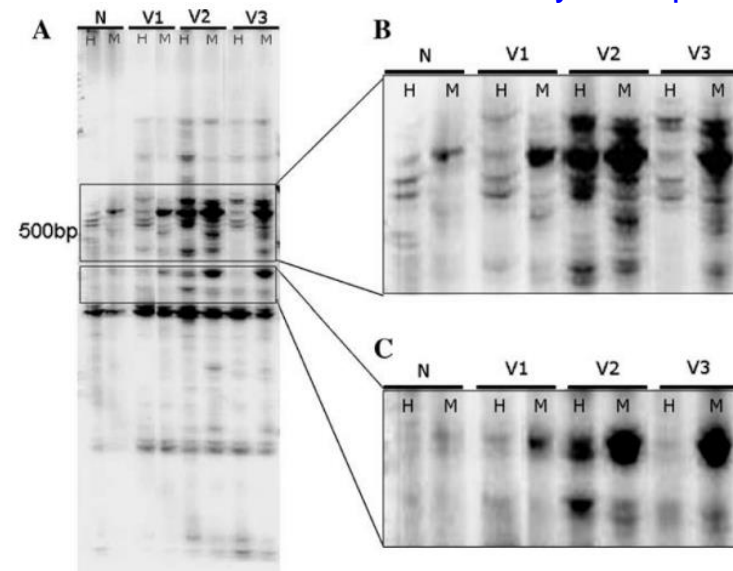


**Figure 1.** Normal and variant flowers of *Doritaenopsis* glenyl 'Labios'. (A) Normal flower. (B) Somaclonal variant (V1)—flowers with malformed lateral sepals with magenta pigmentation. (C) Somaclonal variant (V2)—complete fusion of lateral sepals with labellum. (D) Somaclonal variant (V3)—small flowers with faintly magenta pigmented lateral petals.

## RAPD result



## Methylation pattern analysis



**Figure 3.** (A) Example of methylation pattern detected in normal and somaclonal variants of *Doritaenopsis* glenyl 'Labios' using the primer E + AG/MH-ATG. H and M refer to digestion with *EcoRI* + *HpaII* and *EcoRI* + *MspI*, respectively. (B) and (C) Enlarged view of the boxed areas in (A) indicates DNA methylation polymorphism in the somaclonal variants. N normal, V1, V2, and V3 represent somaclonal variants.



# Morphological changes of flowers in *P. Wedding Promenade*



WP-N



WP-V1



WP-V2



WP-V3



# Morphological changes of flowers in *P. Spring Dancer*



# Morphological characteristics of leaf and flower stalk

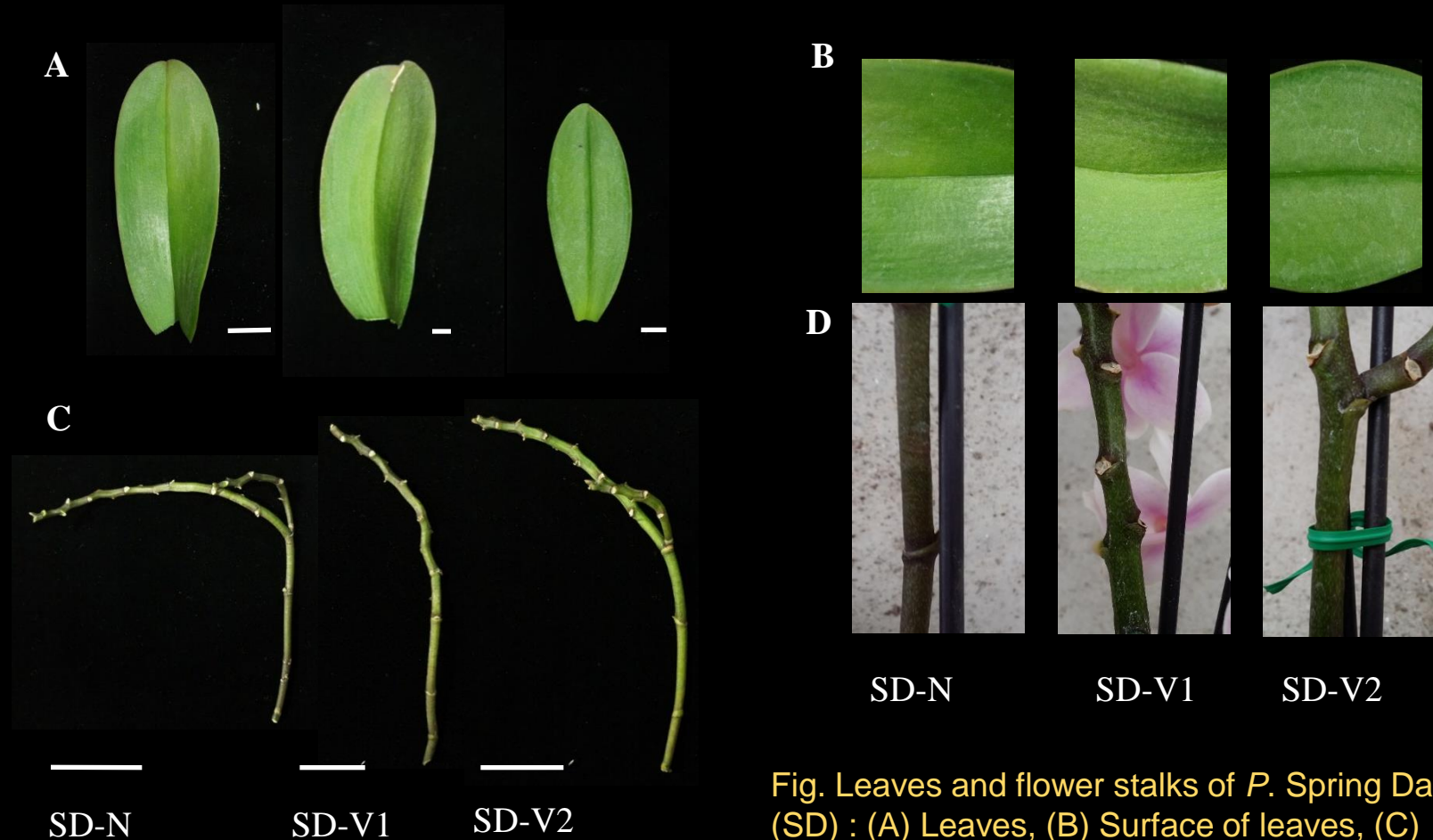


Fig. Leaves and flower stalks of *P. Spring Dancer* (SD) : (A) Leaves, (B) Surface of leaves, (C) Flower stalks, (D) Thickness of flower stalks. (Bar of A,B,D = 1cm, Bar of C = 5cm)



## Morphological characteristics of flowers

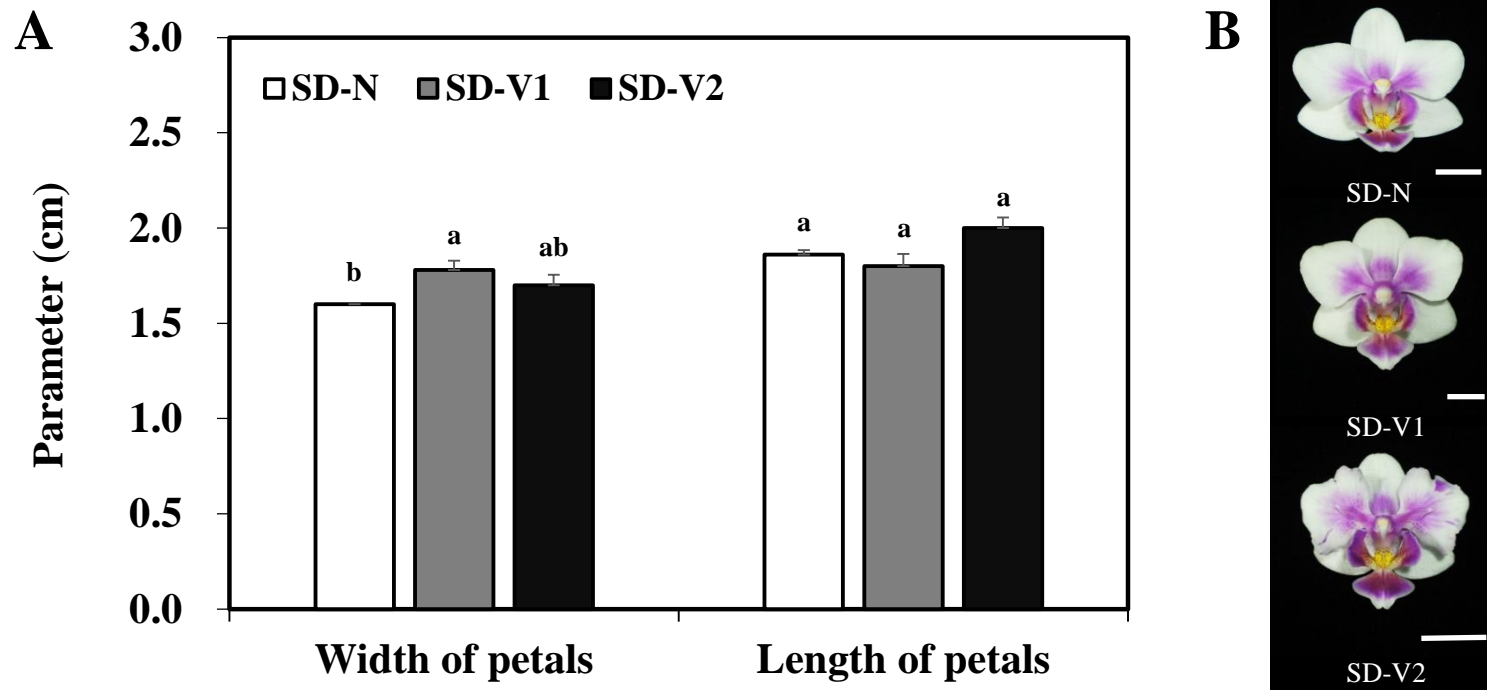


Fig. Width and length of petals(A) and flowers (B) in normal and variants in *P. Spring Dancer* (SD). (Unpublished data)

# Morphological characteristics of leaves

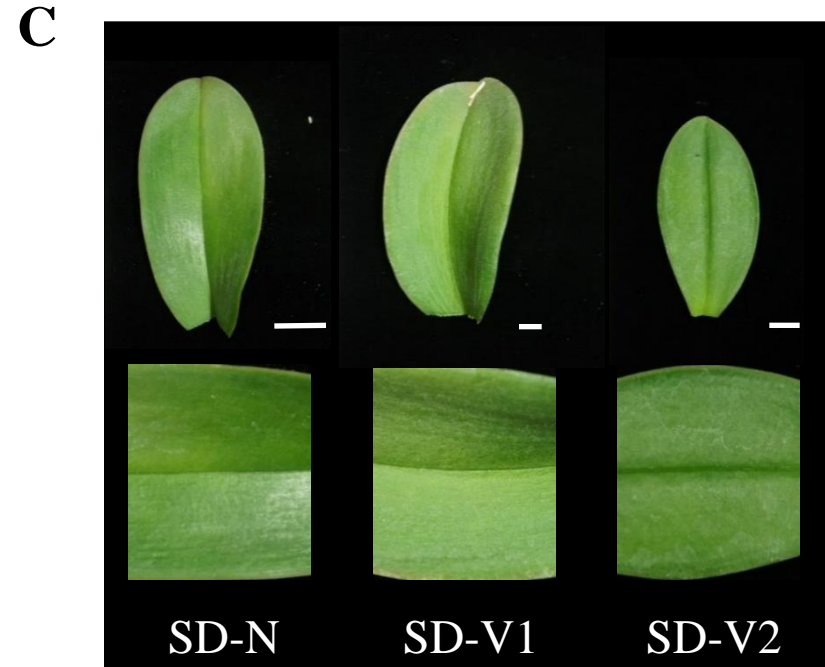
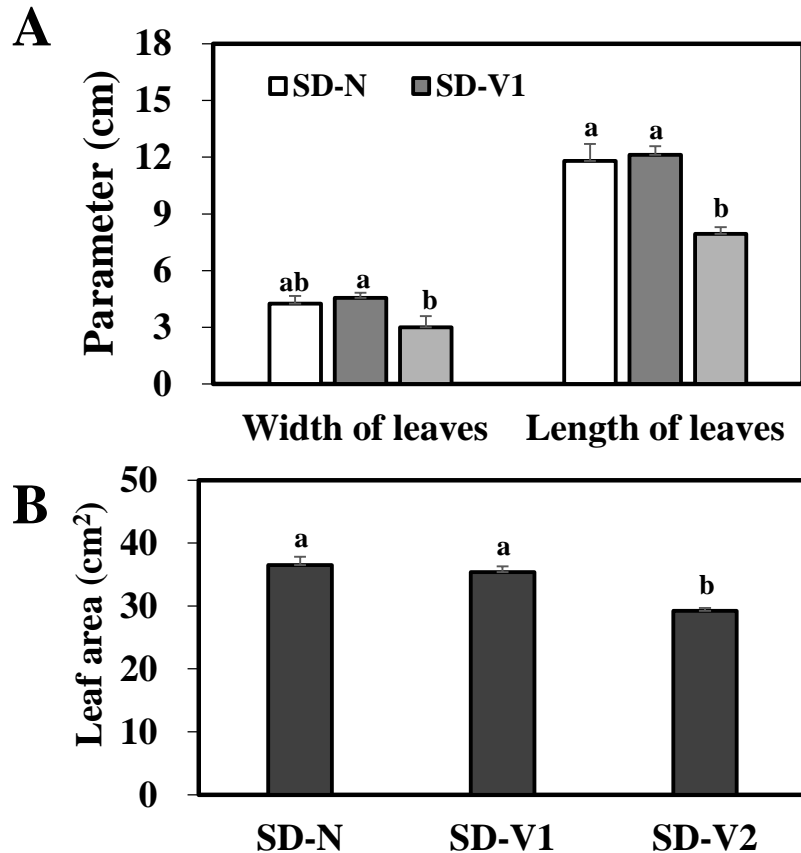
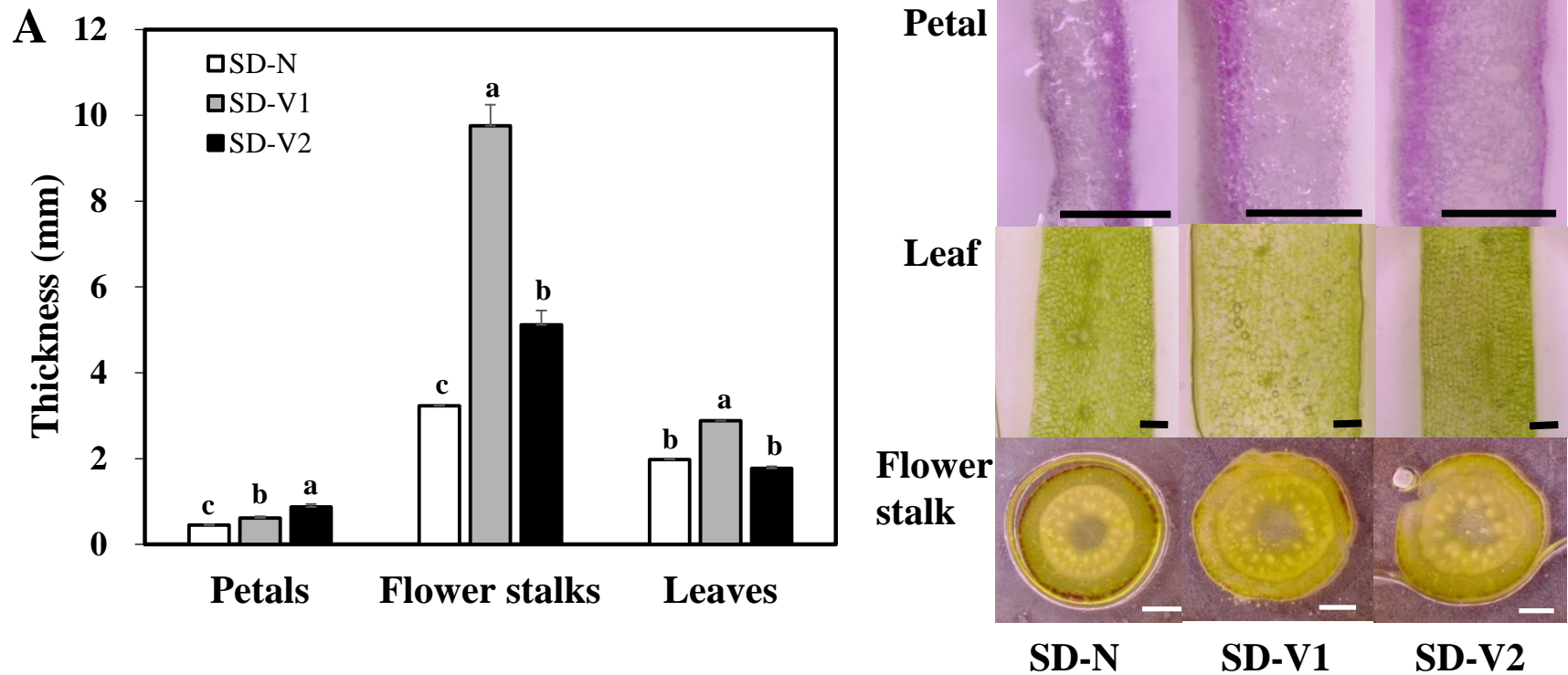


Fig. Width and length of leaves (A) and leaf area(B) and leaves (C) in normal and variants in *P. Spring Dancer* (SD). (Unpublished data)

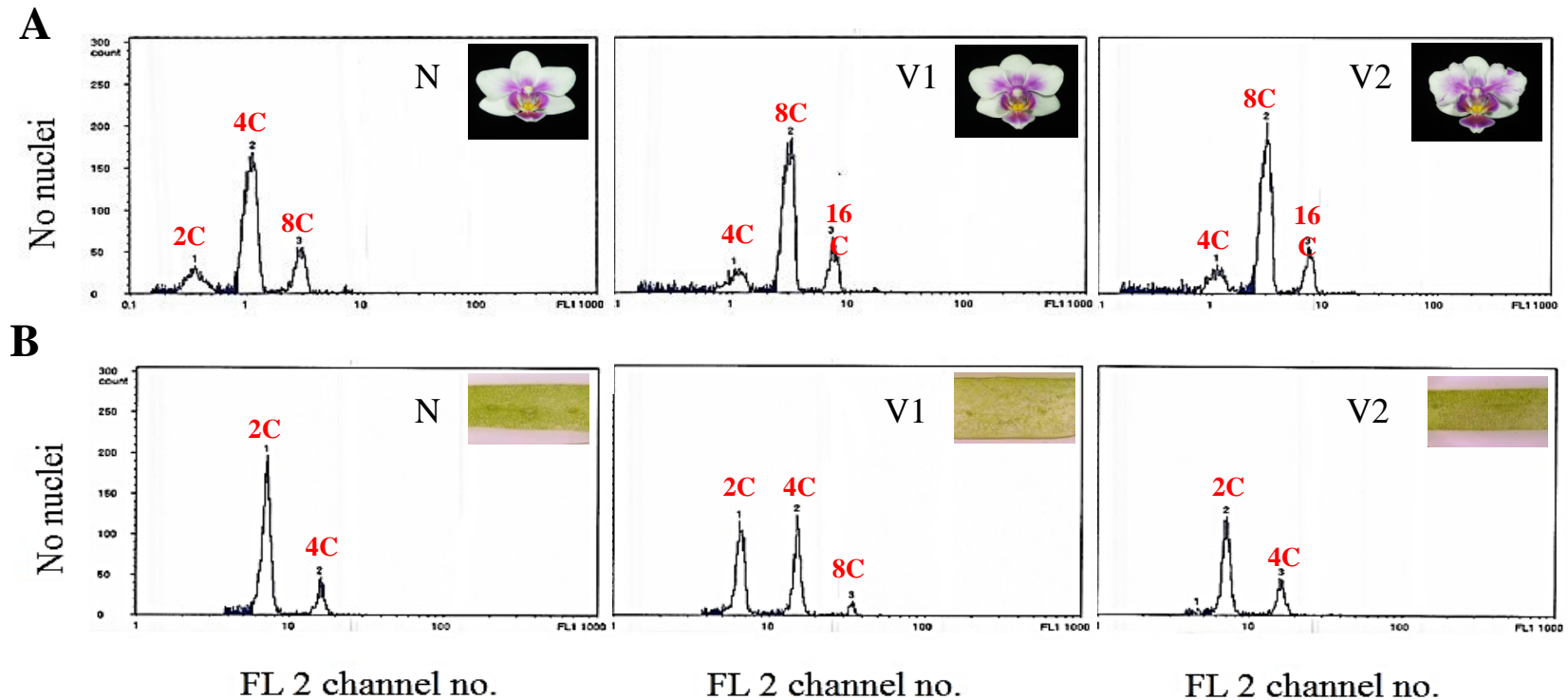
# Morphological characteristics of flowers and leaves



**Fig. Thickness of petal, flower stalk and leaf in *P. Spring Dancer* (SD). (Bar of petal and leaf = 0.5 mm, bar of flower stalk = 1 mm). (Unpublished data)**



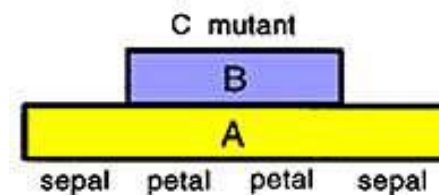
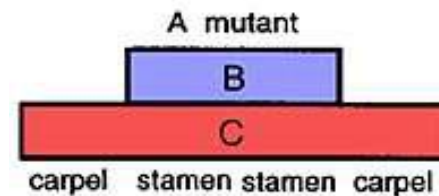
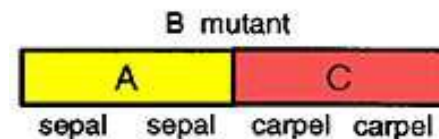
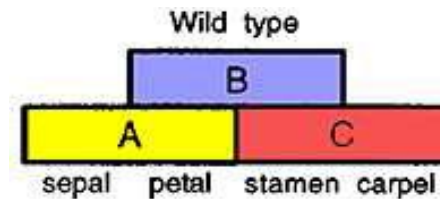
# Ploidy and endoreduplication analysis



**Fig. The nuclear DNA content and distribution of endopolyploid nuclei in petals (A) and leaves (B) of *P. Spring Dancer* (SD). (Unpublished data)**

## What determine floral structure?

- Genetic mechanisms that establish floral organ identity
- MADS-box genes** involved most central genes in floral organ identity



*apetala2*



*pistillata*



*agamous*

## PMADS4 expression in flowers and leaves

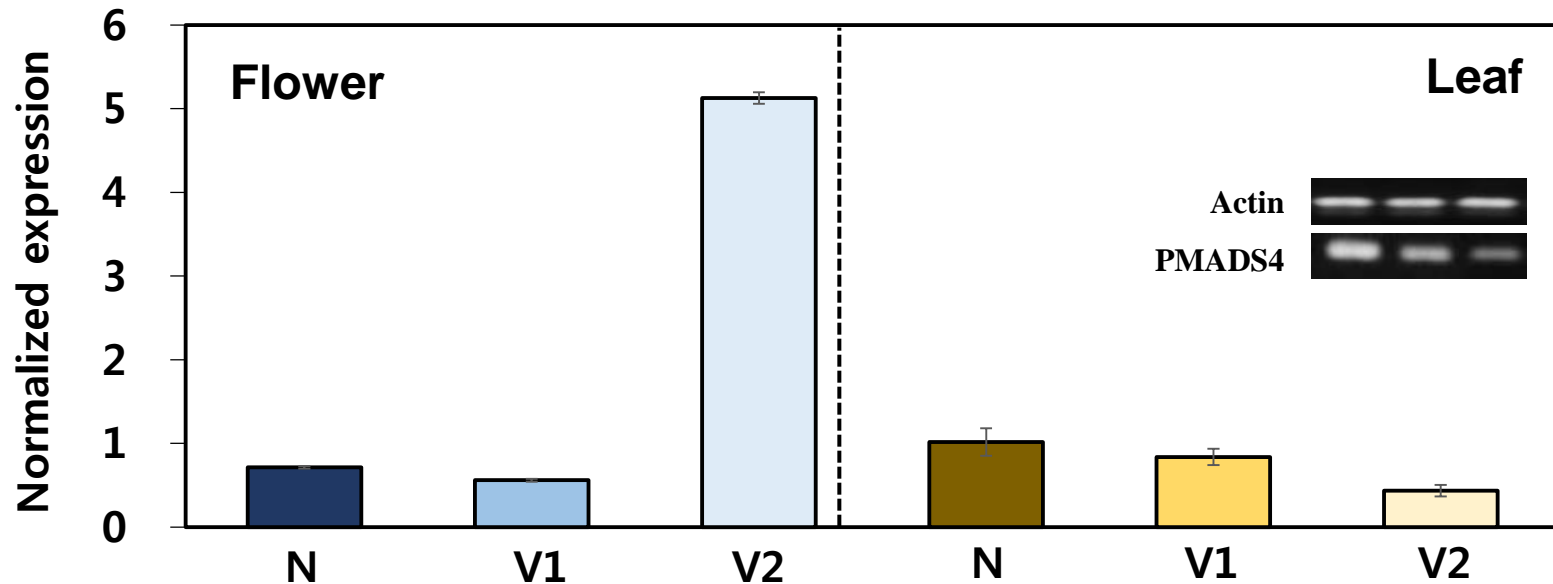


Fig. Real-Time PCR analysis for PMADS4 expression level of normal and variant's flowers and leaves in *P. Spring Dancer* (SD). (Unpublished data)



## PMADS4 expression in flowers

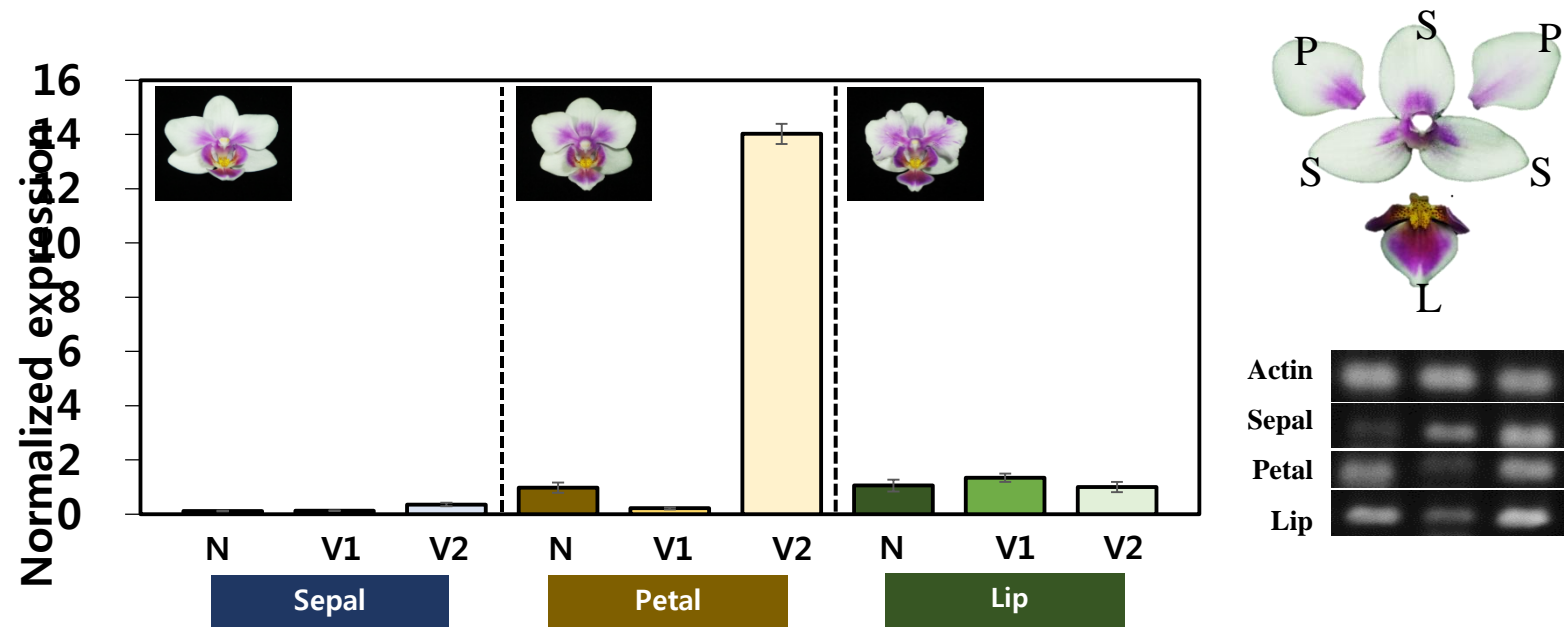


Fig. Real-Time PCR and electrophoresis analysis for PMADS4 expression level of normal and variant's flower organs in *P. Spring Dancer* (SD). (Unpublished data)



# Conclusions

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## Things to be improved in orchid breeding & production

- Stable production of high-quality clones by micrompropagation
- Development of new varieties for shorten juvenile phase
- Year-round culture system by applying hydroponic cultures
- Breeding new varieties by application of biotechnology
- Strict control of disease and insects

# International Orchid Conference

## World Orchid Conference (WOC)



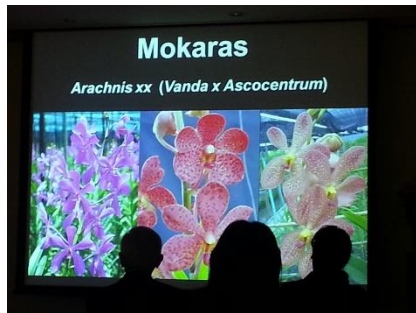
2011 20<sup>th</sup> WOC in Singapore





# International Orchid Conference

## Asia Pacific Orchid Conference (APOC)



2012 APOC in Okinawa, Japan




2016 APOC in Bangkok, Thailand

# International Orchid Conference

## International Orchid Symposium (IOS)



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**Announcement** 🌸  
: All oral presenters must submit their full paper to publish in the *Acta Horticulturae*.  
: Deadline of full paper submission was extended to March 30, 2014.

**IOS2014**  
: [Book of Abstracts](#)  
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2014 2<sup>nd</sup> IOS in Bangkok, Thailand



2018 3<sup>rd</sup> IOS in Korea



Thank you!

