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# 台灣草莓(*Fragaria hayatae* Makino)之植物性狀及其與‘桃園三號’草莓(*Fragaria ×ananassa* Duch.)之種間雜交

# *Fragaria hayatae* Makino: Characteristics and Artificial Hybridization with ‘Taoyuan No. 3’ Strawberry (*Fragaria ×ananassa* Duch.)

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# 中文摘要

台灣草莓(*Fragaria hayatae* Makino)是台灣之特有種，部份文獻認為是黃毛草莓(*F. nilgerrensis* Schlecht.)的一個亞種。但台灣草莓果實紅色且富含花青素，形態上明顯異於黃毛草莓。本研究室曾於小雪山採集得一群形態異於典型台灣草莓，但與黃毛草莓較相近之白果草莓，此群草莓在台灣尚無文獻紀錄。本試驗調查台灣草莓、白果草莓、黃毛草莓及其他草莓屬植物形態特徵及 RAPD 分子標誌之差異，以釐清其間之親緣關係與分類地位。性狀調查結果顯示白果草莓之葉柄及走莖為綠色，花瓣及果實均為白色；台灣草莓之葉柄及走莖為紅色，花瓣為白色基部帶有紅紫色條斑，果實為紅色；黃毛草莓之葉柄為綠色，走莖為紅色，花瓣及果實為白色。白果草莓與台灣草莓之葉片與花之大小以及果實高無顯著差異，而黃毛草莓之葉片、葉柄、花之大小及果高則顯著大於台灣草莓及白果草莓。RAPD 分子標誌分析之結果顯示，白果草莓與台灣草莓的親緣關係非常接近(相似距離 = 0.94)，這兩者與黃毛草莓則有相當程度之差異(相似距離 = 0.43)。綜合形態特徵調查及 RAPD 分子標誌分析結果，台灣草莓應非黃毛草莓之一個亞種，且白果草莓應為台灣草莓之變種而非來自中國的黃毛草莓。

為配合種間雜交之需求，本試驗亦研究溫度與光週對台灣草莓生育之影響。結果顯示低溫會抑制台灣草莓之營養生長，低溫處理結束植株移至溫暖環境數週後，低溫處理者之營養生長反而較旺盛。以 15/10℃或 10/5℃配合 10 小時日長處理 6 至 10 週後，25%-50%之植株可開花。以 15/10℃配合 14 小時日長或 10 小時日長以及 15/5℃配合 10 小時日長處理 6 週後，8.3%之台灣草莓可開花。由於開花植株百分比低於 50%，台灣草莓之最合適開花誘導條件仍待進一步研究。

台灣草莓(2x)與栽培種草莓(*F.* ×*ananassa*)‘桃園三號’(8x)進行互交，以台灣草莓為母本時，雜交結果率為 73%，而以栽培種草莓‘桃園三號’為母本，雜交結果率為 41%。以栽培種草莓‘桃園三號’作為母本得到的雜交種子之發芽率為 34%，另一

組合之種子無法發芽。成功存活的 21 個雜交後代中，7 株可開花結果，但果實多為畸形。畸形果可能是授粉不完全之結果，或可能是成功雜交得到 5 倍體之證據。

# Abstract

*Fragaria hayatae* Makino is endemic to Taiwan. It was considered as a subspecies of *F. nilgerrensis* Schlecht. in some literature, but *F. hayatae* is characterized with red fruit and anthocyanin in all parts of the plant, and it is significantaly distinct from *F. nilgerrensis* in morphological characteristics. A white-fruited strawberry population that is morphologically distinct from typical *F. hayatae* but similar to *F. nilgerrensis* was found in Shiaoshueshan by our lab, this white-fruited form was never reported in Taiwan. Morphological characteristics and RAPD markers were studied to clarify the relationship among *F. hayatae*, the white-fruited strawberry, *F. nilgerrensis*, and some other *Fragaria* species. The white-fruited strawberry had green petiole, green runner, white petal, and cream-white colored fruit which were distinct from the red petiole, red runner, white petal with purplish-red blush at base, and red fruit of *F. hayatae*. *Fragaria nilgerrensis* and the white-fruited strawberry both lack of anthocyanin coloration in petiole, petal, and fruit, but *F. nilgerrensis* had red runners whereas the white-fruited strawberry had green runners. Despite the color of the plants, the white-fruited strawberry and *F. hayatae* was similar in size of leaf, petiole, flower, and fruit height. *Fragaria nilgerrensis* was significantly larger than *F. hayatae* and the white-fruited strawberry in size of leaf, petiole diameter, and size of flower. RAPD marker analysis indicated closer relationship between the white-fruited strawberry with *F. hayatae* (similarity index = 0.94) rather than *F. nilgerrensis* (similarity index = 0.43). We suggested that the white-fruited strawberry should be a mutant of *F. hayatae* and not *F. nilgerrensis* from China.

For the need of interspecific hybridization, the effects of temperature and photoperiod on growth and flowering in *F. hayatae* were studied. Cool temperature

slowed down the vegetative growth rate during treatment, but accelerated the growth after transferring the plants into warm temperature condition for several weeks. Flowers were initiated in plants treated under 15/10℃ or 10/5℃ under 10 hour day length for 6-10 weeks, and plants treated under 15/10℃ 14 hour day length, 15/10℃ 10 hour day length, and 15/5℃ 10 hour day length for 6 weeks. The rate of plants flowering was 8.3% to 50% under the above condition, and the optimal inductive condition for flower initiation in *F. hayatae* awaits further investigation.

Reciprocal cross was made between *F. hayatae* (diploid) and the cultivated strawberry 'Taoyuan No. 3' (octoploid) to study the interspecific hybridization compatibility. 34% of seeds from *F. ×ananassa* 'Taoyuan No. 3' × *F. hayatae* germinated while no seed germinated in the other combination. Seven out of the 21 survived hybrid seedlings bloomed and bore fruits. The fruits were usually misshaped, which should result from partial pollination, or might be the evidence of obtaining pentaploids from successful hybridization.

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# Introduction

*Fragaria hayatae* Makino, also called as *F. nilgerrensis* Schlecht. ex J. Gay subsp. *hayatae* in some studies, is the only endemic *Fragaria* species in Taiwan. *Fragaria hayatae* is characterized with anthocyanin in all parts of the plant (Hancock, 1999), which results in brownish red petiole, brownish red runner, red fruit, and purplish-red blush at the base of white petal. In a field trip collecting *F. hayatae*, one population of strawberry with green petiole, green runner, cream-white fruit and white petal was found by our lab in Shiaoshueshan (小雪山). We called the newly found strawberry "white-fruited strawberry".

*Fragaria hayatae* was recorded in "Flora of Taiwan" (Ohashi, 1993). However, it was classified as a subspecies of *F. nilgerrensis* by Staudt (1989; 1999), and in Flora of China (Li et al., 2003). According to the characteristics described by Staudt (1999), *F. nilgerrensis* was characterized with yellowish, brownish or even reddish petiole, runner with or without anthocyanin, white to cream-colored fruit, and white petal, which is significantly different from *F. hayatae*. We considered *F. hayatae* to be distinct from *F. nilgerrensis*. It may be that the white-fruited strawberries were brought by birds from China or they might be a mutant of *F. hayatae* or even a new species of *Fragaria*. In order to solve this problem, morphological characteristics of the white-fruited strawberry, *F. hayatae*, *F. nilgerrensis* and *F. vesca* were observed and surveyed, and the DNA marker of some diploid *Fragaria* species was analyzed to unravel the relationship and taxonomic position of *F. hayatae* and the white-fruited strawberry population.

*Fragaria hayatae*, being the only endemic *Fragaria* species of Taiwan, was never used in commerce despite its useful horticultural characteristics such as good taste

(Hayata, 1908) and significantly different odor from the cultivated strawberry. In order to understand the growth habit of *F*. *hayatae*, the effects of a series of temperature and photoperiod were studied.

Incorporation of traits from lower ploidy *Fragaria* species to the cultivated strawberry (octoploid) could be accomplished by artificially doubling chromosome numbers following interspecific hybridization (Hancock and Luby, 1993; Harbut and Sullivan, 2004). Hence reciprocal crosses were made between *F*. *hayatae* (diploid) and the cultivated strawberry ‘Taoyuan No. 3’ (octoploid) to study the interspecific hybridization compatibility.

# Literature Review

## 1. Taxonomy and Distribution of the Strawberry Species

Strawberry belongs to the family Rosaceae, subfamily Rosoideae, tribe Potentilleae and genus *Fragaria* L. Among tribe Potentilleae, *Duchsnea* Smith and *Potentilla* L. are the closest to the genus *Fragaria*. Over 20 species (Table 1) are recognized in the *Fragaria* genus, which is divided into four fertility groups mainly associated with ploidy levels including diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), hexaploid (2n = 6x = 42), and octoploid (2n = 8x = 56) species (Darrow, 1966; Hancock, 1999; Folta and Davis, 2006). The basic chromosome number in *Fragaria* is x = 7 (Staudt, 1989).

*Fragaria* species are distributed throughout the holarctic zone with a few species spread into the tropics (Fig. 1). With the exception of the diploid *F. vesca* that is distributed in both Eurasia and America, all species are confined to a single continent. Central Asia and the Far East are the two centers of diversity for the diploid species. The tetraploid species are limited to east and southeastern Asia. The only hexaploid species is originated in Europe. The octoploid species are mainly distributed in North and South America, but one species is endemic to the Far East (Southern Kuriles) (Staudt, 1989).

Six diploid *Fragaria* species were used in this study. Their characteristics are described as follow.

1.) *Fragaria vesca* L.

There are four subspecies in this group: subsp. *vesca* is distributed in woods of Europe and Asia; subsp. *americana* is distributed in woods of eastern North America to British Columbia; subsp. *bracteata* is distributed in woods of western North America;

and subsp. *californica* is distributed in California (Hancock, 1999).

*Fragaria vesca* is a perennial herbaceous plant. The plant height is 5-30 cm. Leaves are green and 3-foliolate, rarely pinnately 5-foliolate. Shape of leaflets is obovate, elliptic or broadly ovate and 1-5 cm long. Inflorescences are corymbiform, with 2-4 (or 5) flowers. Inflorescences are about the same or taller than leaf petioles. Flowers are bisexual and hermaphroditic, approximately 1.3 cm wide, white, and with 5 petals. Ovoid fruits are highly aromatic and flesh is soft. Although some white fruits form, most of the plants have red fruits when mature. Achenes are raised or superficial. Runnerless forms exist (Hancock, 1999; Li et al., 2003).

2.) *Fragaria viridis* Duch.

This strawberry is distributed in Europe, eastern and central China and Canary islands.

*Fragaria viridis* is a perennial herb. Plants are 15-25 cm tall. Leaves are ternately compound, dark green, with ovate to elliptic leaflets. Inflorescences are erect with 4-10 flowers, and are often exserted above than leaves. Flowers are hermaphroditic and petals overlap. They are yellowish green when blooming then turn white. Fruits are obloid or globose, and the color is light green when mature. Fruits have firm texture and are fragrant. Achenes are yellowish green, in shallow pits or level with surface or superficial (Deng and Lei, 2005; Lei et al., 2006).

3.) *Fragaria mandschurica* Staudt

This species of strawberry is distributed in the Russian Far East, extending westward to Lake Bajkal, Mongolia, Manchuria, and North Korea.

*Fragaria mandschurica* is a perennial herb. Plants are 5.0-26.0 cm ($\bar{x}$ = 14.9) tall. Leaves are ternately compound, bright green, and with rhombic-ovate to obovate cuneate leaflets. Terminal leaflets are 32.0-68.0 mm ($\bar{x}$ = 49.1) long, and length-width

index is 1.24-2.14 ($\bar{x}$ = 1.54). Inflorescences mostly surpass leaves, with 2.0-12.0 ($\bar{x}$ = 4.9) flowers. Flowers are bisexual, hermaphroditic, and 13.0-29.0 mm ($\bar{x}$ = 20.5) in diameter with 5-7 petals ($\bar{x}$ = 5.2) which are white with yellow claw. Fruits are ovoid to broadly ovoid, 9.0-23.0 mm ($\bar{x}$ = 12.6) long, 8.0-26.0 mm ($\bar{x}$ = 11.2) wide, and color only in skin (Royal Horticultural Society (R. H. S.) Color Chart Red 45B-A to 46A when ripe). This strawberry has juicy flesh, melty texture, slightly acidulous with pleasing, and sometimes strong fruity flavour and taste. Achenes are yellow to light brown when mature, and they are in shallow pits or superficial. *Fragaria mandschurica* closely resembles the tetraploid *F. orientalis* (Staudt, 1989), but it can be morphologically distinguished by that the former is hermaphroditic and the latter is dioecious and trioecious (Staudt, 2003).

4.) *Fragaria pentaphylla* Lozinsk

This species is distributed in the Sino-Himalayan region.

*Fragaria pentaphylla* is a perennial herb. Plants are 2.3-29.0 cm ($\bar{x}$ = 12.3) tall. Leaves are ternately compound, and dark green. The proximal part of petiole is mostly with two or rarely up to four accessory leaflets, which are smaller and less toothed than ternate ones. Shape of leaflets is elliptic to narrowly elliptic. Terminal leaflets are 1.0-5.0 cm ($\bar{x}$ = 2.9) long and the length-width index is 1.10-2.09 ($\bar{x}$ = 1.59). Inflorescences are mostly shorter than leaves, with 1-5 flowers ($\bar{x}$ = 2.1). Flowers are bisexual, hermaphroditic, and 13.0-27.0 mm ($\bar{x}$ = 21.4) in diameter, with 5 -7 white petals. Fruits are ovate to widely ovate, 7-24 mm long, 8-18 mm wide, and color only in skin (R. H. S. color chart: Red 44A and B and Red 45 B when ripe). Fruits are slightly juicy, with spongy texture, but nearly without prominent smell and taste, somewhat acidulous. Achenes are reddish brown when mature, deeply imbedded or in shallow pits. There is a forma alba distributed on Mt. Gyala Peri and North of Tsangpo Gorge,

southeast Tibet. The fruits of this form have widely depressed ovoid shape, and the color is white to cream colored (R. H. S. Color Chart: Yellow-white 158 B-C when ripe). The taste of fruits is quite pleasant, sweetish caramel-like. Other characteristics vary in the range of the typical forma (Staudt and Dickoré, 2001).

5.) *Fragaria nilgerrensis* Schlecht.

This strawberry is mainly distributed in the temperate zone of mountainous regions of the Nilgiri Hills, southwestern India, the Khasi Hills, northeastern India, East Himalaya, northeastern Burma, northern Vietnam and Southwest and Central China.

*Fragaria nilgerrensis* is a perennial herb with 2.2-23.5 cm in height. Leaves are ternately compound, dark green. Terminal leaflets are subcircular or broadly obovate, 17.0-54.0 mm long, length-width index 0.61-1.74 ($\bar{x}$ = 1.17). Inflorescences are below, equaling or above the leaves, with 1-13 flowers ($\bar{x}$ = 8.5). Flowers are hermaphroditic, 13-20 mm ($\bar{x}$ = 16.4) in diameter, with 5 (occasional 6-7 in primary flowers) white petals. Fruits are subglobose to depressed subglobose or slightly conoidal, white to cream colored skin and flesh (R. H. S. Color Chart: Yellow 4D, Yellow 8D, Yellow 11D), slightly hairy, distinctly fruity, of a somewhat sweetish taste and strong fruity flavor. Achenes are yellowish brown, usually deeply imbedded. Runners are with or without anthocyanin (Staudt, 1999).

6.) *Fragaria hayatae* Makino

This species is not recorded from outside Taiwan.

*Fragaria hayatae* is a perennial herb with 2.5-15.7 cm in height. Leaves are ternately compound, terminal leaflets are 10.5-30.5 mm long, 10.0-23.5 mm wide, length-width indices are 1.05-1.40 ($\bar{x}$ = 1.18). Inflorescences are with 2-5 flowers, which are bisexual, 7-16 mm ($\bar{x}$ = 14.2) in diameter with 5 petals. Petals are white and clawed with anthocyanin. Fruits have pinkish to red skin (R. H. S. Chart: Orange Red

31 D, Orange Red 32 D, Orange Red 33 C and D), and are slightly hairy. The cortex and pith are white to cream colored. Fruits have no distinct taste and smell. Achenes are yellowish brown to reddish brown, and imbedded. Runners are usually with anthocyanin (Staudt, 1999).

## 2. Brief History of Strawberry Domestication

*Fragaria vesca*, the alpine or wood strawberry, was the first strawberry domesticated in the Old World. It was originally cultivated in gardens by Romans and Greeks. This strawberry was cultivated all across Europe by the 1300s and had reached its widest popularity in 1500s and 1600s before the introduction of strawberry species from the New World. It is now generally restricted to home gardens and most of the varieties grown are everbearers (Darrow, 1966; Hancock, 1999; Hancock et al., 2008).

*Fragaria moschata*, the musky-flavored strawberry was planted in European gardens by the late 15$^{th}$ century. Its fruit was used by English, Germans and Russians. *Fragaria viridis*, the green strawberry, was also cultivated for ornamental use across Europe at that time. Neither *F. moschata* nor *F. viridis* is of current commercial importance (Darrow, 1966, Hancock, 1999, Hancock et al., 2008).

*Fragaria virginiana*, the Virginian or scarlet strawberry, was introduced from Canada and Virginia to Europe, possibly by Jacques Cartier, and it had thus replaced the dominant role of *F. vesca* in the 1600s. All clones imported to Europe were of wild origin as the North American aboriginals did not cultivate strawberries. The first *F. virginiana* imported from Canada often bear small fruit and were green where the fruit was under shading. Those imported from Virginia made an impact in the horticulture industry. Because of their large fruit size, high yields and deep red color, *F. virginiana* was known as the scarlet strawberry (Hancock, 1999).

*Fragaria chiloensis* was domesticated 1000 years ago by indigenous Chilean

Mapuches and was spread widely by the Spanish during the colonization period (Hancock et al., 2008). One Chilean clone of *F. chiloensis* was brought to Europe in the early 1700s by Captain Amédée Frézier, a French spy, but the strawberries he introduced were all female and had insufficient hardiness to be widely grown. *Fragaria chiloensis* became more import after *F.* ×*ananassa* was accidentally born. It is currently grown in a small extent in Chile, although it was once grown widely, and it was replaced by *F.* ×*ananassa* since the late 1800s (Hancock, 1999; Hancock et al., 2008).

Unusual seedlings appeared in Brittany (a former province of France) and other gardens in Europe with unique combinations on morphological characteristics after introduction of *F. chiloensis*. The French botanist Antoine Nicholas Duchesne (1766) determined that they were hybrids of *F. chiloensis* × *F. virginiana* and named them as *F.* ×*ananassa* due to the fragrant of fruits similar to pineapple (*Ananas*). The dessert strawberry, *F.* ×*ananassa*, now dominates cultivation of the strawberries (Hancock, 1999).

## 3. Strawberry Species in Taiwan

*Fragaria hayatae*, the Hayata's strawberry or Taiwan strawberry, is the only species of *Fragaria* native in Taiwan (Ohashi, 1993). It is endemic and does not belong to any threatened category of the IUCN list (Naruhashi et al., 1999). Taiwan strawberry is distributed at 2,000 to 3,700 m altitude in the Central Mountain Region, and it is usually found in somewhat moist, open places such as exposed slopes along road cut, hiking trails or mountain meadow (Naruhashi et al., 1999).

*Fragaria hayatae* was first discovered at Mountain Morrison (Yushan) by T. Kawakami and U. Mori during their collection journey in October 1906. Kawakami noted "the fruit of this *Fragaria* is very delicious" (Hayata, 1908). The morphology was described, but the species name was not given due to incompleteness of specimen (lack

of flower) at this collection (Chen, 1995). It was later named as variety minor of *F. vesca* by Hayata (*F. vesca* L. var. *minor* Hayata) (Kawakami, 1910) and its descriptions were recorded in "Materials for a Flora of Formosa" at 1911. The complete Latin description was given by Makino, who published it as *F. hayatai* Makino (Makino, 1912; Chen, 1995) and this was the scientific name recorded in "Flora of Taiwan" (Ohashi, 1993).

The taxonomy and scientific name of the Taiwan strawberry was controversial. Staudt (1999) examined herbarium specimens and live materials of *F. nilgerrensis* Schltdl. ex J. Gay and *F. hayatae* cultivated in Berlin, Cologne and Merzhausen, Germany. He addressed that *F. hayatae* should be a subspecies of *F. nilgerrensis* and made a combination of the Taiwan strawberry as *F. nilgerrensis* Schltdl. ex J. Gay subsp. *hayatae* (Makino) G. Staudt. The subspecies *hayatae* was distinguished by having anthocyanin in all parts of plants, even the berries (Staudt, 1989). The Taiwan strawberry was categorized as *F. nilgerrensis* var. *nilgerrensis* in Flora of China, although Ikeda and Ohba (two of the three editors of the *Fragaria* section of Flora of China) believed that the plants from Taiwan should be separated as *F. hayatae*, because they differd from *F. nilgerrensis* in having 1(-3)-flowered inflorescences and the shapes of petals are obovate to broadly obovate, white with a reddish purple base. Whereas *F. nilgerrensis* had (1 or)2-5(or 6)-flowered inflorescences and the shape of petals were orbicular, white throughout (Li et al., 2003 ).

## 4. Breeding Potential of the Wild Strawberries

Since 1960, the majority of the genetic makeup of strawberry cultivars in North America came from only seven nuclear and ten cytoplasmic sources (Sjulin and Dale, 1987; Dale and Sjulin, 1990). This germplasm base also predominated in the international breeding programs (Hancock et al., 1993). Narrow genetic base may lead

to lethal inbreeding effects and lack of diversity to adapt to new environment (Hancock and Luby, 1993). The cultivated strawberry came from an accidental cross of *F. chiloensis* and *F. virginiana*, but little use of the native germplasm has been made by breeders until recently (Hancock et al., 1993). Disease resistance, stress adaptability, and characteristics in wild strawberries can be used to expand the germplasm of the cultivated strawberry and improve its pleasant characteristics (Hancock and Luby, 1993). The key to use native germplasm in plant breeding is to catalog their horticultural useful traits (Hancock et al., 2003). Wild clones of *Fragaria* species were collected and evaluated for germplasm conservation and for utility in strawberry breeding.

Species with the same ploidy level can often be successfully crossed and all octoploid *Fragaria* species were completely interfertile (Hancock and Luby, 1993). *Fragaria chiloensis* and *F. virginiana*, the octoploid species, which were the progenitors of cultivated strawberry, are the suitable materials for improving its genetic base. Although the lower ploidy strawberries were more difficult to be crossed with the octoploid *F.* ×*ananassa* (Hancock and Luby, 1993), they were valuable for holding potential to improve modern strawberry cultivars by introducing traits such as unique flavors and disease resistance while increasing genetic diversity (Harbut and Sullivan, 2004).

## 4.1. The octoploid species

The first step of utilizing wild strawberries should be collection of germplasm. Possible useful traits could be speculated by the original habitat of the collection site. Staudt (1999) and Hancock et al. (2001a) assumed that there were horticulturally useful genes in the native *Fragaria* since they distributed in a broad geographical range covering various biotic and abiotic stress environment. Native *F. virginiana* was

collected for representatives of North America from Pacific Northwest in 1985 and Northern Rocky Mountains in 1989 (Luby et al., 1992). The diverse habitat of which *F. virginiana* was collected, ranged from dry pine forests to wet meadows. It suggested that certain accessions could possess resistance to drought or water-saturated soil. Some *F. virginiana* accessions were found near timberline where growing seasons were 6-8 weeks, with frost or snow occurring any time of the year indicating possible cold hardiness or blossom frost tolerant resource. Collections from sites with alkaline soils may be sources of higher pH (Luby et al., 1992). Similarly, *F. chiloensis* clones were collected with representatives of its wide geographical range and important horticultural traits like very large fruit, resistance to powdery mildew, red stele caused by *Phytophthora fragariae*, leaf spot, aphids, and two-spotted spider mites were observed (Hancock et al., 2001a). A bulk collection representing all octoploid strawberry species in North and South America was evaluated by Hancock et al. (2003). In this study, *F. chiloensis* was generally superior in crown number, fruit weight, soluble solids and seed set while *F. virginiana* was superior for runner production, peduncle length, fruit number, fruit color, and winter hardiness.

After selecting elite accessions of the wild strawberries, the performance test should be conducted at various sites to affirm if the characteristics were truly genotypic or resulted from interaction between genotype and environment. The work of Hancock et al. (2001b) demonstrated that fruit characteristics could be assessed in one single site since the fruit weight, skin color, flesh color and firmness coincided throughout the five sites (Maryland, Oregon, Minnesota, Michigan, and Pennsylvania). However, multiple sites were necessary to predict physiological adaptations and disease resistance since the percentage bed fill, foliar disease incidence, 50% bloom date and number of flowering cycles showed interactions between genotype and location (Hancock et al., 2001b).

A subsequent step was to cross the elite accessions of wild strawberries with the cultivated strawberry or another elite wild strawberry accession for evaluation of the hybrid progeny and the transferability of traits from the parents. It was reported that fruits of hybrid from the cross of wild *F. virginiana* with *F. ×ananassa* were generally too soft and often irregular in appearance, but the fruits displayed high level of fertility, high flavor and highly productive behavior (Hancock et al., 2001a). Hancock et al. (1993) and Luby et al. (2008) considered that reconstruction of *F. ×ananassa* using superior clones of *F. chiloensis* and *F. virginiana* might be more efficient than simply backcrossing to *F. ×ananassa* because the hybrid of wild strawberries possessed much higher proportion of unique genes that will be available for recombination in later generations.

## 4.2. The lower ploidy species

*Fragaria* species occupied various environments and they should carry some horticultural useful traits (Hancock et al., 2008). *Fragaria vesca* (2x) was often found in the same habitats as *F. virginiana* but frequently occupied drier and coarser sites where *F. virginiana* was absent. It suggests that *F. vesca* might be a source of extreme drought tolerance (Luby et al., 1992). *F. moschata* (6x) was found under heavy shade (Hancock and Luby, 1993). Harbut and Sullivan (2004) indicated that a species adapted to shade might maintain higher $CO_2$ assimilation rate, and it could be beneficial for production in greenhouse, low light areas and high plant populated areas.

Disease resistance can be found in some lower ploidy *Fragaria* species. Resistance to *Phytophthora cactorum* (crown rot or leather rot on fruit) was found in several *F. vesca* clones and its hexaploid and decaploid derivatives indicated that the wood strawberry might be a source of crown rot resistance and this ability was inheritable (Gooding et al., 1981). Xue et al. (2005) screened eleven non-octoploid *Fragaria*

species for resistance to *Xanthomonas fragaria* Kennedy and King, the bacterial angular leaf spot which may reduce yield for up to 75%. Their results indicated that some accessions of *F. pentaphylla* (2x) and *F. moschata* (6x) either showed no symptoms (highly resistant), hypersensitive reactions (resistant), or restricted water-soaked lesions (moderately resistant) and the two species harbored diversified resistant source. Bors and Sullivan (1997) observed immunity to aphids and leaf diseases in *F. nilgerrensis*, and winter hardiness and excellent leaf disease resistance in *F. moschata*.

Some useful fruit characteristics can be found in the lower ploidy strawberries. Fruit of *F. viridis* (2x) had a spicy, cinnamon-like flavor (Bors and Sullivan, 1997); the flavour of mature fruit of *F. nilgerrensis* (2x) was described as similar to melons or peaches (Oda et al., 1990 in Noguchi et al., 2002), apricots and/or bananas (Staudt et al., 1975); *F. moshchata* tasted like 'Concord' grape when grown in the greenhouse (Bors and Sullivan, 1997). These strawberries could add new elements to typical strawberries and have the potential in aroma breeding. *Fragaria pentaphylla* had very bright red and firm fruits (Bors and Sullivan, 1997) which would be ideal for strawberry shipping (Harbut and Sullivan, 2004). *Fragaria nilgerrensis* subsp. *hayatae* (*F. hayatae*) was found to have anthocyanins in all parts of the plant (Hancock, 1999), and this unique characteristic may lead to high antioxidant capacity (Harbut and Sullivan, 2004).

## 4.3. Crossbility

1.) Crosses between diploids

Four subspecies of *F. vesca* were used as female parent to cross with four diploid *Fragaria* species (*F. nilgerrensis*, *F. nubicola*, *F. pentaphylla* and *F. viridis*) in the study of Bors and Sullivan (2005a). Although the rate of fruit set varied from 39% (*F. vesca* × *F. nilgerrensis*) to 89-100% (other combinations), hybrids were obtained from all combination (Bors and Sullivan, 2005a), indicating that crosses between these

diploid species were generally successful.

2.) Crosses between diploids and hexaploids

Interspecific hybridization was administrated by Evans (1974) using *F. vesca*, *F. viridis*, *F. nubicola*, *F. nilgerrensis*, and *F.* (*vesca* × *viridis*) as female plants to cross with *F. moschata* (6x) and only two seedlings were obtained from the 43 pollinated flowers and the two seedlings died before true leaves formed. This result indicated crossing barriers between these species. However, the diallel crosses of *F. moschata* with *F. nubicola* and *F. viridis* in the study of Bors and Sullivan (2005b) were more successful. They got 1.4 healthy plants/ pollination in the combination of *F. moschata* × *F. viridis*, 3.3 healthy plants/ pollination in *F. nubicola* × *F. moschata* and 0.1 healthy plants/ pollination in *F. viridis* × *F. moschata*. The success rate was raised probably because the germination technique was improved using *in vitro* culture (Bors and Sullivan, 2005b).

3.) Crosses between diploids and octoploids

Interspecific hybridization was conducted in numerous studies and clear crossing barriers were observed (Evans, 1974; Li et al., 2000; Marta et al., 2004). In *F.* × *ananassa* 'Honeoye' × *F. vesca* 'Changsen', a relative low number of hybrid seedlings (84 hybrid seedlings from 303 seeds) was obtained. The germination rate of *F. vesca* pollen on *F.* ×*ananassa* stigmas was low, some germinated pollens did not penetrate the stigma. Elongation of pollen tubes in the style was irregular and the growth of embryos and endosperms was aberrant (Li et al., 2000). In the study of Marta et al. (2004), pollen tube growth was observed to arrest in the first-third of the style and it produced only two aborted seeds in the cross of *F.* ×*ananassa* and *F. vesca*. In the reciprocal cross, 35 seeds were obtained, but the germination rate was only 14% (5 seeds) and seedlings died shortly after germination (Marta et al., 2004). The work of Li

et al. (2000) and Marta et al. (2004) revealed pre-zygotic and post-zygotic barriers between the interspecific hybridization of octoploids and diploids.

The above studies suggest that crosses in the same ploidy level are much easier than interploidy hybridizations. Although species at lower ploidy levels were more difficult to cross with *F.* ×*ananassa*, they had not been ignored by plant breeders (Hancock and Luby, 1993). Incorporation of traits from lower ploidy *Fragaria* species into the cultivated strawberry had been accomplished by artificially doubling chromosome numbers and making numerous crosses (Hancock and Luby, 1993).

Evans (1977) came up with the system called synthetic octoploid (SO system) in which germplasms of 2x, 4x, and 6x *Fragaria* species were incorporated into octoploid hybrids. In this system, *Fragaria* species of the lower ploidy were crossed to obtain tetraploid hybrids and the hybrids were further treated with colchicine resulting in octoploid hybrids that contained various germplasms (Evans, 1977; Harbut and Sullivan, 2004). It bypassed ploidy level differences and facilitated introgression of 2x, 4x and 6x species into the cultivated strawberries (Evans, 1977; Bors and Sullivan, 2005).

The use of this method has led to two SO clones, Guelph SO1 (Evans, 1982a) and Guelph SO2 (Evans, 1982b). Guelph SO1 originated from colchicine treated tetraploid hybrids between *F. moschata* (6x) and *F. nubicola* (2x). It was a staminate clone possessing late flowering, upright flower stalks, high number of flower stalks and its flavor, aroma and flesh color of fruit were distinctive to *F. moschata* (Evans, 1982a). The origin of Guelph SO2 came from crossing the amphidiploid (4x) hybrid of *F. vesca* (2x) and *F. viridis* (2x) with *F. moupinensis* (4x) and the chromosome of the interspecific hybrid was doubled again to form the synthetic octoploid strawberry. SO2 was a staminate clone and had a reasonable resistance to powedery mildew, leaf scorch and leaf blight (Evans, 1982b). SO1 and SO2 were evaluated by means of outcrossing

(recurrent selection) for their potential to contribute horticultural useful traits in strawberry improvement. The yield and berry weight of some hybrid progenies were improved to be as good as or greater than the average of the check cultivars within 3-5 generations (Sangiacomo and Sullivan, 1994).

*Fragaria* ×*ananassa* 'Toyonoka' (female parent) and *F. nilgerrensis* 'Yunnan' (male parent) were crossed, their hybrid chromosome number was doubled and then backcrossed to *F.* ×*ananassa* 'Pajaro' in the study of Noguchi et al. (2002) in order to breed a new aromatic strawberry. This hybrid strawberry performed aroma of peach, light pink skin and soft flesh and was registered as Kurume IH No.1 in Ministry of Agriculture, Forestry and Fisheries of Japan at 2005. Some decaploid strawberries had been produced and released from crosses of *F.* ×*ananassa* and *F. vesca*, namely 'Spadeka', 'Annelie' and 'Sara' (Bauer, 1979 and Trajkovski, 1997).

The works of Bauer (1979), Evans (1982a, 1982b), Sangiacomo and Sullivan (1994), Trajkovski (1997) and Noguchi et al. (2002) verified possibility to incorporate germplasms of lower ploidy *Fragaria* into the cultivated strawberries via using the species of higher ploidy level as female parent when possible, or try to increase the chromosome complement of the species at lower level, or to use means such as embryo culture mentioned by Evans (1974).

## 5. Application of Morphological Traits in *Fragaria* Species

It was reported that morphological characteristics can be used for determining interspecific relationship (Harrison et al., 1997; Sargent et al., 2004), intraspecific relationship (Catling and Porebski, 1998) and cultivar identity (Dale, 1996; Nielsen and Lovell, 2000). The guidelines of International Union for the Protection of New Varieties of Plants (UPOV) provide detailed descriptions covering general habit, leaf, flower and fruit characteristics of strawberries (Annonymous, 1995; Annonymous, 2008) which all

are useful for plant breeders.

Forty-four morphological traits were evaluated on three subspecies of *F. virginiana* (subsp. *glauca*, subsp. *platypetala* and subsp. *virginiana*) and *F. chiloensis* subsp. *lucida* collected in North America. The results of principal component analysis based on genetic distances successfully separated the four subspecies into four distinct groups (Harrison et al., 1997). Sargent et al. (2004) examined quantitative and qualitative morphological characteristics of eight diploid *Fragaria* species (*F. daltoniana*, *F. iinumae*, *F. nilgerrensis*, *F. nipponica*, *F. nubicola*, *F. pentaphylla*, *F. viridis* and *F. vesca*). After summarizing the 14 quantitative characteristics by the principal component analysis, the diploid *Fragaria* species were separated into three distinctive groups, *F. vesca*, *F. nilgerrensis* and the rest of *Fragaria* species. Although the quantitative morphological characteristics did not give clear resolution to some species, they could be distinguished by qualitative characteristics such as bright pink fruit of *F. daltonia* and tertiary leaflets of *F. pentaphylla* (Sargent et al., 2004).

Fourteen morphological characteristics were used to measure 95 plants representing the four subspecies of *F. chiloensis* (subsp. *lucida* and subsp. *pacifica* from North America, subsp. *chiloensis* from South America and subsp. *sandwicensis* from Hawaii) in order to provide a better resolution to the intraspecific relationship. The Hawaiian subspecies, *F. chiloensis* subsp. *sandwicensis* was entirely distinct from the other subspecies in having longer leaflets and longer hairs on the undersurface of the leaflets and more numerous leaflet veins. The South American subsp. *chiloensis* differed from the North American subspecies in having mostly 6-10 petals whereas the latter have 5-6 (rarely 7) petals (Catling and Porebski, 1998).

To distinguish 32 common strawberry cultivars grown in North America, a key of mostly vegetative characteristics was developed by Dale (1996) based on observation

and the UPOV guideline. The key was aimed for initial identification of varieties when fruits and flowers are not available. Nielsen and Lovell (2000) indicated that vegetative characteristics (leaf blistering, length: breadth ratio, base shape and teeth shape of the terminal leaflet) and reproductive characteristics (petal spacing, length : breadth ratio of petal, calyx : corolla ratio, fruit size, fruit length : breadth ratio, fruit shape, width of band without achenes on fruit, insertion of achenes on fruit and insertion of calyx on fruit) could be used for identifying strawberry cultivars cultivated in Auckland, but it is insufficient to use the vegetative or reproductive characteristics alone for identifying cultivars since they may share either floral or leaf characteristics.

## 6. Application of RAPD Markers in *Fragaria* Species

Expression of morphological traits could be affected by environmental factors such as climate conditions or cultivation procedures (Hu, 2001; Kuras, et al., 2004). While traditional identifications of cultivars were based on morphological traits, Nielsen and Lovell (2000) had pointed out the necessity of using alternative method for definitive identification such as molecular markers because it was difficult to put together a key based only on morphological markers. The dendrogram generated from morphological traits and molecular traits of strawberry cultivated in Argentina were not correlated (García et al., 2002) also demonstrated morphological traits alone was not sufficient in differentiating cultivars.

Analysis of molecular markers could be assessed at any developmental time and any organ, only few samples are needed for multiple analysis, DNA samples could be conveniently kept for long term use and procedures are standardized (Lin, 2001).

The technique of random amplified polymorphic DNA (RAPD) was first reported by Williams et al. in 1990. The DNA fragments were amplified by polymerase chain reaction (PCR) using short synthetic primers (generally 10 bp) of random sequence. The

amplified products were separated by gel electrophoresis and polymorphisms were detected as the presence or absence of particular band size. RAPD is quick and easy to assay. Only low quantities of template DNA is required since PCR is involved. No sequence data is needed because the random primers are commercially synthesized and available. The primer length is short and the annealing temperature is relative low which might result in mismatch of primer and sequence when complementary sequence does not exist, therefore the experiment should be repeated to check consistency. Standardized experimental procedures are required to prevent the low reproducibility nature of RAPD. The markers of RAPD are dominant and non locus-specific, the bands can not be interpreted in terms of loci and alleles, and fragments of similar size may not be homologous (Lin, 2001; Hu, 2001; Spooner et al., 2005).

RAPD markers were reported to be useful for determination of genetic relationship in the genera level or the species level. In a study analyzing relationship between *Fragaria*, *Potentilla* and *Duchesnea* of northwest Argentina, the phenogram obtained from RAPD characteristics revelaed that *F. vesca* and *F.* ×*ananassa* cluster together, whereas *Potentilla tucumanensis* and *Duchesnea indica* form a separate cluster. These results were identical to the phenogram generated from morphological and anatomical characteristics (Ontivero et al., 2000). Three subspecies of *F. chiloensis* were evaluated for their varation and genetic relationship by RAPD in the study of Porebski and Catling (1998). The results indicated a clear division between the North American subspecies and the South American subspecies by the similarity index of 0.16, on the other hand, the two North American subsp. *lucida* and subsp. *pacifica* were less separated from the similarity index of 0.64-0.88.

There were reports about application of RAPD markers used for cultivar identification in *Fragaria* species. Thirteen RAPD primers generating 37

genotype-specific bands could be used for cultivar identity for six main varieties of *F.* ×ananassa (‘Camarosa’, ‘Sweet Charlie’, Selva’, ‘Milsei Tudla’, ‘Chandler’, and ‘Pájaro’) cultivated in Argentina (García et al., 2002). When RAPD and ISSR (Inter Simple Sequence Repeat) were assessed for suitability of determining strawberry relationship, cultivars sharing the same pedigree were usually clustered together in the dendrogram generated from both of the molecular markers (Kuras et al., 2004). In the study of Milella et al. (2006), 65 genotypes of strawberry collected from Etna mountain of Italy and one cultivar ‘Madane Moutot’ which was possibly the ancestral genotype of the Etna strawberries were evaluated and their close genetic relationship was assured via RAPD markers.

RAPD markers were found to be linked to specific genes and these markers could facilitate the breeding process (marker-assisted breeding). Two Japanese culativars ‘Ever Berry’ (everbearing type) and ‘Toyonoka’ (Junebearing type) and their $F_1$ cross hybridization progeny were analyzed for RAPD markers linked to the everbearing gene (controlled by a single dominant gene), and five markers were found to construct a likage map, with OPE07-1 and OPB05-1 being closest to the everbearing gene (Sugimoto et al., 2005). Seven RAPD markers were found to be linked to the *Phytophthora fragariae* resistant gene (*Rpf1*) in the cultivated strawberry by bulk segregant analysis, with OPO-08A and OPO-16A being closest to the *Rpf1* gene (Haymes et al., 1997).

## 7. Growth and Development of Strawberry

Strawberry is a perennial plant usually described as herbaceous but was actually a true woody plant by the evidence of secondary xylem in roots and crowns (Darnell et al., 2003). The plant body is comprised of a rosette central stem or crown from which leaves, roots, runners (stolons) and inflorescences emerge (Hancock, 1999). The crown

is terminated by a bud generally containing 5 to 7 developing leaves enclosed within the stipules of the last emerged leaf (Guttridge, 1985). At the top of each leaf along the crown is an axillary bud (Hancock, 1999) which produce runners under long day conditions and develop into branched crowns or remain dormant under short day conditions (Konsin et al., 2001). Inflorescences emerge from the apical meristem of the crown while the uppermost axillary bud continues the vegetative extension of the crown (branched crowns). Flower initiation may occur in the branched crowns when there are more than two leaf primordia and the environment is flower-inductive (Guttridge, 1985; Hytönen et al., 2004).

The cultivated strawberry was traditionally categorized by fruiting behavior as Junebearers and everbearers. Junebearers produce a single flush of flowers every year (single cropping). Everbearers produce several flushes of flowers in the growing season (multiple cropping). The flowering of strawberry is greatly influenced by temperature and photoperiod. Photoperiod requirement for flowering in strawberry can be divided into three groups which were long day plants, short day plants and day-neutral plants. Junebearers are considered as facultative short day plants, with flower initiation under short day if the temperature is above about 15℃ or irrespective of the day length if the temperature is below 15 ℃ (Guttridge, 1985; Hancock, 1999; Taylor, 2002). Everbearers are considered as long day plants because their flowers initiate under long day conditions when temperature is moderate (Hancock, 1999; Darnel et al., 2003). Day-neutral plants were introduced by Bringhurst and Voth (1980) (in Taylor, 2002) to describe multiple cropping cultivars derived from crosses between Junebearing types and *F. virginiana* subsp. *glauca* (everbearing type). Day-neutrals are relatively insensitive to day length for flower initiation (Darnel et al., 2003). Strict categorization is difficult due to the continuum of photoperiodic responses observed in different

genotypes (Darnell et al., 2003).

The physiology of the perennial cycle in June-bearing strawberry was described by Battey et al., (1998). The temperature was cool and the day length was short after August, hence vegetative growth gradually slowed down and flower initiation was triggered. While the temperature got colder in November, strawberry plants underwent a dormant period until next spring, when the temperature was warm and the day length was long, the vegetative growth was recovered and the flower buds which were already differentiated bloomed and set fruit through March to August.

Wen (1984) had reviewed that strawberry seedlings are able to accept the stimulus of short day and low temperature for flower differentiation when there are more than four true leaves and for runner plants, there should be more than four to five expanded leaves. In the study of Verheul et al. (2006) comparing the performance of *F.* × *ananassa* 'Korona' at 4, 8 or 12 weeks of plant age after flower inductive treatment, flowers were induced in 4-week-old runner plants with only three to four leaves. The number of inflorescence and total number of flowers increased as the plant age was increased.

Sønsteby and Nes (1998) investigated the critical number of short day cycles necessary to induce flower in four cultivars of strawberry ('Korona', 'Elsenta', 'Bounty' and 'Senga Sengana') under three temperature region (9℃, 15℃ and 21℃). For 'Korona' and 'Elsanta', 16 short day cycles were required for flower initiation when the temperature was 15℃, while longer short day cycles were required for lower and higher temperatures. In 'Bounty' and 'Senga Sengana', flowers were inducted almost irrespective of short day treatment. Twelve days of inductive cycle was required for 'Chandler' under 9 hours of short day and 16℃, while a longer inductive cycle of 19 days was required under night interruption (Zhang et al., 2000). Verheul et al. (2006)

investigated the optimum short day cycles (14, 21 and 28 days) for flower induction in ‘Korona’ and found that 28 short day treatment resulted in highest number of inflorescences and flowers per plant.

# Materials and Methods

## 1. Morphological Variations among *Fragaria hayatae*, White-fruited Strawberry and other *Fragaria* Species

### 1.1. Plant materials and management

Ten accessions of *F. hayatae* (H1, H2, H4-8, H10, H12 and H13), one accession of white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, *F. ×ananassa* were used in this experiment. *F. hayatae* and white-fruited strawberry were collected from central mountain regions of Taiwan during 2006-2008 (Table 2). All strawberries were obtained as runner plants except *F. vesca*, which was grown from seeds purchased from Known-You Seed Company (Kaohsiung, Taiwan). The plants were cultivated in the outdoor bench of Department of Horticulture, National Taiwan University. All strawberries were planted in 3 in. red plastic pots with King Root Plant Medium #1 : peat moss =1:1 as medium. Bagasse compost (Taiwan Sugar Co., Tainan, Taiwan) was mixed in the medium in 1:10 by volume. Slow release fertilizer Hi-Control No. 1 (N:P:K=14:12:14, Taiwan Horticultural Co., Ltd, Taipei, Taiwan) was used one month after the runner plants rooted. The plants were fertilized with 1000× soluble fertilizer Wonder Grow (N:P:K=20:20:20, Taiwan Horticultural Co., Ltd, Taipei, Taiwan) every week and irrigated with tap water when needed. 500× potassium bicarbonate ($KHCO_3$, AG168 Co., Ltd., Taipei, Taiwan) and 500× narrow range oil (paraffin oil, AG168 Co., Ltd., Taipei, Taiwan) were sprayed when needed to prevent powdery mildew, spider mites and aphids.

### 1.2. Characteristic measurement

Morphological characteristics and conduction method were referred from UPOV document TG/22/9 (1995), TG/22/10 (2008) and Sargent et al. (2004). The characteristics and standards of measurement were listed in Table 3 and 4. More than ten plants from each collection were used for observation.

## 1.3. Data analysis

The means of the characteristics were compared to test for significant difference using General linear model (GLM program) and Duncan's multiple range test (SAS version 8.01, SAS Institute Inc., Cary, N.C., USA). For clustering analysis, these data were first standardized by subtracting the accession mean from the grand mean for each characteristic then divided by the characteristic standard deviation. Euclidean distant coefficient matrix was derived from the standardized morphological characteristic means. The matrix was analyzed using the unweighted pair-group method with arithmetic mean (UPGMA) (SAHN clustering program, NTSYS-pc version 2.11L, Applied Biostatistics Inc., NY, USA). For principle coordinate analysis, the standardized morphological characteristic matrix were used to derive Pearson product-moment correlation coefficient matrix from which the principle components were extracted and projected in two or three dimensions (EIGEN, PROJ, MXPLOT and MOD3D programs, NTSYS-pc version 2.11L, Applied Biostatistics Inc., NY, USA).

Euclidean distant coefficient:

Eij = $\sqrt{\sum_k \left(X_{ki} - X_{kj}\right)^2}$

Eij : the taxonomic distance between individual i and j

Xki: the value of the kth variable of individual i

Xkj: the value of the kth variable of individual j

# 2. Molecular Variations among *Fragaria hayatae*, White-fruited

Strawberry, and Other *Fragaria* Species

## 2.1. DNA samples

Fourteen accessions of *F. hayatae* Makino and one accession of white-fruited strawberry collected from central mountain regions of Taiwan during 2006-2008 were listed in Table 2. *F. vesca* L. seedlings were sown from the seeds, which were purchased from Known-You Seed Co. (Kaohsiung, Taiwan). *F.* ×*ananassa* 'Taoyuan No. 3' was purchased from Shen-Neng Chang's farm at Hsinchu, Taiwan. DNA samples of *F. nilgerrensis* Schlecht., *F. mandschurica* Staudt, *F. pentaphylla* Lozinsk. and *F. viridis* Duch. were provided by Institute of Horticulture, Jiangsu Academy of Agricultural Science, Nanjing, China. *Potentilla matsurae* was gathered from Hohuanshan and included in this study as outgroup.

## 2.2. DNA Extraction

DNA samples were extracted by a modified CTAB method (Torres et al., 1993; Lin, 2004). One or two young leaves (ca. 0.1 g fresh weight) were cleansed with RO water and pat dried by paper towel and fixed with liquid nitrogen. Liquid nitrogen fixed leaves were grounded into fine powder with ceramic mortar and pestle. 1 ml of grinding buffer (2% (w/v) CTAB, 0.1 M Tris-HCL pH 8.0, 20 mM Na-EDTA pH 8.0, 1.4 M NaCl, 0.4% (w/v) β-mercaptoethanol) was added into the mortar before the sample thawed and continued grinding until material became slurry. The slurry was transferred into 1.5 ml eppendorf, inserted into Styrofoam float and incubated in 60℃ water bath for one hour, then removed from water bath and let cool to room temperature. Sufficient (ca. 800 μL) chloroform: isoamyl alcohol solution (24:1) was added into the eppendorf. The eppendorf was vortexed until color of mixture appeared uniform, and was centrifuged at 10,000 x g for 5 minutes to separate phases. The upper (aqueous) phase

was transferred into a new 1.5 ml tube and the lower (chloroform) phase was discarded. The CI extraction steps were repeated until the aqueous solution became clear.

Ice-cold 95% ethanol was added to the sample tube and kept in a freezer for 10 minutes. When white precipitate appeared at the solvent interface, the tube was inverted gently several times to allow complete mix of the solvents. DNA should appear as a vitreous blob. The tube was centrifuged in 10,000 x g for 5 minutes. The supernatant was discarded with caution not to lose the DNA pellet. 1 ml of ice-cold 70% ethanol was added to the tube and kept in freezer for 10 minutes. The tube was centrifuged in 10,000 x g for 5 minutes. The supernatant was discarded and residual ethanol was removed by pipette. The DNA pellet was dried in laminar flow for 20 minutes and then dissolved in 50 μl TE (Tris-EDTA, 10 mM Tris-HCl, 1 mM Na-EDTA, pH 8.0) in a refrigerator overnight.

50 μl RNase solution was added to each sample tube, and was gently pipetted up and down 10 times. The tubes were incubated at 37℃ water bath for 30 minutes to allow digestion of RNA.

## 2.3. Quality check and quantification of DNA samples

10 μl DNA was mixed with 990 μl of sterilized deionized water, after transferred to a quartz cuvette, absorbance at wavelength 260 nm, 280 nm and 320 nm were measured using a UV photospectrometer. Samples with $A_{260}/A_{280}$ fallen between 1.7-2.0 represent high purity of DNA and were further checked for integrity.

10 μl of DNA was loaded on $2\%_{(w/v)}$ agarose gel with 0.5× TBE buffer and electrophoresis was conducted under 100 V for 30 minutes. Samples occur at high molecular weight representing intact DNA were used for polymerase chain reaction.

Concentration of DNA was determined with following equation: [OD260 × 50 μg/mL × 1 mL] / 10 (μg/μL) and was adjusted to 10 ng/μl and then kept in 4℃ for

later use.

## 2.4. RAPD-PCR

A set of 100 random 10-mer UBC set # 1 primers (University of British Columbia, Vancouver, B.C., CA) were used for PCR reactions. PCR amplifications were carried out in 25 μl reaction mixtures containing 25 ng of DNA template, 1× PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 3 mM $MgCl_2$, 200 μM dNTPs (Applied Biosystems, Forster, CA, USA ), 0.5 μM primer, and 2 units of *Taq* DNA polymerase (Invitrogen, Life technologies, Carlsbad, CA, USA). Each reaction was overlaid with about 20 μl of mineral oil to prevent evaporation. The reactions were performed in thermocycler (GeneAmp PCR System 2700, Applied Biosystems, Forster, CA, USA) programmed for initial denaturation at 94℃ for 3 min, followed by 40 cycles of denaturation at 94℃ for 30 sec, annealing at 38℃ for 30 sec, extension at 72℃ for 1 min and finished with a final extension at 72℃ for 5 min. The amplified products were kept in 4℃ until use. Two replicates for all plants were repeated for each primer to check consistency.

## 2.5. Electrophoresis analysis

Following amplification, 15 μl of amplified products were mixed with 1 μl of 6× loading dye and separated by electrophoresis in 1.5% (w/v) agarose gel with 0.5× TBE (44.5 mM Tris Base, 45 mM Boric acid, 1 mM Na-EDTA, pH 8.0) buffer under 100 V and 400 A for 120 min. The gel was then stained by ethidium bromide (0.5 μg/ml) for 20 min, destained in RO water for 20 min and then photographed under illumination with ultraviolet radiation.

## 2.6. Data analysis

A DNA fragment was judged a scorable band if it was of sufficient intensity and differed from neighboring bands. Only reproducible bands were chosen for data analysis.

RAPD markers were used to build a binary matrix by scoring as present (1) or absent (0). The scorable amplification products were used to evaluate genetic similarity by estimating Jaccard coefficient. Cluster analysis was performed using the UPGMA method (SAHN clustering program, NTSYS-pc version 2.11L, Applied Biostatistics Inc., NY, USA.). For principle coordinate analysis, the similarity matrix was transformed by double center method, from which the principle components were extracted and projected in two dimensions (DECENTER, EIGEN, MXPLOT programs, NTSYS-pc version 2.11L, Applied Biostatistics Inc., NY, USA).

Jaccard coefficient:

Sij = Nij / (Ni + Nj + Nij)

Ni: No. of bands which individual i possess but individual j doesn't

Nj: No. of bands which individual j possess but individual i doesn't

Nij: No. of bands which individual i & j both possess

# 3. Effects of Temperature and Photoperiod on Growth and Flowering of *Fragaria hayatae*

## 3.1. Plant materials

Mother plants of *F. hayatae* were collected from Meifung, Highland Experimental Farm, NTU and cultivated in outdoor bench of Department of Horticulture, NTU. The runner plants were cut off from the mother plants and rooted in 3 in. red plastic pots two months before the experiment. They were taken care of as described in section 1.1. Runner plants that had more than four fully expanded leaves were used in the study.

## 3.2. Experimental design

Two experiments were conducted. The purpose of experiment 1 was to understand the effects of temperature and duration of treatment on growth and flowering in *F.*

*hayatae*. Experiment 1 started from the end of January, 2008. In this experiment, runner plants were treated under 10 hour light and 14 hour continuous dark at two temperature condition that were day/night temperature 15/10℃ and 10/5℃, and five treatment duration which were 2, 4, 6, 8 and 10 weeks. The plants were cultivated in 6000 lux growth chamber. Totally there were 10 treatments, including 2 day/night temperature x 5 duration of treatment, each treatment contained 8 plants. Following the treatment, runner plants were moved to a growth chamber with natural day light in 20/15℃.

The purpose of experiment 2 was to understand the effects of day length and day/night temperature on vegetative and reproductive growth of the Taiwan strawberry. Experiment 2 started from mid January, 2009. Runner plants were treated under 15/10 ℃ and 14 hour day length, 15/10℃ and 10 hour day length, and 15/5℃ and 10 hr day length for 6 weeks. The plants were cultivated in 10,000 lux growth chamber. Following the treatment, 12 runner plants for each treatment were moved into a growth chamber under 20/15℃ and 14 hour day length. A control group was made by putting the plants in the out door bench of Department of Horticulture, NTU.

At start of the experiments, all runners were removed. Number of leaves, petiole length, terminal leaflet length and width of the last fully expanded leaf were recorded. Leaf area was predicted from a regression formula of terminal leaflet length × terminal leaflet width, and the method was followed by Wen (1984). Growth was monitored by weekly observations on number of leaf, number of runner, number of flower, petiole length, terminal leaflet length and width of the last fully expanded leaf. In experiment 1, growth was monitored throughout the experiment, but in experiment 2, growth was not recorded in the duration of treatment to prevent from interference of flower formation. Petiole length was measured from the base to the trifoliate attachment zone. Runners were removed weekly after they were recorded.

## 3.3. Data analysis

Data was analyzed with the GLM program of SAS (SAS for windows version 9.1; SAS Institute, Cary, N.C.). Comparison of means was accomplished by Duncan's multiple range test at the $P = 0.05$ level of significance.

# 4. Interspecific Hybridization between *Fragaria hayatae* and *F.* ×*ananassa*

Reciprocal crosses were performed in *F.* ×*ananassa* 'Taoyuan No. 3' and *F. hayatae* during January to April, 2008. More than 10 flowers were pollinated in each combination. Three flowers of each *Fragaria* species were left unpollinated to determine the effectiveness of emasculation. Pollen was collected by detaching flowers from the plants, removing sepals and petals, and placed overnight in small dishes. After the anthers dehisced, pollen was shaken off, sealed and stored in the inner chamber of a glass dish with desiccant in the outer chamber at 4℃. Female plants were emasculated at the 'white bud stage' using a pair of tweezers to remove the ring of sepals, petals and anthers surrounding the receptacle. Pollination was made right after emasculation and 24 hr later with a small paint brush. The pollinated receptacle was covered by paper bag to prevent foreign pollen. Once the receptacle began to swell, the paper bag was removed to allow normal development of the fruit.

Seeds from crosses were collected by tweezers, washed, allowed to dry in room temperature before storing in PE bag at 4℃. Seeds were sown on a petri dish which was covered with moistened filter paper. The germination test was conducted in a growth chamber with day/night temperature at 15/10℃ and light was provided from 8:00 to 20:00. Germinated seeds were transferred to 3 in. plastic pots containing peat moss as substrate and were planted 5 inch plastic pots with King Root medium # 1: peat moss =

1:1 as substrate when transference was necessary.

The number of fruit set, average number of seeds per fruit, germination rate of the seeds and survival rate of the seedlings were recorded.

# Results and Discussion

## 1. Native Habitat of *Fragaria hayatae* and Discovery of the White-fruited Strawberry

Collection of *F. hayatae* in this study was from central mountain range at the altitude between 2200-3100 m (Table 2 and Fig. 2). *Fragaria hayatae* was found in Tianchih (天池), Yakou (埡口) and Shianyang (向陽) on the South Cross-Island Highway (南橫公路), Kuanshanlingshan (關山嶺山), Shiaoshueshan (小雪山), Hohuanshan (合歡山), Meifung Farm (梅峰), Yushan (玉山) and Alishan (阿里山) (Table 2 and Fig. 2). It was not found in Taipingshan (太平山) although there was record of specimens in Herbarium of National Taiwan University, nor in Guanwu (觀霧) Forest Recreation Area. Taiwan strawberry was usually distributed by open places such as roadside or traffic lane, in sunny or partial shaded environment as mentioned by Chen (1995) and Naruhashi et al. (1999). The native habitat of *F. hayatae* was shown in Fig. 3 and it could be grown on loamy soil, detritus or cracks between rocks. The Taiwan strawberry was found to form independent populations or was accompanied by other herbaceous or shrubs such as *Plantago asiatica*, *Potentilla matsumurae*, and *Rubus taiwanicolus*.

White-fruited strawberry was found in a field trip to collect *F. hayatae* in Shiaoshueshan by our lab. It was distinct from the Taiwan strawberry in having green petiole (Fig. 4B), green runner (Fig. 4B), white petal (Fig. 5B left and 5D) and cream-white colored fruit (Fig. 6C). *F. hayatae* was characterized by having red petiole (Fig. 4A), red runner (Fig. 4A), white petal with purplish red blush at base (Fig. 5A left top, 5B right, and 5C), and red fruit (Fig. 6A right and 6B). The white-fruited

strawberry was found to form independent population or was found to be mixed with the Taiwan strawberry. This strawberry was not found elsewhere and was not recorded in “Flora of Taiwan”.

## 2. Morphological Characteristics of *Fragaria hayatae*, White-fruited Strawberry and Other *Fragaria* Species

The means of the morphological characteristics of *F. hayatae*, white-fruited strawberry, and other *Fragaria* species compared by ANOVA and Duncan’s multiple range test were presented in Table 5, 6 and 7. The cultivated strawberry had an average leaflet length of 5.81 cm, average leaflet width of 5.30 cm and average leaf area of 52.26 $cm^2$ and was the largest leaf among the other tested *Fragaria* species. The wood strawberry, *F. vesca*, with leaflet length of 4.50 cm, leaflet width of 3.43 cm and leaf area of 26.41 $cm^2$, was the second large leaf among the five *Fragaria* species. The average leaflet length, width, and leaf area of *F. nilgerrensis* was 3.39 cm, 2.63 cm and 15.23 $cm^2$, respectively, which was significantly larger than *F. hayatae* ($\bar{x}$ = 2.42 cm, 2.14 cm and 9.08 $cm^2$, respectively) and white-fruited strawberry ($\bar{x}$ = 2.17 cm, 1.93 cm and 7.50 $cm^2$, respectively). Although the leaf size characteristics of *F. hayatae* were slightly larger than the white-fruited strawberry, the difference was not significant (Table 5).

The average leaflet length/ width ratio of *F. vesca* and *F. nilgerrensis* was 1.31 and 1.29, respectively, which was significantly larger than the cultivated strawberry ($\bar{x}$ = 1.11), *F. hayatae* ($\bar{x}$ = 1.13), and white-fruited strawberry ($\bar{x}$ = 1.12), indicating a longer leaf shape of the former two and a more rounded shape of the latter three strawberries (Table 5). The serrate ratio among cultivated strawberry ($\bar{x}$ = 0.74), *F. nilgerrensis* ($\bar{x}$ = 0.72), *F. hayatae* ($\bar{x}$ = 0.70), and white-fruited strawberry ($\bar{x}$ =

0.72) was not significantly different and was evidently larger than *F. vesca* ($\bar{x}$ = 0.60). The average of widest length ratio among the five *Fragaria* species ranged within 0.44-0.49 and was not significantly different (Table 5).

Terminal leaflet shape of *F. hayatae* was usually obovate (84%), some orbicular (15%), and occasionally broadly elliptic (2%). White-fruited strawberry had a similar component of leaflet shape to *F. hayatae* as 89% obovate and 11% orbicular. Terminal leaflet shape of *F. nilgerrensis* was obovate (100%); *F. vesca* was mostly obovate (69%), some broadly elliptic (23%), and a few orbicular (8%); the cultivated strawberry was obovate (60%) to orbicular (40%). Shape of terminal leaflet base was acute to obtuse for *F. hayatae* (62% and 38%) and white-fruited strawberry (46% and 54%). It was all obtuse for *F. nilgerrensis* (100%), 92% acute and 8% obtuse for *F. vesca*, and 80% obtuse with 10% of acute and rounded for the cultivated strawberry. *Fragaria hayatae* and white-fruited strawberry had similar shape of leaflet apex as emarginated (65% and 56%) to rounded (31% to 41%) with occasional obtuse (4% and 3%). Shape of leaflet apex was rounded (60%) to emarginated (40%) for *F. nilgerrensis*; obtuse (62%) to rounded (31%) and a few emarginated (8%) for *F. vesca*; obtuse (30%) to rounded (40%) to emarginated (30%) for thecultivated strawberry (Table 5, continued 1).

The angle of leaflet base among the five *Fragaria* species arranged in descending order was, the cultivated strawberry (54.4°) > *F. hayatae* (45.6°) = white-fruited strawberry (45.3°) = *F. nilgerrensis* (45.7°) > *F. vesca* (40.8°). Those with larger angle at the leaflet base had higher percentage of obtuse or rounded base, and those with smaller angle usually had higher percentage of acute base. The index of leaflet shape in cross section was between 1.1 and 1.4 among the *Fragaria* species, which indicated the leaflet shape in cross section was usually concave to straight (Table 5, continued 1). The

number of serrate on the terminal leaflet arranged in descending order was *F. nilgerrensis* ($\bar{x}$ = 21.4) > *F. hayatae* ($\bar{x}$ = 19.7) = white-fruited strawberry ($\bar{x}$ = 18.8) = cultivated strawberry ($\bar{x}$ = 17.6) > *F. vesca* ($\bar{x}$ = 14.3) (Table 5, continued 2).

Average petiole length and longest petiole length of the cultivated strawberry was 13.18 cm and 14.84 cm respectively, which was significantly longer than other strawberries. Average petiole length ($\bar{x}$ = 10.47 cm) and longest petiole length ($\bar{x}$ = 11.45 cm) of *F. vesca* ranked second and was significantly longer than rest of the strawberries. These two characteristics were not statistically different among *F. nilgerrensis*, *F. hayatae*, and the white-fruited strawberry, but the former two was slightly larger than the latter. Petiole diameter of the *Fragaria* species arranged from large to small was the cultivated strawberry ($\bar{x}$ = 2.39 mm), *F. nilgerrensis* ($\bar{x}$ = 1.58 mm), *F. hayatae* ($\bar{x}$ = 1.43 mm), *F. vesca* ($\bar{x}$ = 1.36 mm) and the white-fruited strawberry ($\bar{x}$ = 1.31 mm). Petiolet length of the cultivated strawberry ($\bar{x}$ = 0.55 cm) and *F. vesca* ($\bar{x}$ = 0.49 cm) was significantly longer than that of *F. nilgerrensis* ($\bar{x}$ = 0.41 cm) and *F. hayatae* ($\bar{x}$ = 0.37 cm), and the latter two were statistically larger than white-fruited strawberry ($\bar{x}$ = 0.26 cm) (Table 5, continued 2).

The flower morphological characteristics of the tested strawberries were listed in Table 6. Petal length ($\bar{x}$ = 10.05 mm), width ($\bar{x}$ = 10.22 mm) and corolla diameter ($\bar{x}$ = 23.30 mm) of the cultivated strawberry was the largest, *F. nilgerrensis* was ranked as the second large ($\bar{x}$ = 8.78 mm, 7.08 mm and 19.65 mm), and the rest was ranked as third with petal length ranging between 5.43-5.67 mm, petal width 4.50-4.99 mm and corolla diameter 13.25-14.12 mm. Petal length/width ratio of *F. hayatae* ($\bar{x}$ = 1.21), the white-fruited strawberry ($\bar{x}$ = 1.28), *F. nilgerrensis* ($\bar{x}$ = 1.24), and *F. vesca* ($\bar{x}$ = 1.14) indicated relatively longer petal whereas the cultivated strawberry ($\bar{x}$ = 0.98) possessed relatively shorter petal. Receptacle diameter listed from largest to smallest

was the cultivated strawberry ($\bar{x}$ = 5.04 mm), *F. nilgerrensis* ($\bar{x}$ = 4.66 mm), the white-fruited strawberry ($\bar{x}$ = 4.16 mm), *F. hayatae* ($\bar{x}$ = 3.81 mm), and *F. vesca* ($\bar{x}$ = 2.80 mm), respectively. The order of calyx diameter was similar to receptacle diameter and was 20.57 mm for the cultivated strawberry, 17.16 mm for *F. nilgerrensis*, 16.65 mm for the white-fruited strawberry, 13.28 mm for *F. hayatae*, and 11.61 mm for *F. vesca*. Size of calyx in relation to corolla indicated that the calyx was usually about the same or larger than corolla in the white-fruited strawberry ($\bar{x}$ = 2.7) while the calyx was mostly smaller or about the same size as the corolla in *F. hayatae* ($\bar{x}$ = 1.74), *F. nilgerrensis* ($\bar{x}$ = 1.6), *F. vesca* ($\bar{x}$ = 1.1), and the cultivated strawberry ($\bar{x}$ = 1.1).

There were usually five petals per flower in the tested *Fragaria* species, however, *F. nilgerrensis* ($\bar{x}$ = 5.6), the cultivated strawberry ($\bar{x}$ = 5.5), and *F. vesca* ($\bar{x}$ = 5.33) had higher opportunity to possess more than five petals while this incidence was lower in *F. hayatae* ($\bar{x}$ = 5.12) and the white-fruited strawberry ($\bar{x}$ = 5.0). Average number of flowers per inflorescence was 4.9 in the cultivated strawberry, 3.1 in *F. vesca*, 2.52 in the white-fruited strawberry, 1.36 in *F. nilgerrensis*, and 1.33 in *F. hayatae*. This indicated that the latter two species were usually solitary flower whereas flowers were usually clustered in an inflorescence in the former three species. The average total number of flowers was 8.3 for the cultivated strawberry, 7.3 for the white-fruited strawberry, 6.8 for *F. nilgerrensis*, and 6.5 for *F. vesca*, and were all significantly more than *F. hayatae* ($\bar{x}$ = 3.6). Petal arrangement in *F. hayatae* and the white-fruited strawberry was free ($\bar{x}$ = 1.0 and 1.0 respectively), usually free but sometimes touching in *F. nilgerrensis* ($\bar{x}$ = 1.1) and *F. vesca* ($\bar{x}$ = 1.2), and mostly touching but sometimes overlapping in the cultivated strawberry ($\bar{x}$ = 2.1). Except *F. hayatae* had purplish red blush at the base of white petal, the rest *Fragaria* species had white petal. The inflorescences of *F. nilgerrensis* were above the foliage ($\bar{x}$ = 3.0), at the same

level to above the foliage in white-fruited strawberry ($\bar{x}$ = 2.7) and *F. hayatae* ($\bar{x}$ = 2.2), and mostly at the same level but occasional beneath the foliage for the cultivated strawberry ($\bar{x}$ = 1.9) and *F. vesca* ($\bar{x}$ = 1.8) (Table 6, continued 1).

The morphological characteristics of fruit in the investigated *Fragaria* species were listed in Table 7. The height, width, and weight of berry was largest in the cultivated strawberry, which were 25.28 mm, 22.25 mm and 4.75 g, respectively. *Fragaria vesca* was ranked second as 20.10 mm, 11.28 mm and 0.89 g, respectively. Berry height (ranged from 8.42 mm to 10.46 mm) and berry weight (ranged from 0.29g to 0.48 g) in the white-fruited strawberry, *F. nilgerrensis*, and *F. hayatae* was not significantly different while the berry diameter of the white-fruited strawberry ($\bar{x}$ = 9.68 mm) and *F. nilgerrensis* ($\bar{x}$ = 12.57 mm) was significantly larger than *F. hayatae* ($\bar{x}$ = 9.68 mm). The width of band without achenes were wider in *F. vesca* ($\bar{x}$ = 2.6), moderate in the white-fruited strawberry ($\bar{x}$ = 2.0) and the cultivated strawberry ($\bar{x}$ = 2.0), and narrower in *F. hayatae* ($\bar{x}$ = 1.0) and *F. nilgerrensis* ($\bar{x}$ = 1.0). The rank of berry ratio arranged in descending order was *F. vesca* ($\bar{x}$ = 1.79) > the cultivated strawberry ($\bar{x}$ = 1.15) = *F. hayatae* ($\bar{x}$ = 1.09) > *F. nilgerrensis* ($\bar{x}$ = 0.78) = the white-fruited strawberry ($\bar{x}$ = 0.71). This coincided with the more slender shape of fruit in *F. vesca* and the more flat shape of fruit in the white-fruited strawberry and *F. nilgerrensis*. The berry shape of Taiwan strawberry was obloid ($\bar{x}$ = 73%) to globose ($\bar{x}$ = 27%), and it was usually obloid ($\bar{x}$ = 71%) and sometimes reniform ($\bar{x}$ = 14%) or globose ($\bar{x}$ = 14%) in the white-fruited strawberry and *F. nilgerrensis*. *Fragaria vesca* significantly differed from the other investigated diploids with usually ovoid ($\bar{x}$ = 73%), sometimes rhomboid ($\bar{x}$ = 18%) or globose ($\bar{x}$ = 9%) shape of berry. The cultivated strawberry was usually conical ($\bar{x}$ = 50%), sometimes obloid ($\bar{x}$ = 25%) or globose ($\bar{x}$ = 25%). Color uniformity in white-fruited form strawberries was even and

between even to slightly uneven in the red-fruited forms. Except a few calyx were raised in *F. vesca*, all other *Fragaria* species had calyx that level with the fruit.

Overall, the cultivated strawberry, being an octoploid, was largest among all of the size-related characteristics (terminal leaflet length, width, leaf area, petiole length, width, longest petiole length, petiolet length, petal length, width, diameter in corolla, receptacle and calyx, berry height, width and weight), and these were the most distinguishable characteristics for *F.* ×*ananassa* 'Taoyuan No. 3'. Nevertheless, the diploid *F. vesca* usually ranked second in size except the characteristics of petiole diameter, petal length, petal width, diameter in corolla, receptacle and calyx. *Fragaria vesca* could also be easily distinguished from others by its shorter length of serrate region on the terminal leaflet, gracile petiole, slender leaf and ovoid berry shape. The size and shape characteristics of leaf, petiole, flower, and the size of fruit were generally non-significantly different between the Taiwan strawberry and the white-fruited strawberry, yet the white-fruited strawberry was distinct from the Taiwan strawberry in its white petal, more flowers, more number of flowers per inflorescence, cream-white-colored and more flatted shape of fruit, and the Hayata strawberry were characterized with white petal with purplish red blush at the base, red-colored and more rounded shape of fruit. There were some resemblance between the white-fruited strawberry and *F. nilgerrensis* such as the petal and fruit were both without anthocyanin, the size and smaller height/ diameter ratio of the berry, the length and longest length of petiole, but the size of leaf, length/ width ratio of terminal leaflet, width of petiole, length of petiolet, and size of petal and corolla were significantly larger in *F. nilgerrensis*. The Taiwan strawberry differed from *F. nilgerrensis* by similar characteristics that separated the white-fruited strawberry from *F. nilgerrensis* (except the width of petiole and length of petiolet) and characteristics that separated the Taiwan

strawberry and the white-fruited strawberry.

The results of principal component analysis for *F. hayatae*, the white-fruited strawberry, *F. nilgerrensis*, *F. vesca* and *F. ×ananassa* ‘Taoyuan No. 3’ using 19 vegetative morphological traits were given in Table 8. The first three principal components represented 82.01% of the total variation. The first principal component (PC1) accounted for 48.71% of the total variance, and had high contributing factors from morphological characteristics associated with the size of the leaf and petiole, which were terminal leaflet width, leaf area, leaflet length, leaflet length to the widest point, longest petiole length, petiole length, petiole diameter, petiolet length and leaflet length to the first tooth. According to these nine characteristics, the *Fragaria* populations were separated into three groups, *F. hayatae*, the white-fruited strawberry, and *F. nilgerrensis* were categorized in one group because they all possessed relatively small leaf and short petiole. *Fragaria ×ananassa* ‘Taoyuan No. 3’ was categorized in one group for its large plant size, and *F. vesca* was categorized in a third group as its plant size was between the two groups.

The second principal component (PC2) accounted for 23.51% of the total variance, and had high contributing factors from characteristics mainly associated with the shape of the leaf, namely serrate ratio, shape of terminal leaflet, number of serrate, length/ width ratio of terminal leaflet, shape of terminal leaflet apex, and angle of terminal leaflet base (Table 8). From these six characteristics, *F. vesca* was separated to one group for its larger leaflet ratio, more slender shape of leaflet, fewer number of serrate, smaller serrate ratio, and narrow angle of leaflet base. *Fragaria ×ananassa* ‘Taoyuan No. 3’ was separated into another group for its obovate to rounded shape of leaf, rounded leaflet apex and broader angle of leaflet base. *Fragaria hayatae*, the white-fruited strawberry, and *F. nilgerrensis* were included in one group due to similar

serrate ratio, shape of leaflet, shape of leaflet apex, and angle of leaflet base.

Figure 7 illustrates the accessions distribution in the first two principal components and showed the separation of the 14 accessions studied. *Fragaria vesca* and *F.* × *ananassa* 'Taoyuan No. 3' were most distantly separated from other *Fragaria* populations while *F. hayatae*, the white-fruited strawberry, and *F. nilgerrensis* were grouped together. Although the result indicated a close relationship among *F. hayatae*, the white-fruited strawberry, and *F. nilgerrensis*, the white-fruited strawberry and *F. nilgerrensis* were plotted at two extremes of the group, showing their differences in some extent. Among the accessions of *F. hayatae*, those collected from adjacent areas were generally plotted near by, indicating their closer relationship, for example H12 and H13 were both gathered from Alishan, and H1 and H2 were gathered from Tianchih and Yakou on the Southern Cross-Island Highway. It was worth of notice that, H6 and W (the white-fruited strawberry), which were both collected from Shiaoshueshan, were plotted close when using these 19 vegetative characteristics. H7 (Hohuanshan 1) and H10 (Yushan) were more distant from other Taiwan strawberry because H7 possessed the largest plant size while H10 was the smallest.

The result of the clustering analysis was shown in Figure 8. Using a critical dissimilarity coefficient 7.86, the *Fragaria* populations could be separated into three groups. The first group contained only one species, *F.* ×*ananassa* 'Taoyuan No. 3', which separated with others at the dissimilarity coefficient 10.26. The second group was *F. vesca*, which separated at the dissimilarity coefficient 7.86. The remaining group was comprised of *F. hayatae*, the white-fruited strawberry, and *F. nilgerrensis*. Among the third group, the white-fruited strawberry was very closely related with *F. hayatae* rather than *F. nilgerrensis*, since W (the white-fruited strawberry) was grouped with H6 (*F. hayatae* from Shiaoshueshan) at the dissimilarity coefficient 1.37 and this confirmed

with the result of principal component analysis.

A further principal component analysis using 41 characteristics including both vegetative and reproductive were performed on the five strawberry populations and results were shown in Table 9. The first three principal components explained 51.58%, 31.40%, and 11.72% of the variances, respectively, which together accounted for 94.7% of variances. There were eleven contributing factors that had absolute eigenvector over 0.91 for the first principal component (PC1), they were terminal leaflet length, width and length to the widest point, leaf area, petal length/ width ratio, longest petiole length, berry height, petiole length, arrangement of petals, berry weight, and petiolet length. The second principal component (PC2) had high contributing factors from serrate ratio, receptacle diameter, position of calyx attachment, shape of terminal leaflet base, berry ratio, berry shape, number of serrate, and calyx diameter. The third principal component (PC3) had high contributing factors from leaflet length/ width ratio, widest length ratio, number of petals, petal color, shape of terminal leaflet, and berry color.

The distribution of *Fragaria* accessions by the first three principal components were illustrated in Figure 9. PC1 and PC2 had separated the strawberries into three groups, one with *F.* ×*ananassa* 'Taoyuan No. 3', another with *F. vesca*, and the third with the remaining populations, which was similar to the results of Figure 7. However, the third component had separated *F. nilgerrnsis* from *F. hayatae* and the white-fruited strawberry for the former leaflet ratio was larger than the latter, the more petal number and greater widest length ratio of *F. nilgerrensis*. The white-fruited strawberry was also separated from *F. hayatae* by PC3 since the petal and fruit of the white-fruited strawberry was without anthocyanin while those in the Taiwan strawberry were with anthocyanin.

The results of the clustering analysis were shown in Figure 10. By the dissimilarity

coefficient of 7.57, the accessions could be separated into four groups, one group with *F.* ×*ananassa* 'Taoyuan No. 3', one with *F. vesca*, the third with *F. nilgerrensis*, and the last with *F. hayatae* and the white-fruited strawberry. Although *F. hayatae* and the white-fruited strawberry were clustered in one group indicating their close relationship, they were separated at the dissimilarity coefficient 4.74 indicating their morphological differences.

When using solely vegetative traits for PCA and clustering analysis, *F. vesca* and *F.* ×*ananassa* which were more distantly related to the three other strawberry populations were easily parted, but separation among *F. hayatae*, the white-fruited strawberry, and *F. nilgerrensis* was unsatisfactory. Nielsen and Lovell (2000) had mentioned that solely vegetative or reproductive characteristics were not sufficient for identifying strawberry cultivars since they might share either floral or leaf characteristics. When analyzing with both vegetative and reproductive characteristics, a more clear separation was obtained, showing the importance of using both vegetative and reproductive characteristics when trying to identify strawberry species or cultivars.

## 3. RAPD Characteristics of *Fragaria hayatae*, White-fruited Strawberry and Other *Fragaria* Species

A total of 24 accessions of *Fragaria* species including *F. hayatae*, the white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, *F. mandschurica*, *F. pentaphylla*, *F. viridis*, and *F.* ×*ananassa* 'Taoyuan No. 3' were examined by the RAPD markers, and *Potentilla matsurae* were included as an outgroup (Table 2). 100 UBC set No. 1 primers were pre-examined on three *F. hayatae* accessions, and 47 primers which amplified products were then chosen to be used on all *Fragaria* species. 35 out of 47 primers generated 409 clear and reproducible bands which differentiated the investigated species. Except 8 bands were monomorphic, the rest of 401 bands were polymorphic, and each

primer generated an average of 11 to 12 bands.

The banding patterns generated from the RAPD primers were shown in Fig. 11. Fourteen bands from ten primers were specific for *F. hayatae* and the white-fruited strawberry: UBC 4 (1850, 1700 bp), UBC 16 (1450, 1400 bp), UBC 18 (700 bp), UBC 25 (960, 720 bp), UBC 34 (1350, 770 bp), UBC 43 (2050 bp), UBC 50 (1750 bp), UBC 63 (1000 bp), UBC 65 (1670 bp), UBC 70 (800 bp). Eight bands from four primers were unique to *F. nilgerrensis*: UBC 4 (2200, 1650 bp), UBC 16 (1500, 750 bp), UBC 25 (1750, 750 bp), UBC 34 (1900, 1300 bp). These banding characteristics could be used for distinguishing *F. hayatae*, the white-fruited strawberry, and *F. nilgerrensis*. Due to the property that RAPD primers randomly anneal to the template DNA, the function of the specific size fragments were unknown.

The 2D scatter plot from principal component analysis based on the correlation of 409 bands generated in the *Fragaria* species were presented in Fig. 12. The 14 *F. hayatae* accessions gathered closely together and the white-fruited strawberry nested within this cluster. Among the *Fragaria* species, *F. nilgerrensis* was the closest to the Taiwan strawberry and the white-fruited strawberry. *Fragaria vesca* and *F. viridis* distributed next to each other with *F. mandschurica* plotted close to them. *Fragaria pentaphylla* distributed close to *F.* ×*ananassa* 'Taoyuan No. 3' and was at one extreme of the 2D scatter plot while *Potentilla matsumurae* was at another extreme.

The dendrogram based on UPGMA clustering of Jaccard coefficient derived on the presence-absence data of bands from 35 primers in the *Fragaria* species were shown in Fig. 13. *Potentilla matsurae* was the first to separate from the *Fragaria* species at the similarity index of 0.14. *Fragaria viridis*, *F. vesca*, *F. mandschurica*, *F. pentaphylla*, and *F.* ×*ananassa* 'Taoyuan No. 3' were the second cluster to separate from others at similarity index of 0.23. *Fragaria nilgerrensis* departed from the Taiwan strawberry and

the white-fruited strawberry at similarity index of 0.43. The white-fruited strawberry was separated from *F. hayatae* H6, which was collected from Shiaoshueshan at similarity index of 0.94. Variation of similarity index among the Taiwan strawberries was between 0.81 and 0.99. Those collected from close regions were generally clustered in one group, as H1-H5 (*F. hayatae* from Tianchih, Yakou, Yakou 2, Siangyang, and Kuanshanlingshan, respectively) were distributed near the South Cross-Island Highway and H7-9 (*F. hayatae* from Hohuanshan 1, Hohuanshan 2, and Meifung Farm, respectively) were distributed near Provincial Highway 14 A.

Combining the results of morphological traits and molecular markers, the Taiwan strawberry was distinct from *F. nilgerrensis* and should solely be a species of *Fragaria* rather than a subspecies of *F. nilgerrensis*. Although there were some similar morphological characteristics between the white-fruited strawberry and *F. nilgerrensis*, RAPD markers evidently departed their relative further distance compared to *F. hayatae*. The difficulty of separation between *F. hayatae* and the white-fruited strawberry by molecular characteristics yet they were different in the aspect of morphological characteristics, therefore, the white-fruited strawberry should be considered as a mutant of *F. hayatae*. The white-fruited strawberry and the Taiwan strawberry should both be endemic species of Taiwan.

## 4. Relationship between terminal leaflet and actual leaf area

Relationship among terminal leaflet length, width, and length × width with actual leaf area was linearly correlated at the significance level less than 0.1% (Fig. 14). The rank of relevance coefficient in descending order was terminal leaflet length × width ($p$ = 0.9747), terminal leaflet width ($p$ = 0.9710), and terminal leaflet width ($p$ = 0.9453), respectively. The regression model was y = 1.6694x + 0.1539 for relationship between terminal leaflet length × width and leaf area, y = 6.3716x – 5.2792 for relationship

between terminal leaflet width and leaf area, and y = 5.9752x - 4.7982 for relationship between terminal leaflet length and leaf area. In the equations, y was the predicted leaf area and x was the measured terminal leaflet characteristic. All of the measured terminal leaflet characteristics had high relevance with the actual leaf area, and could all be used to estimate leaf area. In this study, we chose to use terminal leaflet length × width for estimation of leaf area not only because it had the highest relevance among the three measured characteristics, but also the length and width of terminal leaflet could be used as an index of the terminal leaflet shape.

## 5. Effects of Temperature and Photoperiod on *Fragaria hayatae*

From our observation, the Taiwan strawberry cultivated in Taipei thrived from November to April but the growth gradually decreased as the temperature get higher and most of the plants died during mid- to late-summer, so the plants should be transferred to a cool temperature growth chamber, for example under 25℃, for maintenance. A few of the Taiwan strawberry collected from mountain regions during October to February flowered about two months after transplanted in Taipei, but the rate of plants flowering and number of flowers per plant was low. The flowers of the Taiwan strawberry was recorded to bloom from April to July (Lai, 2004), or June to August (Chen, 1995), and the fruits bear from August to October (Chen, 1995) in its native habitat. Thus we assumed that the Taiwan strawberry should be June-bearers which require short day and cool temperature for flower initiation.

We have observed some characteristics such as cold hardiness, aphid tolerance, and mite tolerance in *F. hayatae*, and these could be useful in commercial strawberry cultivation. We attempted to cross the Taiwan strawberry with the cultivated strawberry ‘Taoyuan No. 3’ but flowering of the cultivated strawberry was November to March which did not meet with the Taiwan strawberry. Therefore, finding out the optimal

growth and flowering condition for *F*. *hayatae* and to force it to flower in time for later interspecific hybridization was neccessary. Two experiments were conducted to understand the effects of temperature and photoperiod on growth and flowering in the Taiwan strawberry.

1.) Experiment 1: effects of temperature and duration of treatment on growth and flowering in *Fragaria hayatae*

The plants were treated under short day (10 hour day length) and cool temperature (15/10℃ and 10/5℃) for a period of time (2, 4, 6, 8, and 10 weeks), and then transferred to a warm environment (20/15℃) for further observation. Vegetative growth was restrained when the plants were cultivated under cool temperature. The new leaf and runner formation was slow during treatment, and after transferring to warm temperature (20/15℃), the growth was accelerated. This effect was more significant at 10/5℃ (Fig. 16 and 18) when compared to 15/10℃ (Fig. 15 and 17). Evidence was also supported by shorter petiole length, smaller leaf area, slower rate of leaf and runner formation at the end of 15/10℃ and 10/5℃ treatment, while after several weeks of growth under warm environment, petiole length and leaf area were increased and the rate of leaf and runner formation were accelerated (Table 11).

At the end of the treatments, temperature had no significant influence on petiole length, but had significant effect on leaf area, formation rate of leaf, and formation rate of runner (Table 11). *Fragaria* hayatae grown under 15/10℃ had larger leaf area (13.76 $cm^2$), faster rate of leaf formation (0.64 leaf per week) and runner formation (0.23 runner per week) whereas those grown under 10/5℃ had smaller leaf area (11.08 $cm^2$), slower rate of leaf formation (0.35 leaf per week), and slower rate of runner formation (0.13 runner per week). After several weeks of growth under 20/15℃ with natural day light, temperature significantly influenced on petiole length, leaf area, and

rate of formation in leaf, while no significant effect was found on rate of formation in runner. Petiole length and leaf area was significantly greater when treated in 10/5℃ than 15/10℃, the rate of leaf formation was faster when treated in 15/10℃ rather than 10/5℃, and the treated temperature had no significant effect on rate of runner formation after several weeks of growth in warm temperature.

Duration of treatment had no significant effect on leaf area and rate of leaf formation but had significant effect on petiole length and rate of leaf formation at the end of treatment (Table 11). As the treatment duration was prolonged, petiole length became short and rate of leaf formation slowed down. Although there was no statistically difference among the five duration of treatment on rate of runner formation, plants which underwent 2 weeks of treatment formed more runners than those treated for 4 weeks or up. After transferring to warm temperature for several weeks, the influence of treatment duration was significant. Although it was difficult to give a absolute relationship between the measured vegetative characteristics and the treatment duration, plants treated for 4 or 10 weeks had the longest petiole (8.41 cm; 8.93 cm) and largest leaf area (18.19 $cm^2$; 18.41 $cm^2$), and the leaf formation rate (1.03 leaf per week; 1.02 leaf per week) and runner formation rate (0.83 runner per week; 0.69 runner per week) was fastest in plants treated for 4 or 6 weeks.

The effects of temperature and duration of treatment on flowering was displayed in Table 12. Flowers emerged in plants treated under 15/10℃ for 6 to 8 weeks and 10/5℃ for 6 to 10 weeks. The percentage of plants flowering was 25% for those treated under 15/10℃ condition and 25% to 50% for those treated under 10/5℃. An average of 5.25 flowers emerged in the plants grown under 15/10℃ whereas an average of 1.43 flowers emerged in the plants grown under 10/5℃. It took 9.5 to 12.0 weeks for the Taiwan strawberry to bloom after 6-10 weeks of treatment, which totally took 16.5 to 19.3

weeks for the flower induction and initiation. The results were not satisfactory since only 25% to 50% of plants flowered.

The possibility of the low percentage of flowering plants might result from the fluctuation of temperature since the plants were taken out of the growth chamber for about two hours per week for observation and management. Thus the plants were kept in the growth chamber during treatment except for necessary management in the subsequent experiment to avoid interference from temperature fluctuation.

2.) Experiment 2: Effects of day/night temperature and day length on vegetative growth in *Fragaria hayatae*

The results were presented in Table 13. At the end of the 6-week treatment, the plants grown outdoor formed most leaves (9.3), followed by 15/10℃ 14 hour day length (8.6), 15/10℃ 10 hour day length (7.4), and 15/5℃ 10 hour day length (6.9), respectively. The plants were transferred to 20/15℃ long day (14 hour day length) after treatment, and after 10 weeks of growth, the number of leaves was 11.8 to 15.1 and had no significant difference. The control plants had the largest leaf area (10.60 $cm^2$), following by 15/10℃ short day (9.03 $cm^2$), 15/10℃ long day (7.42 $cm^2$), and 15/5℃ short day (7.08 $cm^2$). After growing under warm temperature and long day for 10 weeks, the largest leaf area was 15/5℃ short day (11.19 $cm^2$), following by 15/10℃ long day (9.30 $cm^2$), 15/10℃ short day (8.53 $cm^2$), and control (8.33 $cm^2$). At the end of the 6-week treatment, control plants had the longest petiole length (5.21 cm), subsequently were 15/10℃ long day (2.26 cm), 15/10℃ short day (2.23 cm), and 15/5℃ short day (2.11 cm). After 10 weeks of growth under 20/15℃ long day, the rank of petiole length from long to short was control (3.94 cm), 15/5℃ short day (3.16 cm), 15/10℃ long day (2.84 cm), and 15/10℃ short day (2.70 cm). At the $6^{th}$ week of treatment the controlled plants produced 5 runners whereas the other plants produced 0.1 to 0.7

runners. After 10 weeks of growth in warm temperature and long day, the number of runners produced on the 16th week for the four groups ranged from 1.0 to 1.8 and was not of statistical difference. The growth rate of new leaf for the plants treated for 6 weeks under cool temperature was about the same as the control group (Fig. 19A). The growth rate of runner was reduced for the first three weeks of transferring from cool temperature to warm temperature condition, and the growth was restored two to three weeks later (Fig 19B).

Following to the above mention, vegetative growth of the plants was suppressed during treatment under cool temperature (15/10℃ and 15/5℃) when compared to the control plants, which were grown outdoor, and this result was similar to experiment 1. There was no significant difference between the control plants and the treated plants after 10 weeks of growth under 20/15℃ long day in number of leaves and petioles. This suggested that the restrain effects of temperature and day length on the treated plants for leaf and petiole formation was released. Leaf area and petiole length of 15/5 ℃ short day was the smallest at the end of the treatment but became the greatest after 10 weeks of growth in warm temperature and long day, this suggested that the growth was restrained at low temperature and short day but was greatly enhanced when the environment was optimal for growth.

No plants flowered in the control group grown outdoor and 8.3% of the treated plants flowered regardless of the temperature and day length (Table 14). Among the three treatments, plants grown under 15/10℃ long day bloomed most number of flowers per plant (10 flowers) and was the earliest to flower (7 weeks after treatment), followed by 15/5℃ short day (6 flowers; 9 weeks after treatment) and 15/10℃ short day (4 flowers; 10 weeks after treatment). Though the rate of plants flowering was low in the treated plants, the treatments were relatively successful since the control plants

did not flower.

The temperature fluctuation from taking plants out of the growth chamber for about 2 hours per week seemed not to be the cause for low percentage of flowering since the flowering percentage was not raised in experiment 2. We have not mastered the optimal conditions for flowering in Taiwan strawberry yet we do know that treatment of temperature lower than 15℃ for 6 to 10 weeks would be inductive for a few plants to flower.

## 6. Interspecific Hybridization between *Fragaria* ×*ananassa* and *F. hayatae*

We thought some characteristics of the Taiwan strawberry such as cold hardiness, aphid tolerance, mite tolerance, and distinct fragrant should be useful in commercial strawberry cultivation. Therefore, we tried to perform interspecific hybridization between *F.* ×*ananassa* 'Taoyuan No. 3' and *F. hayatae*, hoping to obtain some hybrids that possessed good characteristics of *F. hayatae* yet retained the merits of *F.* × *ananassa* 'Taoyuan No. 3'.

The receptacles of the emasculated *F.* ×*ananassa* 'Taoyuan No. 3' and *F. hayatae* did not swell and were dried out, which verified that emasculation was successful (data not shown). The results of the reciprocal cross between *F.* ×*ananassa* 'Taoyuan No. 3' and *F. hayatae* were presented in Table 15. When the cultivated strawberry was used as female plant and the Taiwan strawberry used as pollen donor, 41% of the crosses set fruit. When the Taiwan strawberry was used as female plant and the cultivated strawberry used as pollen donor, 73% of the crosses set fruit. The average seed per fruit was 10 as the cultivated strawberry was used as female and 13 when the Taiwan strawberry was used as female. 34% of seeds from *F.* ×*ananassa* 'Taoyuan No. 3' × *F. hayatae* germinated while none of the seeds from the reciprocal cross had germinated.

Marta et al. (2004) mentioned that development of embryo and endosperm in some hybrid achenes was poor, and in some achenes, the embryo and endosperm was not observed in serial microtome sections. In the study of Bors and Sullivan (2005b), brown shriveled embryos were prevalently observed in normal sized achenes indicating that embryos might be aborted at a stage after the outer seed coat had reached maximum size. Thus the low germination rate in the reciprocal cross between *F.* ×*ananassa* 'Taoyuan No. 3' and *F. hayatae* might be due to abnormal development of embryos and endosperms. But further studies such as microtome section should be conducted before the conjecture could be confirmed. The low germination rate in this study might be improved by *in vitro* culture as in the work of Miller et al. (1992) and Bors and Sullivan (2005b).

Out of the 44 germinated hybrids, only 21 seedlings survived 7 months after sown. Some of the seedlings died before the second true leaf formed, some seedlings were reddish (Fig. 20 C), and some displayed rosette growth (Fig. 20 D). The growth was very slow in the aberrant seedlings (Fig. 20 C and D) when comparing with those that were normal (Fig. 20 A and B). Similar phenomenon was observed in the study of Marta et al. (2004) and Evans (1974), and these were the evidence of post-zygotic barrier of cross hybridization.

Seven out of 21 survived seedlings flowered about five months after they were planted (Table 15). Some of the fruits from the hybrid seedlings were irregular (misshapen fruit) with only a few parts swelled (Fig. 21). Although the chromosome number was not examined yet, the misshapen fruit might be the evidence of successful hybridization instead of selfing since the growth of the strawberry receptacle relied on the stimulation of auxin produced from the fertilized achenes (Nitsch, 1950). The unferitilized achenes probably resulted from abnormal pollen or inviable carpel (Carew

et al., 2003), since the open pollinated fruits of *F.* ×*ananassa* ‘Taoyuan No. 3’ and *F. vesca* cultivated near by were usually normal. The natural pentaploids discovered by Bringhurst and Senanayake (1966) were usually infertile, only a single seed was found on one female flower, and the stainable pollen ranged from 0.6% to 3.3% whereas stainable pollen was 80% to 98.9% in their progenitor diploids and octoploids. The chromosome number of the hybrid seedlings awaits examination before we can be sure if the hybridization was successful. Pollen stainability also awaits examination to inspect whether the occurrence of misshapen fruits resulted from abnormal pollens of the hybrid seedlings.

# Conclusion

From both the morphological and molecular aspect, *F. hayatae* was distinct from *F. nilgerrensis* thus the Taiwan strawberry should be an independent species rather than a subspecies of *F. nilgerrensis*. The white-fruited strawberry was distinguished from *F. hayatae* by having different colored petiole, runner, petal, and fruit while it possessed similar petiole, petal and fruit color as *F. nilgerrensis*. However, the white-fruited strawberry was very closely related to *F. hayatae* from the evidence of similarity index = 0.94, whereas the similarity index between white-fruited strawberry and *F. nilgerrensis* was only 0.43. Therefore, we concluded that the white-fruited strawberry should be a mutant of *F. hayatae*, and it was not *F. nilgerrensis* from China.

Although the flowering seasons were not close, 44 crosses were managed between *F. hayatae* and *F.* ×*ananassa* 'Taoyuan No. 3'. The combination of *F.* ×*ananassa* 'Taoyuan No. 3' × *F. hayatae* gained 21 survived hybrid seedlings. The fruits of the seven flowered hybrid seedlings were usually misshaped, probably resulting from partial pollination, abortion of pollen grain, and might be the evidence of gaining pentaploids from successful hybridization.

According to the growth behavior in its native habitat, *F. hayatae* should be a short day plant, and a temperature and photoperiod study was performed. This study was conducted to maintain the plants in Taipei, and to get the flowers in time for hybridization. Temperature lower than 15℃ with 6-10 weeks of treatment was inductive for flower initiation in *F. hayatae* regardless of long day (14 hour day length) or short day (10 hour day length).

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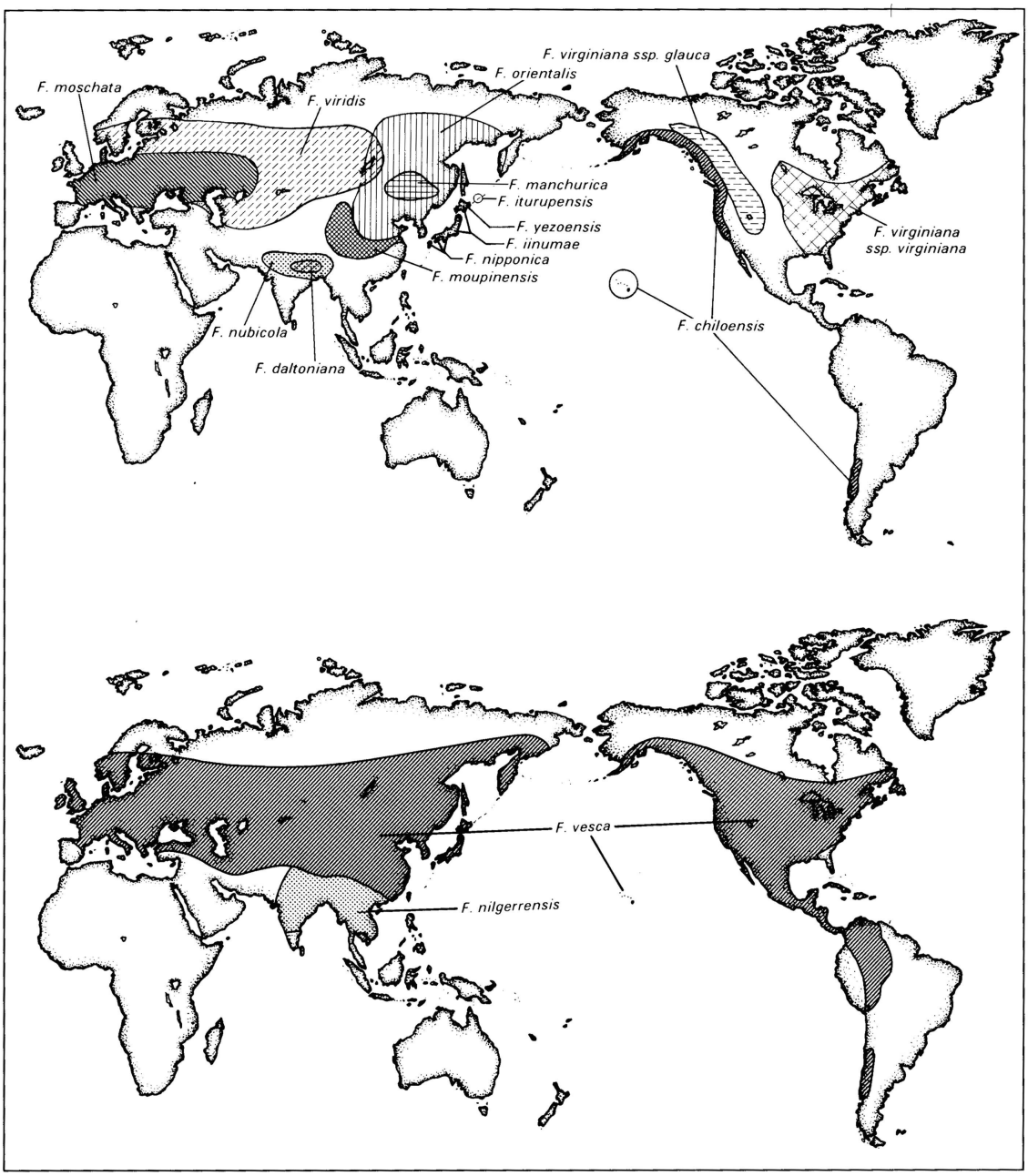


Fig 1. World distribution of *Fragaria* species.

圖 1. 草莓屬植物的世界分布情形

(Hancock and Luby, 1993)

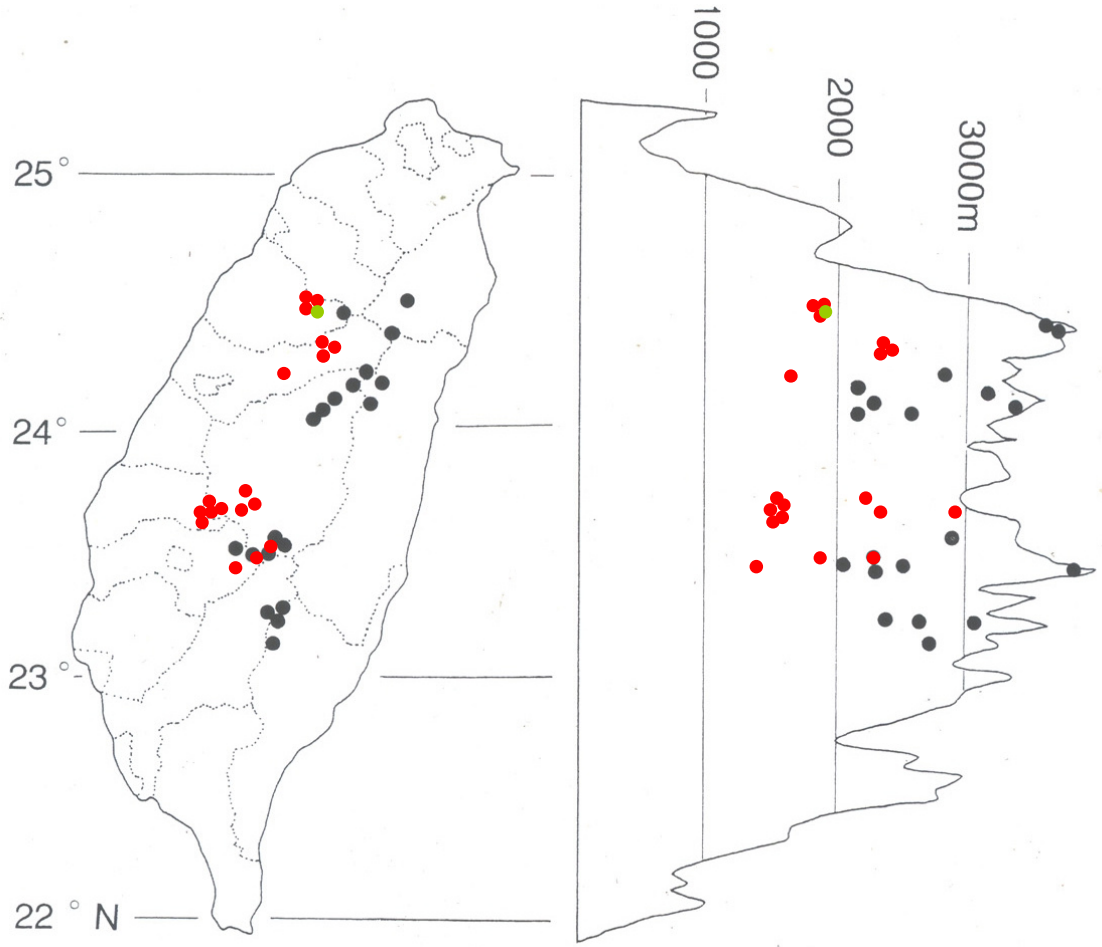


Fig. 2. Distribution of *Fragaria hayatae* in Taiwan based on Naruhashi et al. (1999) and the author's field collection (2006-2008).

圖 2. 台灣草莓於台灣之採集地分佈圖

(Modified from Naruhashi et al., 1999)

- Collection sites in Naruhashi et al., 1999
- Collection sites of Lee and Chen, 2006-2008

Fig. 3. Native habitat of *Fragaria hayatae*.

圖 3. 台灣草莓的原生地狀況

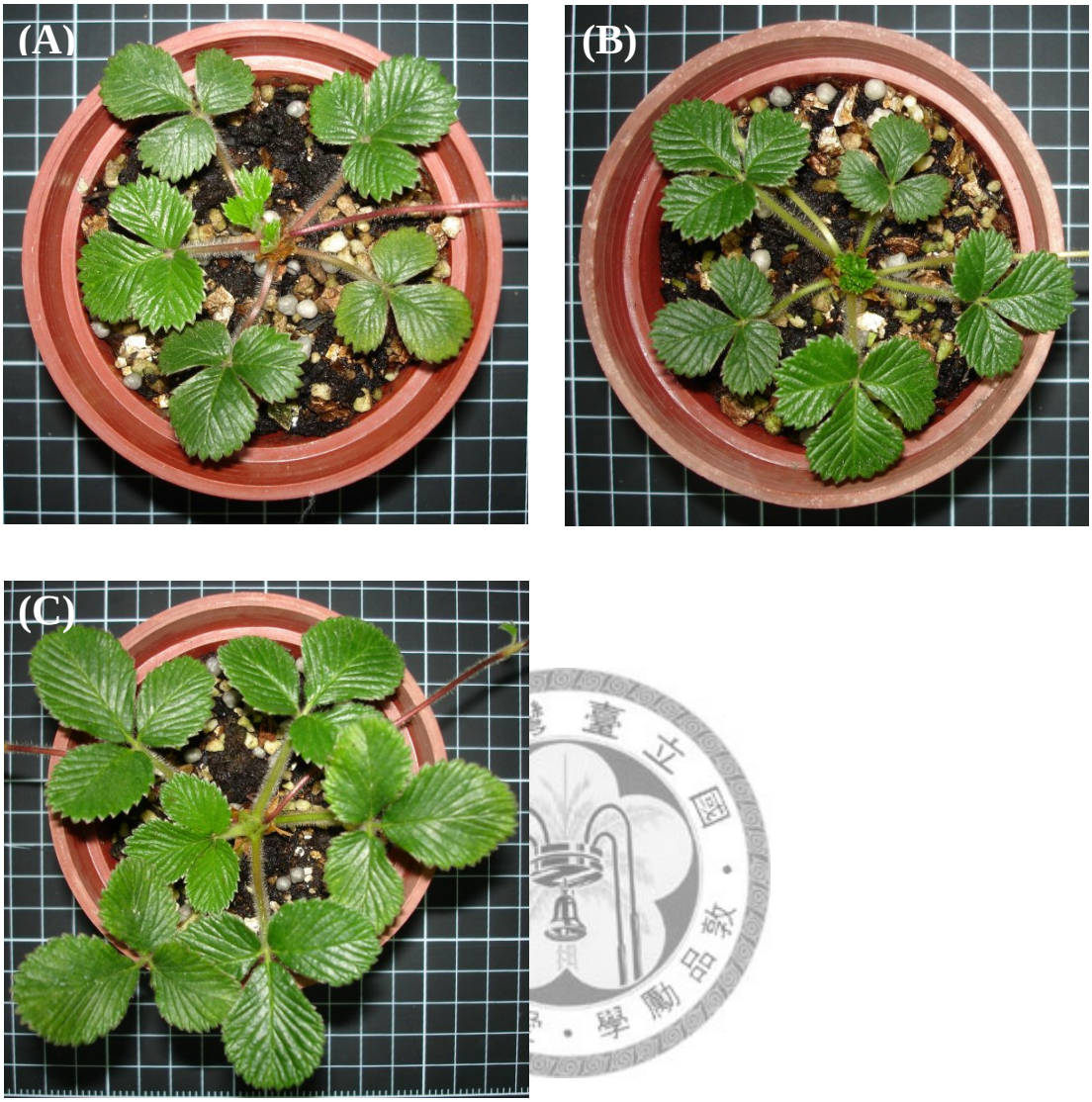


Fig. 4. Plants of *Fragaria hayatae* (A), white-fruited strawberry (B), and *F. nilgerrensis* (C). Each grid on the background is 1 $cm^2$.

圖 4. 台灣草莓(A)、白果草莓(B)以及黃毛草莓(C)之植株。背景每方格面積為 1 平方公分

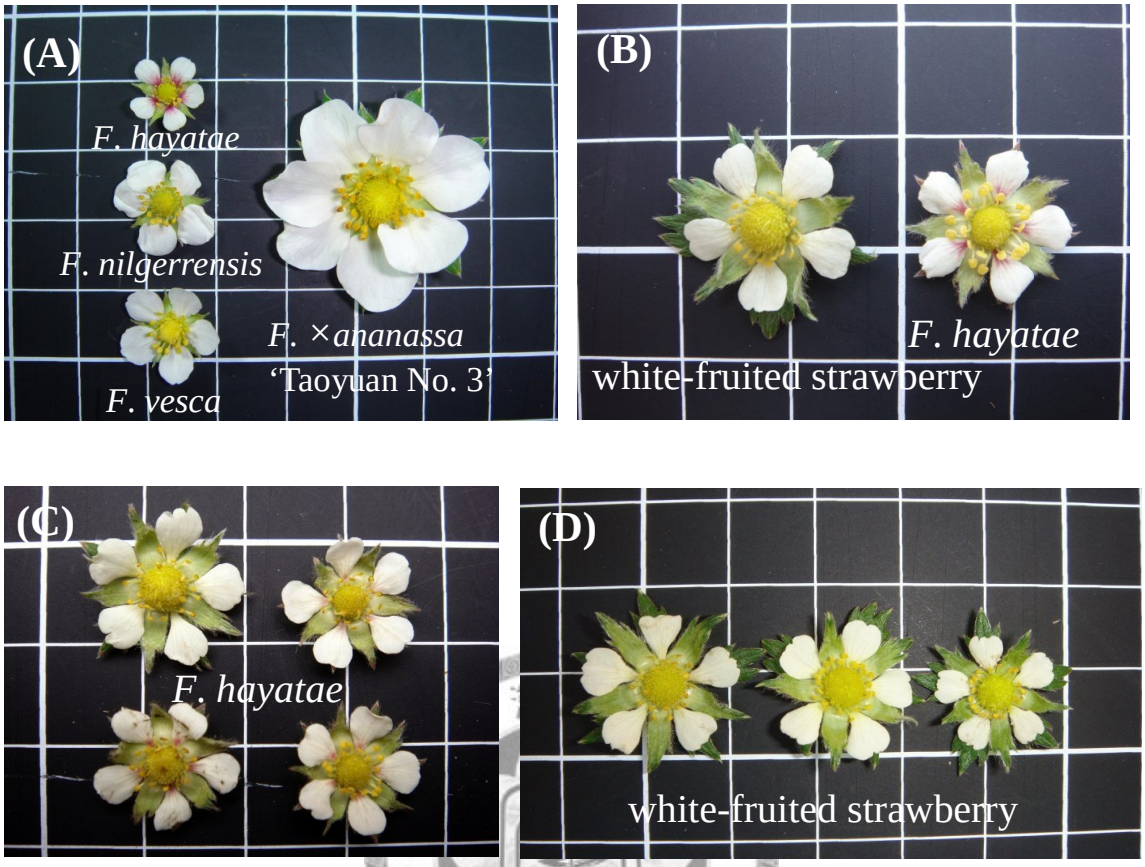


Fig. 5. Flowers of the *Fragaria* species. (A) from top to bottom on the left: *F. hayatae*, *F. nilgerrensis*, *F. vesca*, and on the right: *F. ×ananassa* 'Taoyuan No. 3'. (B) from left to right is white-fruited strawberry and *F. hayatae*. (C) is *F. hayatae* and (D) is white-fruited strawberry. Each grid on the background is 1 $cm^2$.

圖 5. 台灣草莓(A 左上、B 右及 C)、白果草莓(B 左、D)、黃毛草莓(A 左中)、森林草莓(A 左下)與栽培種草莓'桃園三號'(A 右)之花形態。背景每方格面積為 1 平方公分

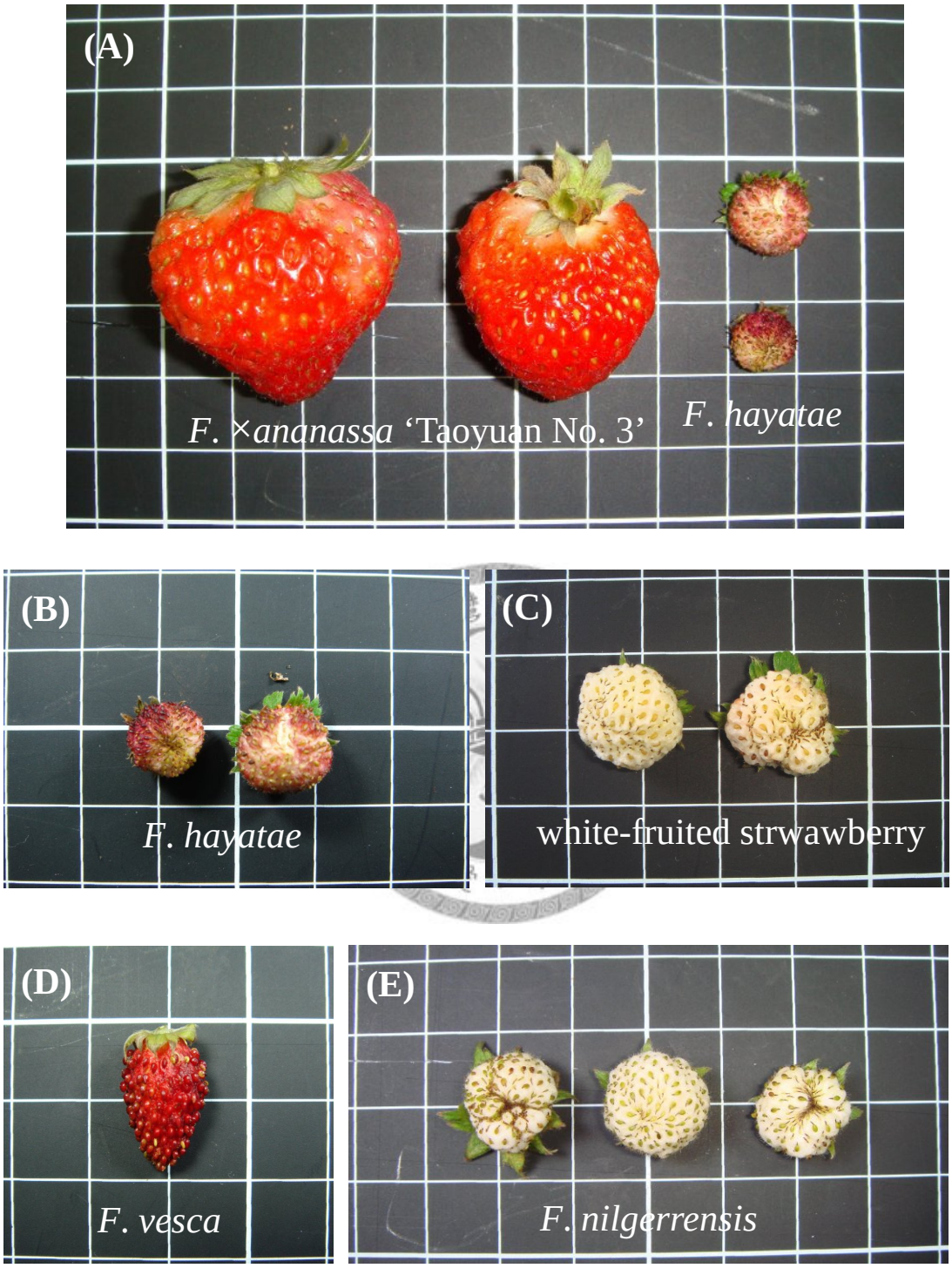


Fig. 6. Fruit of the *Fragaria* species. *Fragaria hayatae* (A right and B), white-fruited strawberry (C), *F. vesca* (D), *F. nilgerrensis* (E) and *F. ×ananassa* ‘Taoyuan No. 3’ (A left). Each grid on the background is 1 $cm^2$.

圖 6. 台灣草莓(A 右及 B)、白果草莓(C)、森林草莓(D)、黃毛草莓(E)以及栽培種草莓‘桃園三號’(A 左)之果實形態。背景每方格面積為 1 平方公分

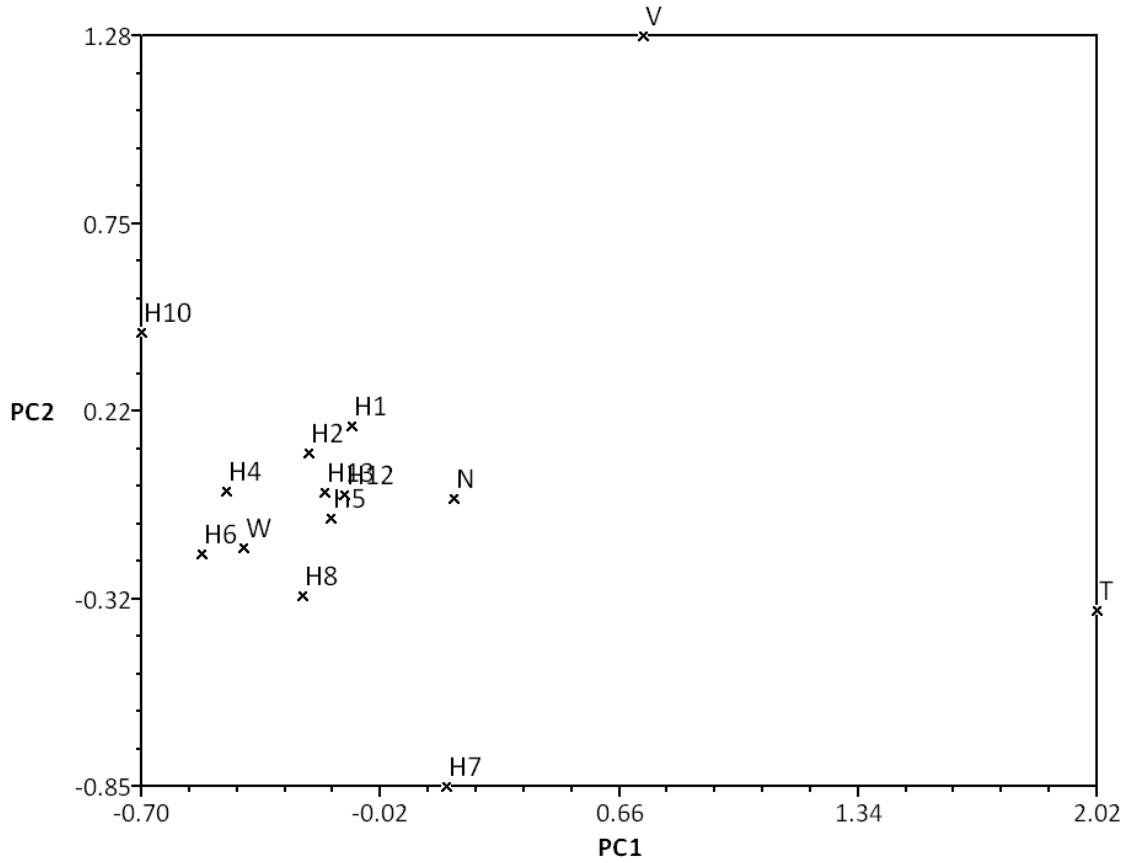


Fig. 7. Principal component analysis 2D scatter plot based on the correlation of 19 vegetative morphological characteristics in *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca* and *F.* ×*ananassa* 'Taoyuan No. 3'.

圖 7. 台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓'桃園三號'以 19 個營養器官形態性狀經主成分分析之平面圖

H1-2, H4-8, H10, H12-13: Accessions of *F. hayatae*, W: white-fruited strawberry, N: *F. nilgerrensis*, V: *F. vesca*, T: *F.* ×*ananassa* 'Taoyuan No. 3'

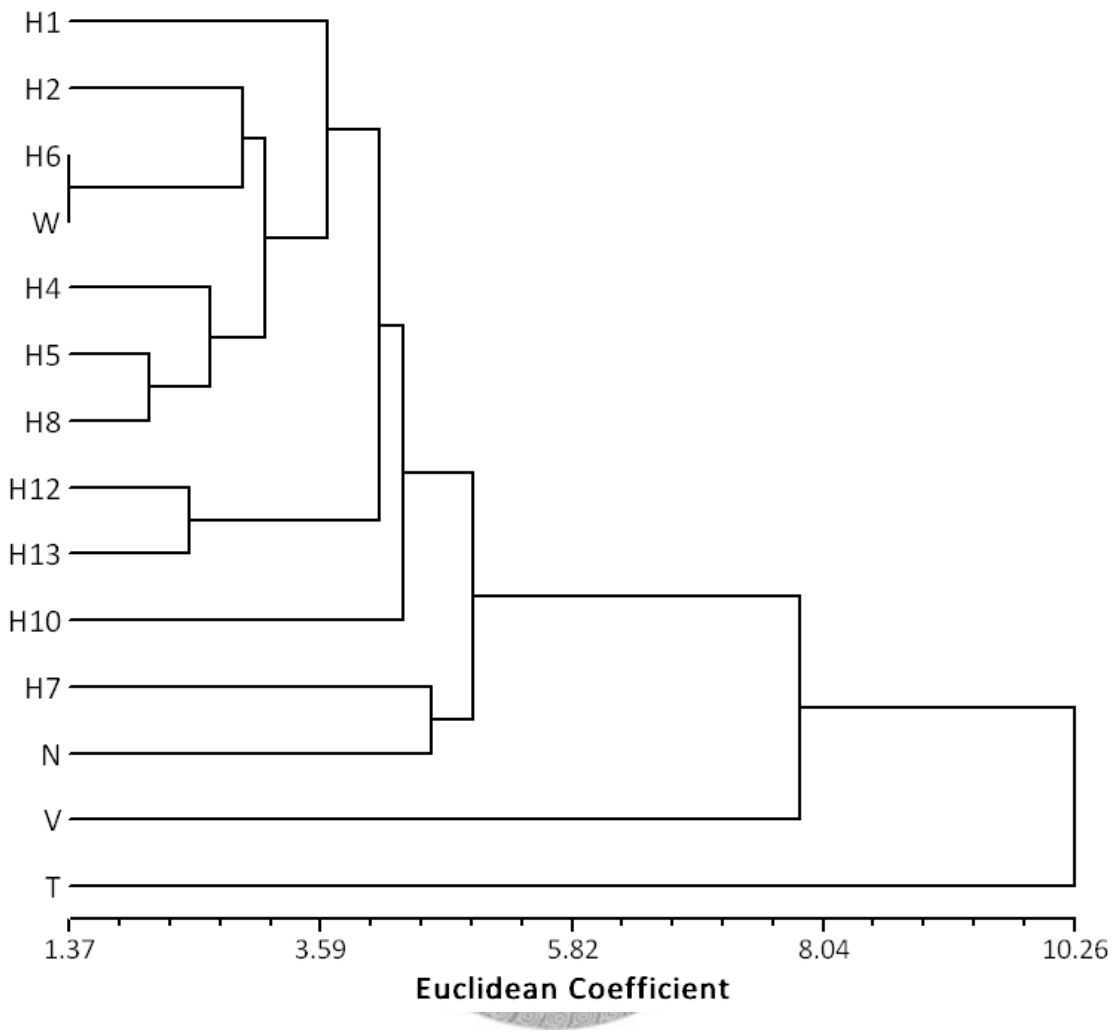


Fig. 8. Dendrogram of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca* and *F.* ×*ananassa* 'Taoyuan No. 3' clustering by UPGMA method based on Euclidean coefficient correlation derived from 19 vegetative morphological characteristics.

圖 8. 台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓'桃園三號'以 19 個營養器官形態性狀經群集分析之聚類樹狀圖

H1-2, H4-8, H10, H12-13: Accessions of *F. hayatae*, W: white-fruited strawberry, N: *F. nilgerrensis*, V: *F. vesca* , T: *F.* ×*ananassa* 'Taoyuan No. 3'

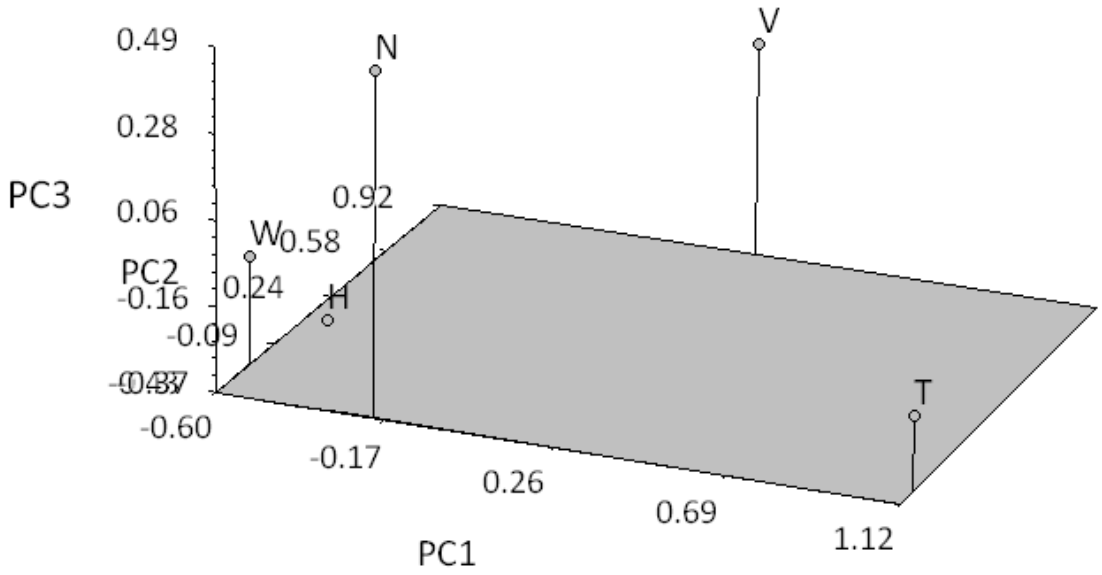


Fig. 9. Principal component analysis 3D scatter plot based on the correlation of 19 vegetative and 22 reproductive morphological characteristics in *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca* and *F.* ×*ananassa* 'Taoyuan No. 3'.

圖 9. 台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓'桃園三號'以 19 個營養器官及 22 個生殖器官形態性狀經主成分分析之立體圖

H: Combination of *F. hayatae* accessions, W: White-fruited strawberry, N: *F. nilgerrensis*, V: *F. vesca*, T: *F.* ×*ananassa* 'Taoyuan No. 3'

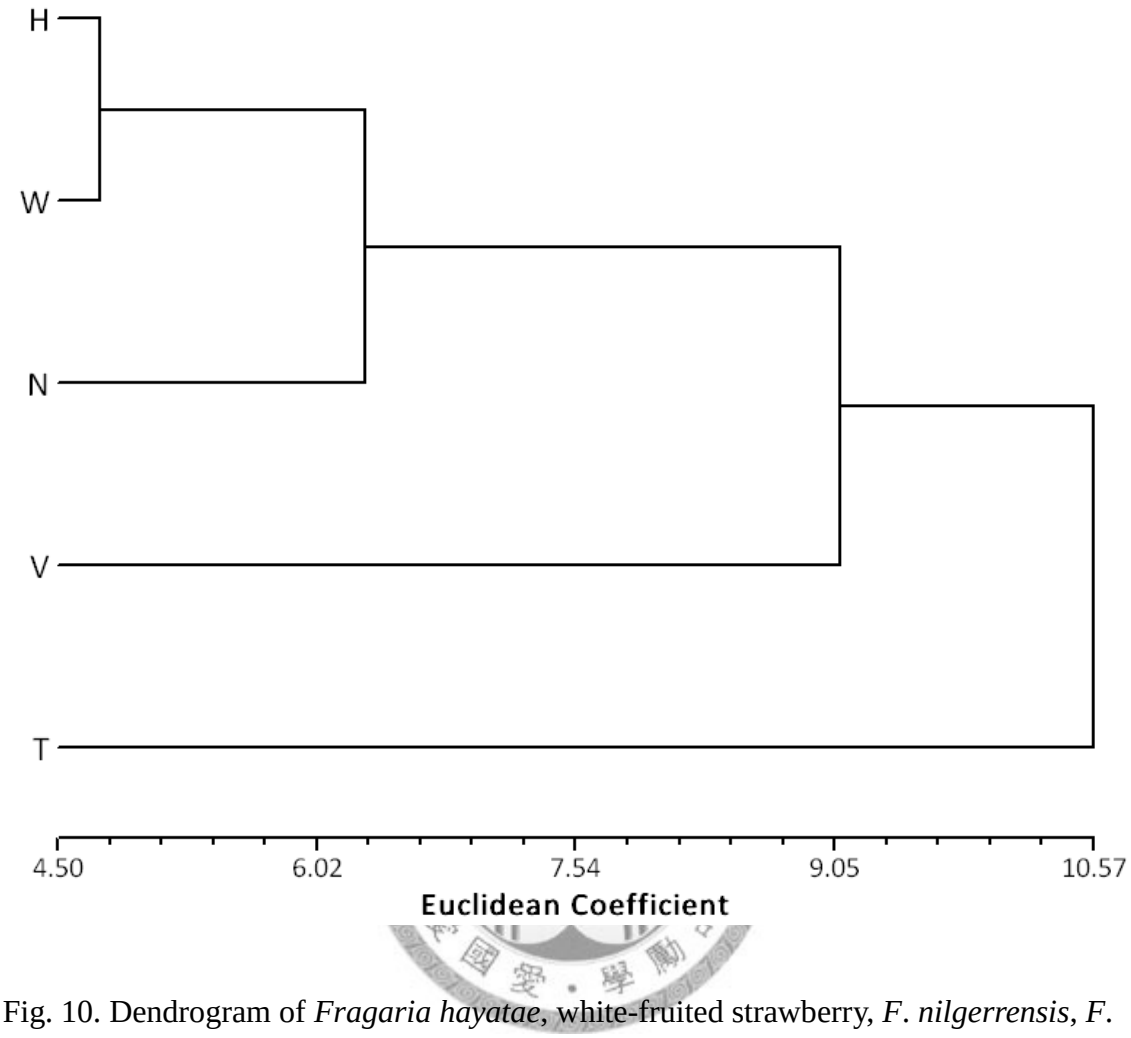


Fig. 10. Dendrogram of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca* and *F.* ×*ananassa* 'Taoyuan No. 3' clustering by UPGMA method based on Euclidean coefficient correlation derived from 19 vegetative and 22 reproductive morphological characteristics.

圖 10. 台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓'桃園三號'以 19 個營養器官及 22 個生殖器官形態性狀經群集分析之聚類樹狀圖

H: Combination of *F. hayatae* accessions, W: White-fruited strawberry, N: *F. nilgerrensis*, V: *F. vesca*, T: *F.* ×*ananassa* 'Taoyuan No. 3'

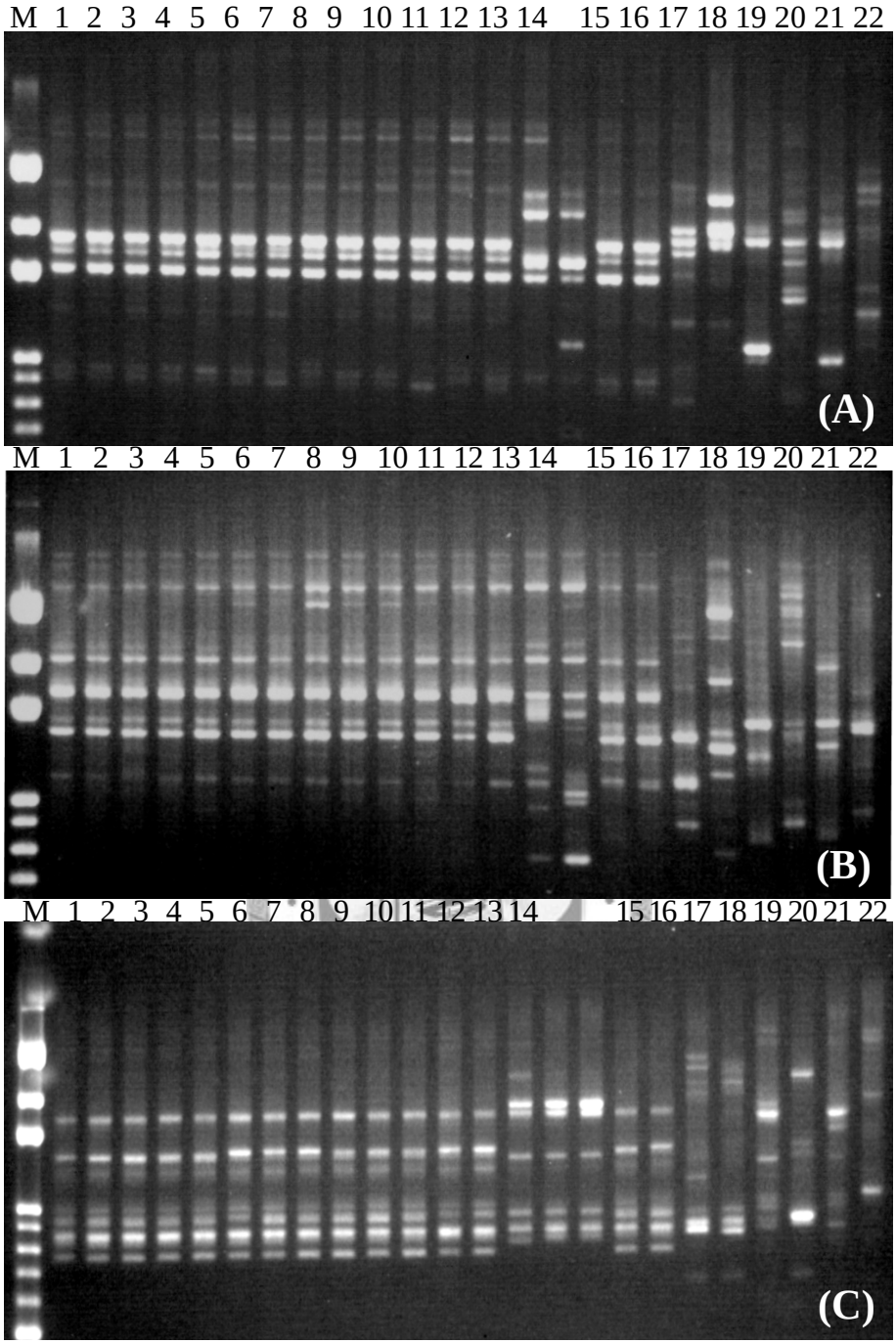


Fig. 11. Polymorphism among *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis* and other *Fragaria* species with primer UBC-4 (A), UBC-16 (B) and UBC-34 (C).

圖 11. 台灣草莓與其他草莓屬植物之 RAPD UBC-4 (A)、UBC-16 (B)及 UBC-34 引子多形性分析圖譜

1-11, 13, 15-16: Accessions of *F. hayatae*; 12: white-fruited strawberry; 14: *F. nilgerrensis*; 17: *F. vesca*; 18: *F. mandschurica*; 19: *F. pentaphylla*; 20: *F. viridis*; 21: *F.* × *ananassa* 'Taoyuan No. 3'; 22: *Potentilla matsurae*; M: DNA markers from top to bottom 3kb, 2kb, 1.5kb, 1kb, 900bp, 800bp, 700bp, 600bp, 500bp

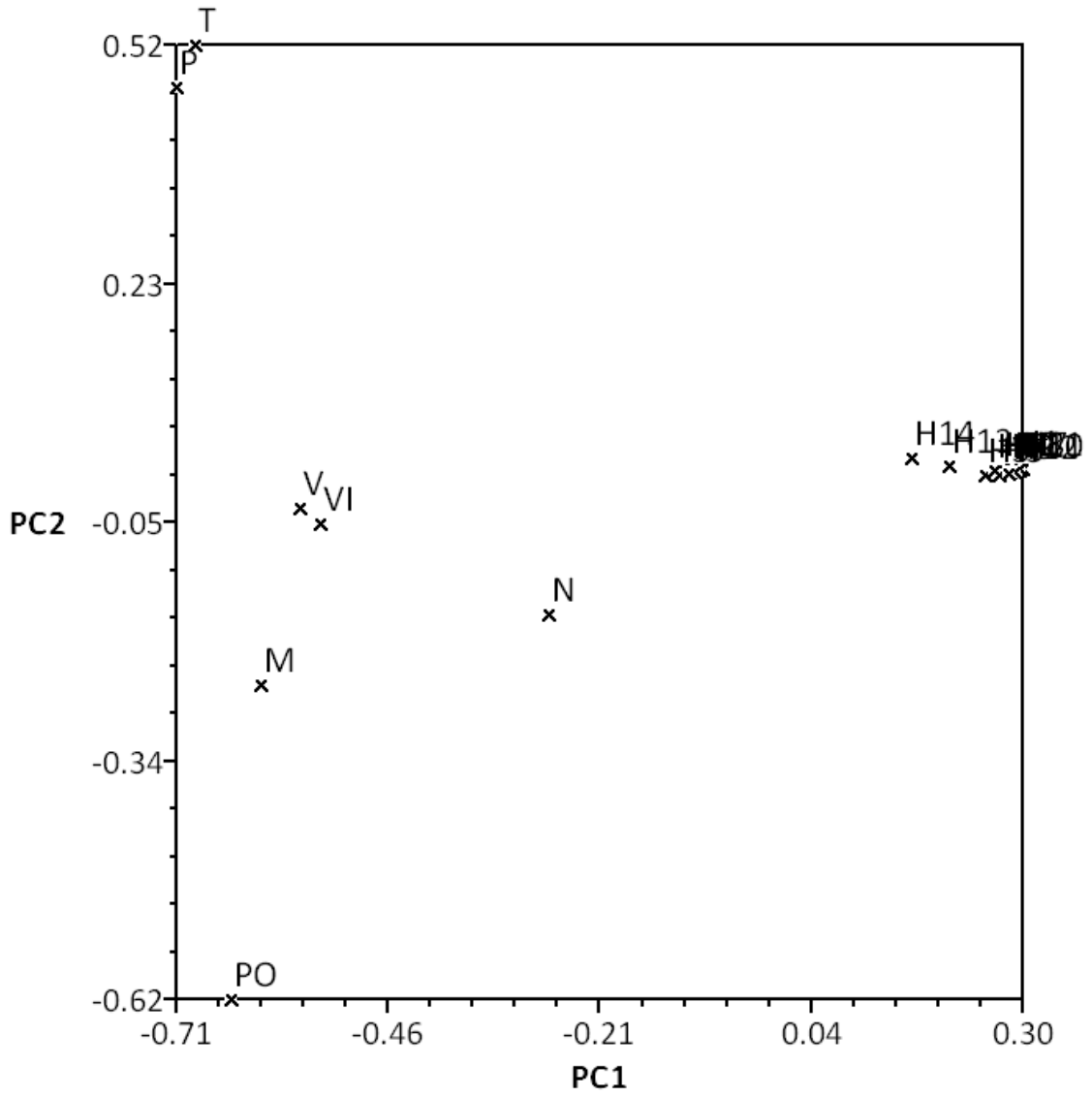


Fig. 12. Principal component analysis 2D scatter plot based on the correlation of 409 bands from 35 primers in *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, *F.* ×*ananassa* 'Taoyuan No. 3' and *Potentilla matsumurae*.

圖 12. 台灣草莓、白果草莓、黃毛草莓、森林草莓、栽培種草莓'桃園三號'及高山翻白草以 409 個條帶性狀經主成分分析之平面圖

H1-H14: Accessions of *F. hayatae*, W: White-fruited strawberry, N: *F. nilgerrensis*, V: *F. vesca*, M: *F. mandschurica*, P: *F. pentaphylla*, VI: *F. viridis*, T: *F.* ×*ananassa* 'Taoyuan No. 3', PO: *Potentilla matsumurae*

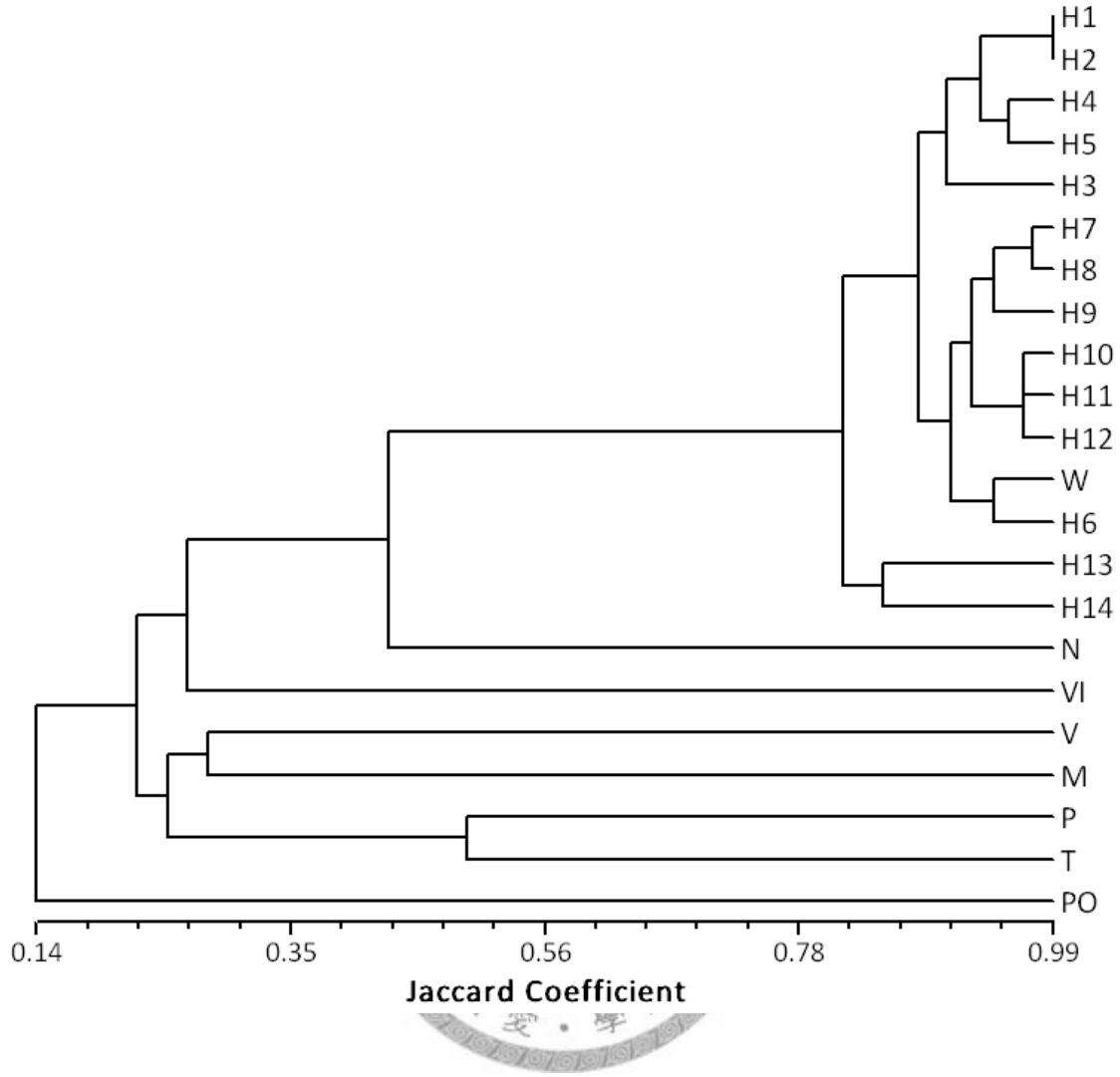


Fig. 13. Dendrogram of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, *F.* ×*ananassa* 'Taoyuan No. 3' and *Potentilla matsumurae* clustering by UPGMA method based on Jaccard coefficient derived from presence-absence data of 409 bands from 35 primers.

圖 13. 台灣草莓、白果草莓、黃毛草莓、森林草莓、栽培種草莓'桃園三號'及高山翻白草以 409 個條帶經 UPGMA 群集分析之聚類樹狀圖

H1-H14: Accessions of *F. hayatae*, W: White-fruited strawberry, N: *F. nilgerrensis*, V: *F. vesca*, M: *F. mandschurica*, P: *F. pentaphylla*, VI: *F. viridis*, T: *F.* ×*ananassa* 'Taoyuan No. 3', PO: *Potentilla matsumurae*

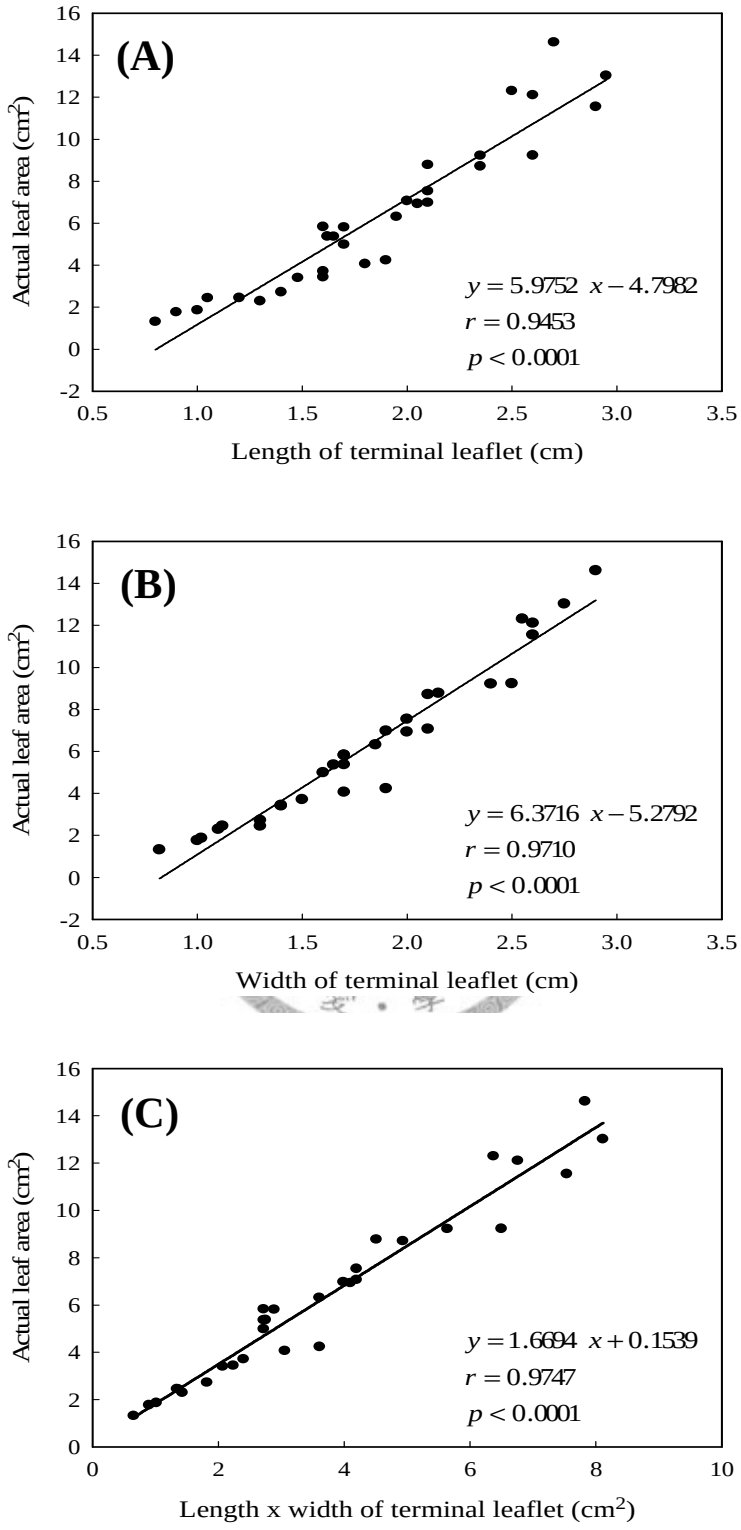


Fig. 14. Relationship between terminal leaflet length (A), terminal leaflet width (B), and terminal leaflet length × width (C) with actual leaf area in *Fragaria hayatae*.

圖 14. 台灣草莓中間小葉長(A)、中間小葉寬(B)及中間小葉長×寬(C)與葉面積之關係

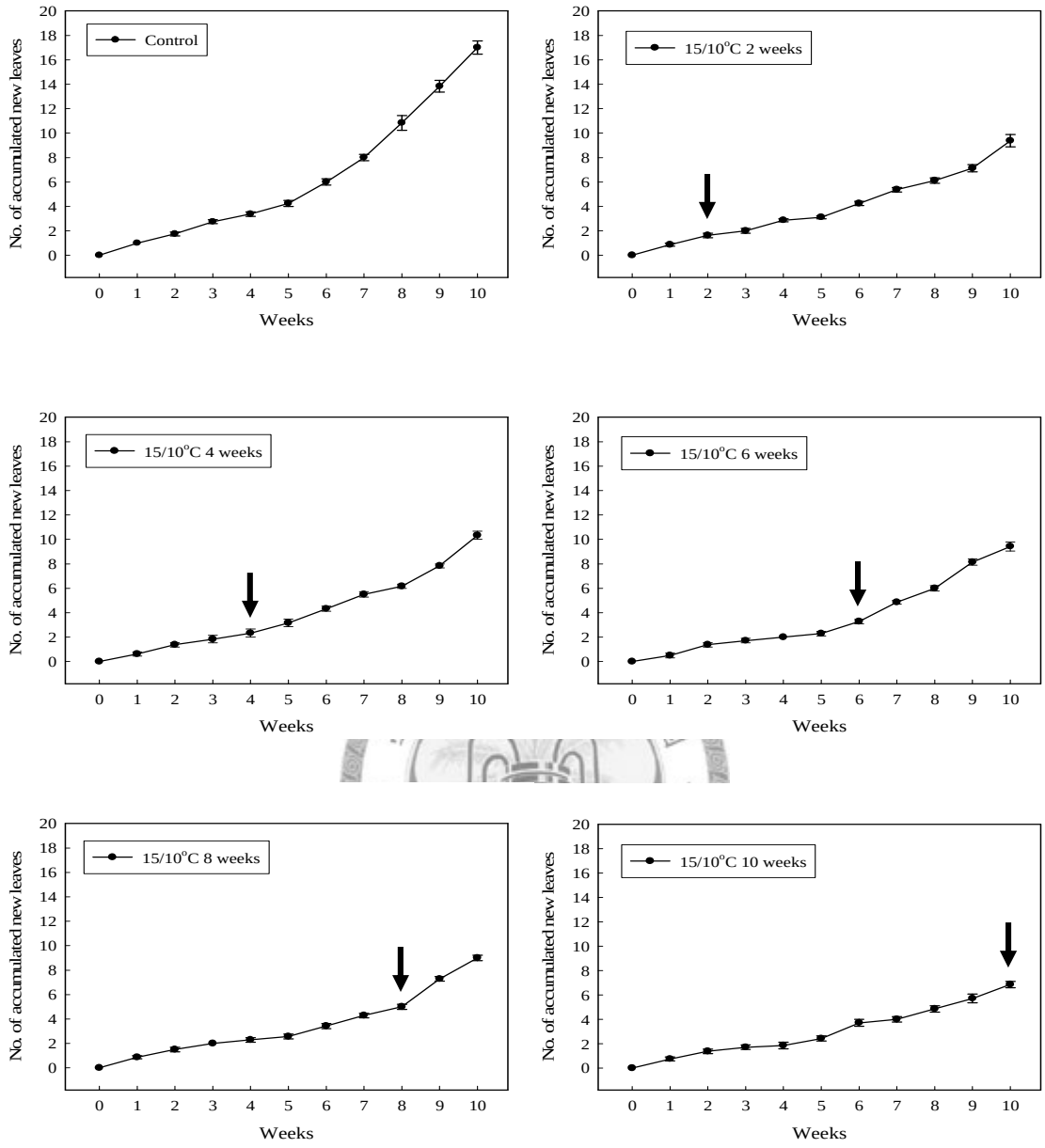


Fig. 15. Effects of temperature and its duration on new leaf formation in *Fragaria hayatae*. Values are means ± S.E. of 8 plants. The arrowheads indicated the ending of treatment and the beginning of 20/15℃ and natural day length.

圖 15. 溫度與處理時間對台灣草莓之新生葉片累積數的影響

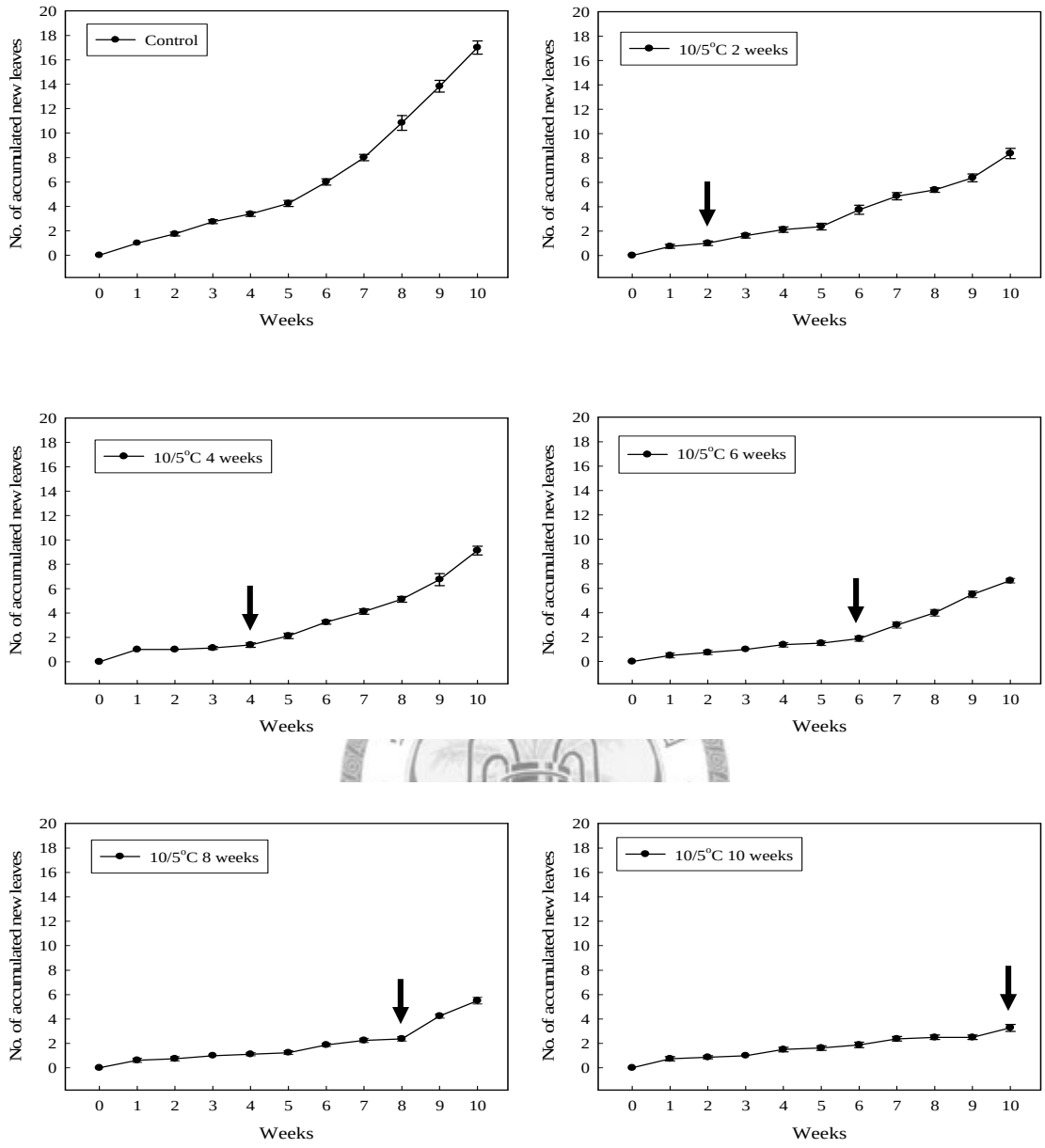


Fig. 16. Effects of temperature and its duration on new leaf formation in *Fragaria hayatae*. Values are means ± S.E. of 8 plants. The arrowheads indicated the ending of treatment and the beginning of 20/15℃ and natural day length.

圖 16. 溫度與處理時間對台灣草莓之新生葉片累積數的影響

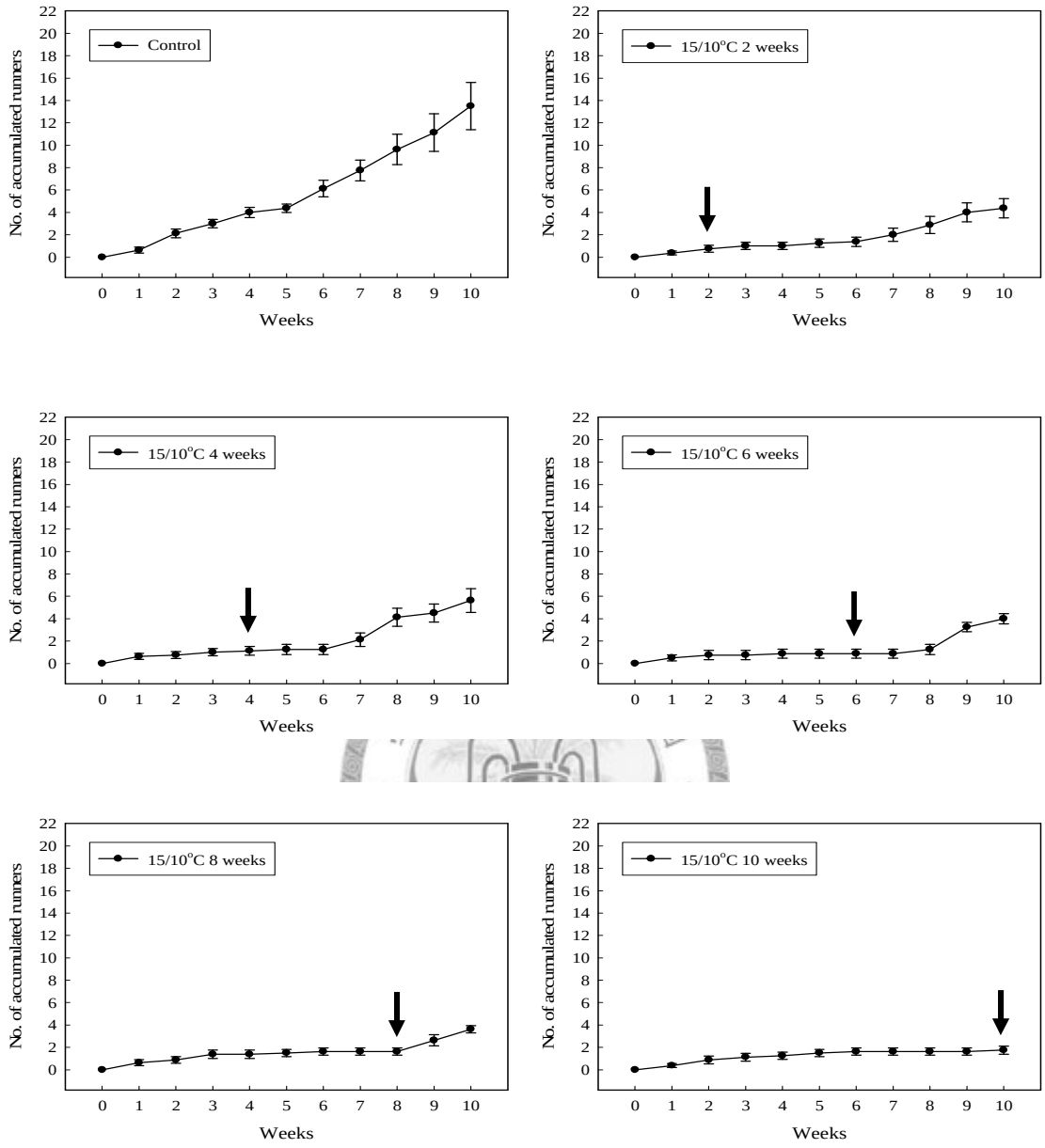


Fig. 17. Effects of temperature and its duration on runner formation in *Fragaria hayatae*. Values are means ± S.E. of 8 plants. The arrowheads indicated the ending of treatment and the beginning of 20/15℃ and natural day length.

圖 17. 溫度與處理時間對台灣草莓之走莖累積數的影響

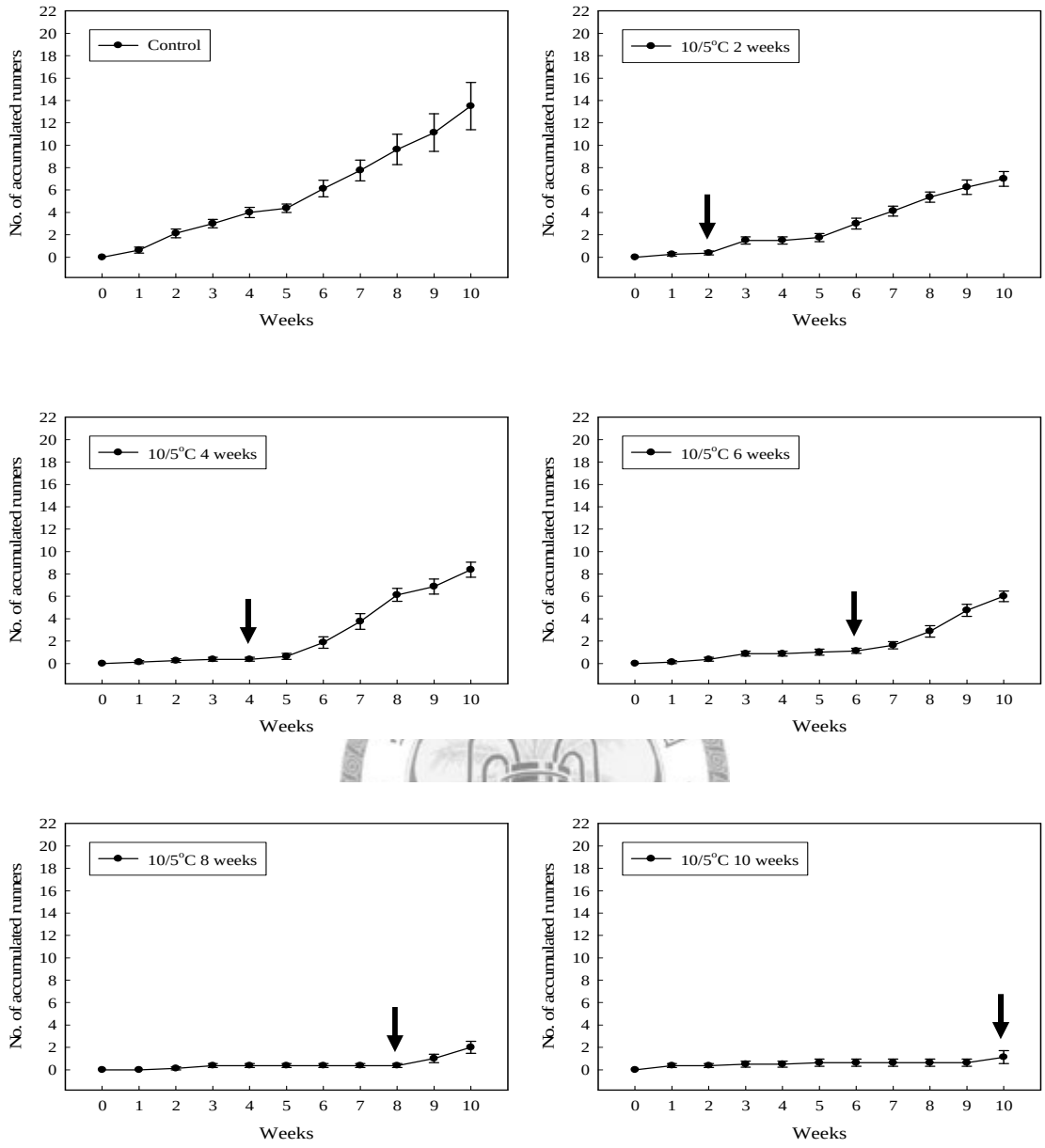


Fig. 18. Effects of temperature and its duration on runner formation in *Fragaria hayatae*. Values are means ± S.E. of 8 plants. The arrowheads indicated the ending of treatment and the beginning of 20/15℃ and natural day length.

圖 18. 溫度與處理時間對台灣草莓之走莖累積數的影響

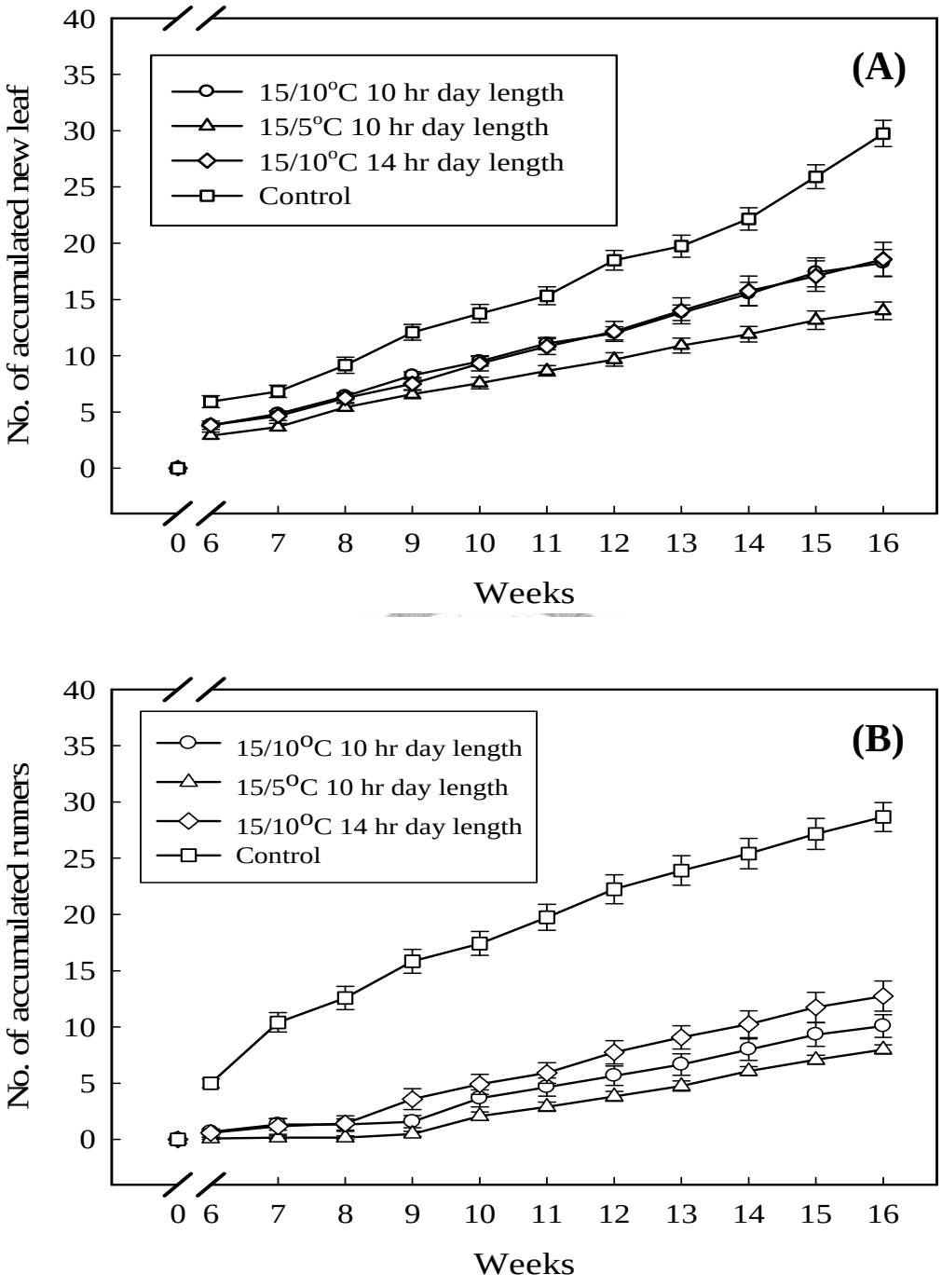


Fig. 19. Effects of day/night temperature and day length on leaves (A) and runners (B) in *Fragaria hayatae*. Values are means ± S.E. of 12 plants.

圖 19. 溫度與日長對台灣草莓新葉(A)及走莖(B)累積數的影響

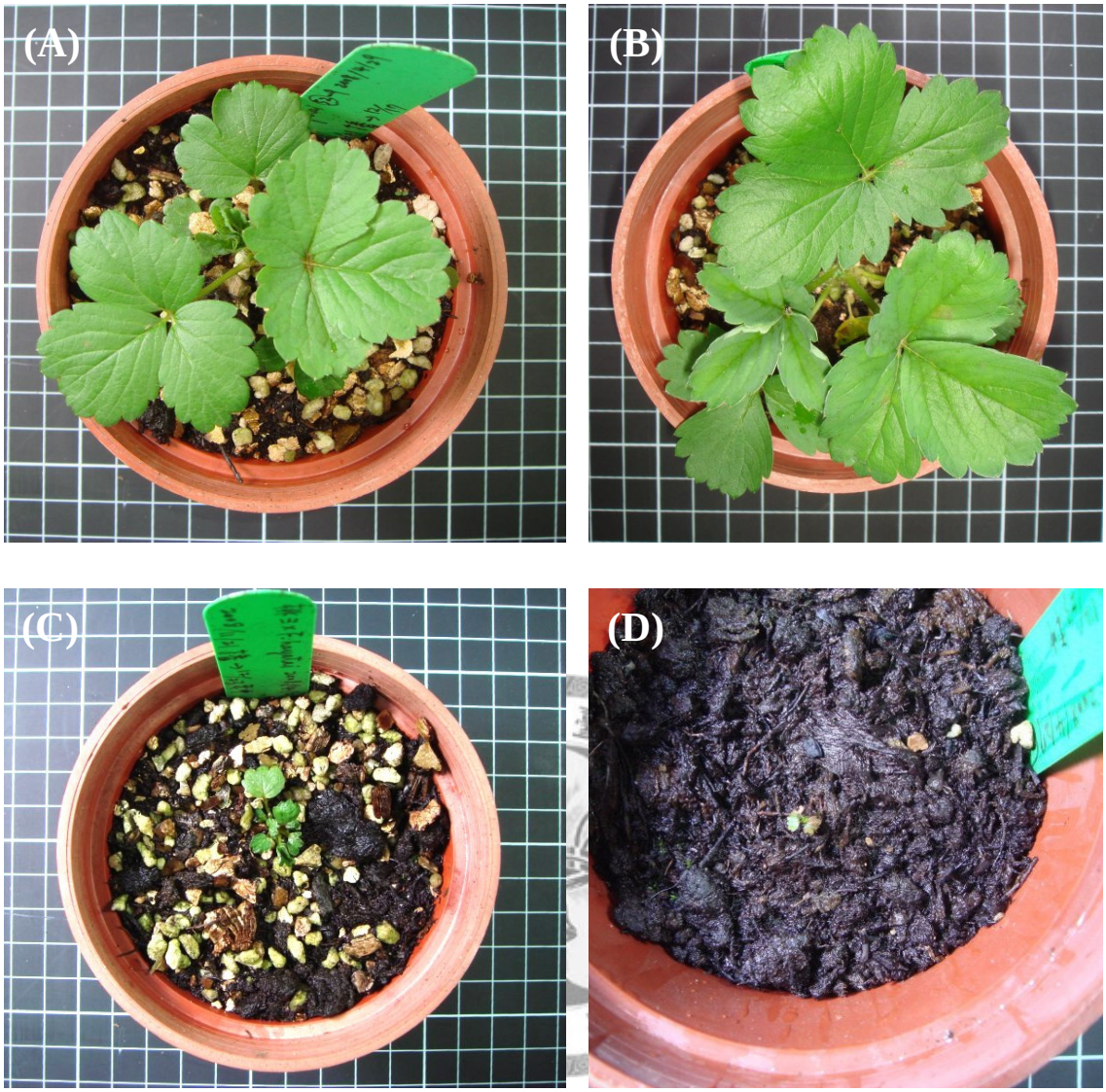


Fig. 20. Hybrid seedlings of *Fragaria* ×*ananassa* ‘Taoyuan No. 3’ × *F. hayatae*, (A) and (B) are normal seedlings while (C) and (D) are aberrant seedlings. Each grid on the background is 1 $cm^2$.

圖 20. 栽培種草莓‘桃園三號’與台灣草莓之雜交後代，(A)與(B)為正常植株，(C)與(D)為異常植株，背景每方格面積為 1 平方公分

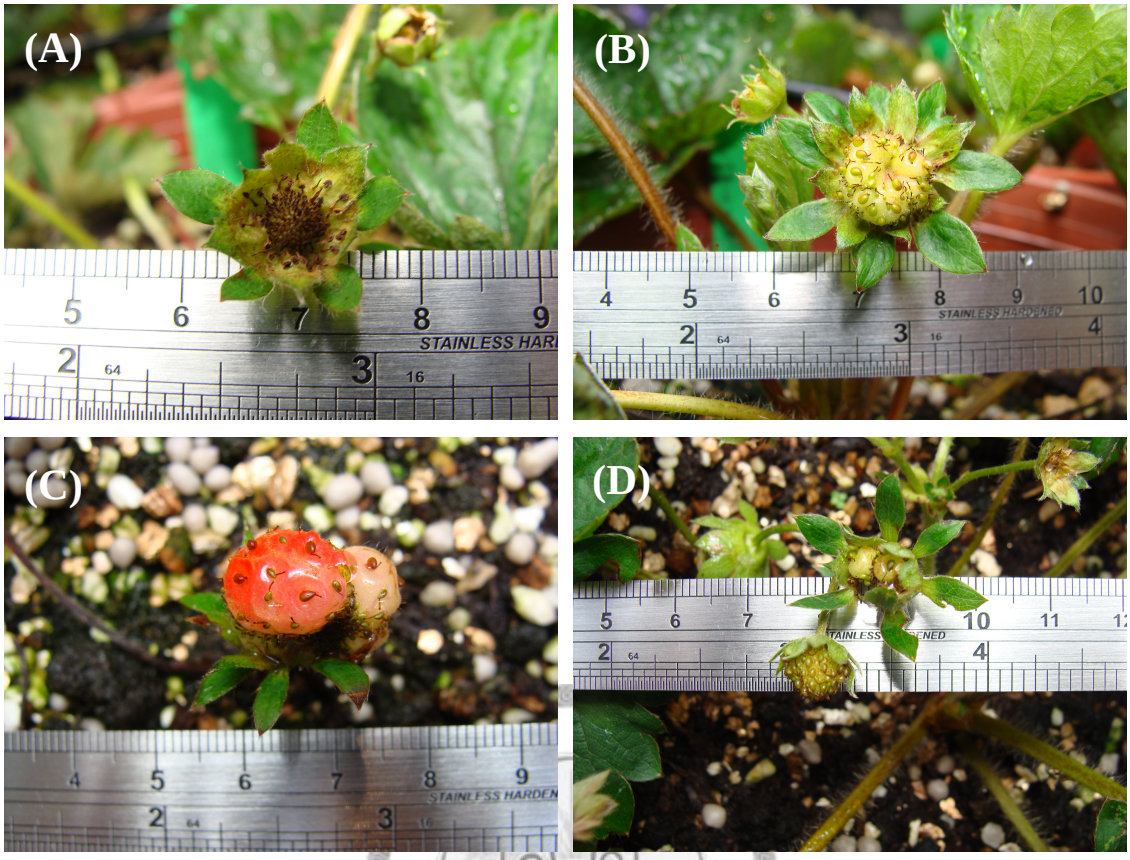


Fig. 21. Fruit of hybrid seedlings from *Fragaria* ×*ananassa* 'Taoyuan No. 3' × *F. hayatae*. Note the unfertilized flower in (A), and partial fertilized fruits in (B), (C), and (D).

圖 21. 栽培種草莓'桃園三號'與台灣草莓雜交後代之果實，(A)為未結實，(B)、(C)及(D)則為部份結實

Table 1. *Fragaria* ploidy level and geographic distributions.

表 1. 草莓屬植物染色體之倍數與地理分佈

| Species | Ploidy | Primary natural geographic range |
|---|---|---|
| *F. vesca* | | |
| subsp. *vesca* | 2x | Europe to Siberia as far as Lake Baikal |
| subsp. *bracteata* | 2x | North America, westward of Rocky Mountains |
| subsp. *americana* | 2x | North America, mainly east of Rocky Mountains |
| subsp. *californica* | 2x | California, Southern Oregon coast |
| *F. viridis* | 2x | Europe to Siberia as far as Lake Baikal |
| *F. yezoensis* | 2x | Japan |
| *F. nipponica* | 2x | Japan |
| *F. nubicola* | 2x | Eastern Himalayan region |
| *F. bucharica* | 2x | Western Himalayan region |
| *F. daltoniana* | 2x | Eastern Himalayan region |
| *F. nilgerrensis* | 2x | Central Asia into China |
| *F. hayatae* / *F. nilgerrensis* subsp. *hayatae* | 2x | Taiwan |
| *F. mandschurica* | 2x | Northeastern Asia |
| *F. pentaphylla* | 2x | Southwest China, Himalayan region |
| *F. gracilis* | 2x | Northwest China |
| *F. iinumae* | 2x | Southern and central Sahalin, Russia, Japan |
| *F. ×bifera* | 2x, 3x | Europe, hybrid between *F. vesca* and *F. viridis* |
| *F. corymbosa* | 4x | Northern China |
| *F. moupinensis* | 4x | Southwest China |
| *F. orientalis* | 4x | Northeastern Asia |
| *F. tibetica* | 4x | Eastern Himalayan region |
| *F. moschata* | 6x | Europe, eastward to Ural Mountains to Lake Baikal |
| *F. chiloensis* | 8x | Western N. and S. America, Hawaii |
| *F. virginiana* | 8x | North America |
| *F. iturupensis* | 8x | Iturup island (Kurile island) |
| *F. ×ananassa* | 8x | widely cultivated |
| *F. ×bringhurstii* | 5x, 6x, 9x | California coast, hybrid between *F. vesca* and *F. chiloensis* |

Literature cited from: Folta and Davis, 2006; Ohashi, 1993.

Table 2. Location of *Fragaria hayatae* and white-fruited strawberry used in this study.
表 2. 本試驗搜集之台灣草莓及白果草莓族群分佈位置

| Accession number | Collection site | Administrative region | Altitude (m) | GPS Position |
|---|---|---|---|---|
| H1 | Tianchih | Taoyuan Township, Kaoshiung County | 2277.0 | N 23° 16’ 39.7” E 120° 54’ 53.8” |
| H2 | Yakou | Haiduan Township, Taitung County | 2743.0 | N 23° 15’ 51.8” E 120° 57’ 42.3” |
| H3 | Yakou 2 | Haiduan Township, Taitung County | 2743.0 | N 23° 15’ 51.8” E 120° 57’ 42.3” |
| H4 | Siangyang | Haiduan Township, Taitung County | 2312.0 | N 23° 15’ 51.4” E 120° 57’ 41.0” |
| H5 | Kuanshanlingshan | Taoyuan Township, Kaoshiung County | 2850.0 | N 23° 15’ 53.0” E 120° 57’ 33.0” |
| H6 | Shiaoshueshan | Dongshih Township, Taichung County | 2586.7 | N 24° 16’ 49.2” E 121° 01’ 33.4” |
| H7 | Hohuanshan 1 | Ren-ai Township, Nantou County | 2771.6 | N 24° 07’ 4.5” E 121° 14’ 13.8” |
| H8 | Hohuanshan 2 | Ren-ai Township, Nantou County | 2813.0 | N 24° 07’ 6.1” E 121° 15’ 3.9” |
| H9 | Meifung Farm | Ren-ai Township, Nantou County | 2157.9 | N 24° 05’13.3” E 121° 10’24.4” |
| H10 | Yushan 1 | Alishan Township, Chiayi County | 3096.0 | N 23° 27’ 50.0” E 120° 55’ 50.0” |
| H11 | Yushan 2 | Alishan Township, Chiayi County | 3402.0 | N 23° 28’ 21.0” E 120° 52’ 22.0” |
| H12 | Alishan 1 | Alishan Township, Chiayi County | 2263.5 | N 23° 30’ 56.1” E 120° 48’ 49.0” |
| H13 | Alishan 2 | Alishan Township, Chiayi County | 2272.0 | N 23° 30’ 49.9” E 120° 48’ 36.9” |
| H14 | Alishan 3 | Alishan Township, Chiayi County | 2274.9 | N 23° 31’ 36.7” E 120° 48’ 57.9” |
| W | Shiaoshueshan | Dongshih Township, Taichung County | 2592.9 | N 24° 16’ 4.7” E 121° 01’ 32.2” |

Table 3. Descriptions of vegetative morphological characteristics used in this study.
表 3. 本試驗所調查之營養形態性狀及調查標準

| Characteristics[z] | Descriptions |
|---|---|
| Terminal leaflet length (cm) | |
| Terminal leaflet Width (cm) | |
| Terminal leaflet length to the widest point (cm) | From base of the terminal leaflet to the widest width along the mid vein |
| Terminal leaflet Length to the first tooth (cm) | From base of the terminal leaflet to first tooth along the mid vein |
| Leaflet length/ width Ratio | Ratio of leaflet length: leaflet width |
| Serrate ratio | Ratio of leaflet length with serrate: leaflet length |
| Widest length ratio | Ratio of length to the widest point: leaflet length |
| Leaf area ($cm^2$) | Area of the trifoliate |
| Shape of terminal leaflet | 1 = broadly elliptic, 2 = obovate, 3 = orbicular |
| Shape of terminal leaflet base | 1 = acute, 2 = obtuse, 3 = rounded |
| Shape of terminal leaflet apex | 1 = obtuse, 2 = rounded, 3 = emarginated |
| Shape in leaflet cross section | 1 = concave, 2 = straight, 3 = convex |
| Shape of incisions of margin | 1 = serrate, 2 = crenate |
| Angle at base (°) | angle between mid vein and the base of terminal leaflet |
| Number of serrate | |
| Petiole length (cm) | |
| Longest petiole length (cm) | |
| Petiole diameter (cm) | |
| Petiolet length (cm) | |

[z]All Characteristics were measured on the last fully expanded leaf.

Table 4. Descriptions of reproductive morphological characteristics used in this study.
表 4. 本試驗所調查之生殖形態性狀及調查標準

| Characteristics | Descriptions |
|---|---|
| Petal length (mm) | |
| Petal width (mm) | |
| Petal length/ width ratio | |
| Corolla diameter (mm) | |
| Receptacle diameter (mm) | |
| Calyx diameter (mm) | |
| Size of calyx in relation to corolla | 1 = smaller, 2 = same size, 3 = larger |
| Number of petals | |
| Number of flowers per inflorescence | |
| Total number of flowers | |
| Arrangement of petals | 1 = free, 2 = touching, 3 = overlapping |
| Petal color | 1 = white, 2 = white with pink blush at base |
| Relative position of inflorescence to foliage | 1 = beneath, 2 = same level, 3 = above |
| Berry height (mm) | |
| Berry diameter (mm) | |
| Berry weight (g) | |
| Berry ratio | Ratio of berry height: berry diameter |
| Berry shape | 1 = reniform, 2 = oblate/obloid, 3 = round/globose, 4 = conical, 5 = bi-conical/rhomboid, 6 = ovate/ovoid, 7 = almost cylindrical/cylindrical, 8 = wedged, 9 = cordiform/cordate |
| Berry color | 1 = without anthocyanin, 2 = with anthocyanin |
| Width of band without achenes | 1 = absent or very narrow, 2 = narrow, 3 = broad |
| Evenness of color | 1 = even or very slightly uneven, 2 = slightly uneven, 3 = strongly uneven |
| Position of calyx attachment | 1 = inserted, 2 = level with fruit, 3 = raised |

Table 5. Leaf morphological characteristics of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, and *F. ×ananassa*.

表 5. 台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓之葉性狀比較

| | Terminal leaflet | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Length (cm) | | Width (cm) | | Length/ width Ratio | | Serrate ratio | | Widest length ratio | | Leaf area ($cm^2$) | |
| ***F. hayatae*** | | | | | | | | | | | | |
| Tianchih | 2.90 d[z y] | A[x] | 2.56 c | A | 1.13 cd | BC | 0.63 ef | E | 0.39 d | C | 12.54 cde | A |
| Yakou | 2.48 ef | C | 2.20 def | B | 1.13 cd | BC | 0.68 cde | CDE | 0.42 bcd | ABC | 9.26 ef | BCD |
| Siangyang | 2.08 g | E | 1.95 ef | C | 1.07 d | CD | 0.66 de | DE | 0.41 cd | BC | 7.04 fg | E |
| Kuanshanlingshan | 2.56 de | C | 2.26 d | B | 1.13 cd | BC | 0.70 bcd | BCD | 0.43 bcd | ABC | 9.98 def | B |
| Shiaoshueshan | 2.12 g | E | 1.91 f | C | 1.11 d | CD | 0.70 bcd | BCD | 0.44 abcd | ABC | 7.18 fg | ED |
| Hohuanshan 1 | 2.85 d | AB | 2.67 c | A | 1.07 d | D | 0.79 a | A | 0.46 abc | AB | 12.96 cd | A |
| Hohuanshan 2 | 2.42 efg | CD | 2.24 de | B | 1.08 d | CD | 0.75 ab | AB | 0.46 abc | AB | 9.24 ef | BCD |
| Yushan | 1.71 h | F | 1.43 g | D | 1.20 b | A | 0.67 cde | CDE | 0.47 abc | AB | 4.27 g | F |
| Alishan 1 | 2.48 ef | C | 2.10 def | BC | 1.19 bc | AB | 0.72 bc | BC | 0.47 abc | AB | 8.93 f | BCDE |
| Alishan 2 | 2.58 de | BC | 2.13 def | BC | 1.21 b | A | 0.72 bc | BC | 0.48 ab | A | 9.43 ef | BC |
| **Average of *F. hayatae*** | 2.42 | | 2.14 | | 1.13 | | 0.70 | | 0.44 | | 9.08 | |
| **White-fruited strawberry** | | | | | | | | | | | | |
| Shiaoshueshan | 2.17 fg | DE | 1.93 f | C | 1.12 cd | CD | 0.72 bc | BC | 0.45 abc | AB | 7.50 fg | CDE |
| ***F. nilgerrensis*** | 3.39 c | | 2.63 c | | 1.29 a | | 0.72 bc | | 0.49 a | | 15.23 c | |
| ***F. vesca*** | 4.50 b | | 3.43 b | | 1.31 a | | 0.60 f | | 0.46 abc | | 26.41 b | |
| ***F. ×ananassa* 'Taoyuan No. 3'** | 5.81 a | | 5.30 a | | 1.11 d | | 0.74 ab | | 0.46 abc | | 52.26 a | |

[z]Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.

[y]Statistical results indicated by lowercase letters were analyzed from all *Fragaria* species

[x]Statistical results indicated by capital letters were restricted to *F. hayatae* and white-fruited strawberry.

Table 5. Leaf morphological characteristics of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, and *F. ×ananassa*. (continued 1)

表 5. 台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓之葉性狀比較(續 1)

| | Terminal leaflet | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shape (%) | | | Shape of base (%) | | | Shape of apex (%) | | | Angle at base (°) | | Shape in cross section | |
| | broadly elliptic | obovate | orbicular | acute | obtuse | rounded | obtuse | rounded | emarg-inated | | | | |
| ***F. hayatae*** | | | | | | | | | | | | | |
| Tianchih | 0 | 100 | 0 | 69 | 31 | 0 | 0 | 8 | 92 | 44.0 cd[z y] | B[x] | 1.3 cde | CD |
| Yakou | 0 | 100 | 0 | 40 | 60 | 0 | 0 | 60 | 40 | 47.1 bc | AB | 1.9 ab | AB |
| Siangyang | 0 | 79 | 21 | 87 | 13 | 0 | 0 | 53 | 47 | 43.9 cd | B | 1.0 e | D |
| Kuanshanlingshan | 0 | 88 | 12 | 67 | 33 | 0 | 0 | 33 | 67 | 45.5 bc | AB | 1.1 de | D |
| Shiaoshueshan | 0 | 82 | 18 | 42 | 58 | 0 | 5 | 36 | 59 | 45.6 bc | AB | 1.7 bc | BC |
| Hohuanshan 1 | 0 | 61 | 39 | 14 | 86 | 0 | 0 | 14 | 86 | 47.9 b | A | 1.0 e | D |
| Hohuanshan 2 | 0 | 88 | 12 | 87 | 13 | 0 | 7 | 40 | 53 | 46.1 bc | AB | 1.0 e | D |
| Yushan | 18 | 73 | 9 | 82 | 18 | 0 | 40 | 20 | 40 | 44.8 bc | AB | 1.5 bcd | BC |
| Alishan 1 | 8 | 92 | 0 | 92 | 8 | 0 | 0 | 14 | 86 | 46.4 bc | AB | 1.4 cde | CD |
| Alishan 2 | 0 | 91 | 9 | 100 | 0 | 0 | 0 | 8 | 92 | 45.1 bc | AB | 2.1 a | A |
| **Average of *F. hayatae*** | 2 | 84 | 15 | 62 | 38 | 0 | 4 | 31 | 65 | 45.6 | | 1.4 | |
| **White-fruited strawberry** | | | | | | | | | | | | | |
| Shiaoshueshan | 0 | 89 | 11 | 46 | 54 | 0 | 3 | 41 | 56 | 45.3 bc | AB | 1.4 cde | CD |
| ***F. nilgerrensis*** | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 60 | 40 | 45.7 bc | | 1.1 de | |
| ***F. vesca*** | 23 | 69 | 8 | 92 | 8 | 0 | 62 | 31 | 8 | 40.8 d | | 1.3 cde | |
| ***F. ×ananassa* 'Taoyuan No. 3'** | 0 | 60 | 40 | 10 | 80 | 10 | 30 | 40 | 30 | 54.4 a | | 1.1 de | |

[z]Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.

[y]Statistical results indicated by lowercase letters were analyzed from all *Fragaria* species.

[x]Statistical results indicated by capital letters were restricted to *F. hayatae* and white-fruited strawberry.

Table 5. Leaf morphological characteristics of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, and *F. ×ananassa*. (continued 2)

表 5. 台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓之葉性狀比較(續 2)

| | Terminal leaflet | | Petiole | | | | | | Petiolet Length | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number of serrate | | Length (cm) | | Longest length (cm) | | Diameter (mm) | | (cm) | |
| ***F. hayatai*** | | | | | | | | | | |
| Tianchih | 19.3 c | C | 6.05 d | B | 6.62 de | BC | 1.48 cd | BC | 0.42 bcd | AB |
| Yakou | 18.3 cd | CD | 5.59 de | BC | 5.79 ef | CD | 1.46 cde | BC | 0.38 d | B |
| Siangyang | 17.9 cd | CD | 5.17 de | BC | 5.61 ef | CD | 1.36 def | CDE | 0.30 e | C |
| Kuanshanlingshan | 18.7 cd | CD | 5.88 de | BC | 5.93 ef | CD | 1.53 bc | B | 0.41 cd | AB |
| Shiaoshueshan | 17.5 d | D | 3.86 f | D | 4.84 f | D | 1.26 f | E | 0.23 e | D |
| Hohuanshan 1 | 24.9 a | A | 7.28 c | A | 7.64 cd | AB | 1.67 b | A | 0.41 bcd | AB |
| Hohuanshan 2 | 21.7 b | B | 5.27 de | BC | 5.56 ef | CD | 1.50 cd | B | 0.39 cd | B |
| Yushan | 14.7 e | E | 3.90 f | D | 5.00 f | D | 1.11 g | F | 0.30 e | C |
| Alishan 1 | 21.3 b | B | 7.84 c | A | 8.55 c | A | 1.43 cde | BCD | 0.46 bc | A |
| Alishan 2 | 22.2 b | B | 7.43 c | A | 8.20 c | A | 1.51 cd | B | 0.42 bcd | AB |
| **Average of *F. hayatai*** | 19.7 | | 5.83 | | 6.37 | | 1.43 | | 0.37 | |
| **White-fruited strawberry** | | | 4.77 ef | CD | 5.67 ef | CD | 1.31 ef | DE | 0.26 e | CD |
| Shiaoshueshan | 18.8 cd | CD | | | | | | | | |
| ***F. nilgerrensis*** | 21.4 b | | 5.39 de | | 6.35 e | | 1.58 bc | | 0.41 cd | |
| ***F. vesca*** | 14.3 e | | 10.47 b | | 11.45 b | | 1.36 def | | 0.49 ab | |
| ***F. ×ananassa* 'Taoyuan No. 3'** | 17.6 cd | | 13.18 a | | 14.84 a | | 2.39 a | | 0.55 a | |

[z]Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.

[y]Statistical results indicated by lowercase letters were analyzed from all *Fragaria* species

[x]Statistical results indicated by capital letters were restricted to *F. hayatae* and white-fruited strawberry.

Table 6. Flower morphological characteristics of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, and *F.* ×*ananassa*.
表 6.　台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓之花性狀比較

| | Petal length (mm) | Petal width (mm) | Petal length/ width ratio | Corolla diameter (mm) | Receptacle diameter (mm) | Calyx diameter (mm) | Size of calyx in relation to corolla |
|---|---|---|---|---|---|---|---|
| ***F. hayatae*** | 5.43 c [z] | 4.54 c | 1.21 ab | 13.25 c | 3.81 c | 13.28 c | 1.7 b |
| **White-fruited strawberry** | 5.69 c | 4.50 c | 1.28 a | 14.12 c | 4.16 bc | 16.65 b | 2.7 a |
| ***F. nilgerrensis*** | 8.78 b | 7.08 b | 1.24 a | 19.65 b | 4.66 ab | 17.16 b | 1.6 b |
| ***F. vesca*** | 5.67 c | 4.99 c | 1.14 b | 13.80 c | 2.80 d | 11.61 c | 1.1 b |
| ***F.* ×*ananassa* 'Taoyuan No. 3'** | 10.05 a | 10.22 a | 0.98 c | 23.30 a | 5.04 a | 20.57 a | 1.1 b |

[z]Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 6. Flower morphological characteristics of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, and *F.* ×*ananassa*. (continued)

表 6. 台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓之花性狀比較(續)

| | Number of petals | Number of flowers per inflorescence | Total number of flowers | Arrangement of petals | Petal color (%) | | Relative position of inflorescence to foliage |
|---|---|---|---|---|---|---|---|
| | | | | | white | white with purplish-red blush | |
| ***F. hayatae*** | 5.1 ab[z] | 1.3 c | 3.6 b | 1.0[y] b | 18 | 82 | 2.2 b |
| **White-fruited strawberry** | 5.0 b | 2.5 b | 7.3 a | 1.0 b | 100 | 0 | 2.7 a |
| ***F. nilgerrensis*** | 5.6 a | 1.4 c | 6.8 a | 1.1 b | 100 | 0 | 3.0 a |
| ***F. vesca*** | 5.3 ab | 3.1 b | 6.5 a | 1.2 b | 100 | 0 | 1.8 c |
| ***F.* ×*ananassa* 'Taoyuan No. 3'** | 5.5 a | 4.9 a | 8.3 a | 2.1 a | 100 | 0 | 1.9 bc |

[z]Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.

[y]1 = free, 2 = touching, 3 = overlapping.

Table 7. Fruit morphological characteristics of *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca*, and *F.* ×*ananassa*.

表 7.　台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓之果性狀比較

| | Berry | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Shape (%) | | | | | | Color (%) | | Width of band without achenes | Evenness of color | Position of calyx attachment |
| | Height (mm) | Diameter (mm) | Weight (g) | Height/ diameter ratio | reni-form | obloid | globose | conical | rhom-boid | ovoid | white | red | | | |
| ***F. hayatae*** | 10.46 c[z] | 9.68 c | 0.29 c | 1.09 b | 0 | 73 | 27 | 0 | 0 | 0 | 0 | 100 | 1.0 c | 1.5 ab | 2.0 b |
| **White-fruited strawberry** | 8.42 c | 11.92 b | 0.36 c | 0.71 c | 14 | 71 | 14 | 0 | 0 | 0 | 100 | 0 | 1.0 c | 1.0 b | 2.0 b |
| ***F. nilgerrensis*** | 9.72 c | 12.57 b | 0.48 c | 0.78 c | 14 | 71 | 14 | 0 | 0 | 0 | 100 | 0 | 1.0 c | 1.0 b | 2.0 b |
| ***F. vesca*** | 20.10 b | 11.28 b | 0.89 b | 1.79 a | 0 | 0 | 9 | 0 | 18 | 73 | 0 | 100 | 2.6 a | 1.9 a | 2.2 a |
| ***F.* ×*ananassa* 'Taoyuan No. 3'** | 25.28 a | 22.25 a | 4.75 a | 1.15 b | 0 | 25 | 25 | 50 | 0 | 0 | 0 | 100 | 2.0 b | 1.8 a | 2.0 b |

[z]Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 8. Results of principal component analysis performed on 19 vegetative morphological characteristics among *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca* and *F. ×ananassa* 'Taoyuan No. 3'.

表 8. 以 19 個營養形態性狀對台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓'桃園三號'進行主成分分析之結果

| Characteristics | Principal component eigenvector | | | |
|---|---|---|---|---|
| | PC1 | PC2 | PC3 | PC4 |
| Terminal leaflet length | 0.98 | 0.14 | 0.00 | 0.02 |
| Terminal leaflet Width | 0.99 | -0.02 | -0.10 | 0.00 |
| Terminal leaflet length to the widest point | 0.97 | 0.15 | -0.11 | -0.04 |
| Terminal leaflet Length to the first tooth | 0.80 | 0.55 | -0.12 | -0.05 |
| Petiole length | 0.93 | 0.13 | 0.10 | -0.24 |
| Petiolet length | 0.79 | 0.13 | 0.28 | -0.42 |
| Longest petiole length | 0.94 | 0.14 | 0.11 | -0.15 |
| Petiole diameter | 0.88 | -0.42 | -0.04 | -0.10 |
| Angle at base | 0.60 | -0.65 | -0.07 | 0.11 |
| Shape of terminal leaflet | 0.41 | -0.81 | -0.27 | 0.07 |
| Shape of terminal leaflet base | 0.47 | -0.51 | -0.09 | 0.46 |
| Shape of terminal leaflet apex | -0.43 | -0.66 | 0.11 | -0.52 |
| Shape of incisions of margin | 0.00 | 0.00 | 0.00 | 0.00 |
| Number of serrate | -0.06 | -0.74 | 0.47 | -0.34 |
| Shape in leaflet cross section | -0.28 | 0.26 | 0.24 | -0.25 |
| Leaf area | 0.99 | 0.04 | -0.10 | 0.07 |
| Leaflet length/ width Ratio | 0.14 | 0.71 | 0.59 | 0.14 |
| Serrate ratio | 0.12 | -0.87 | 0.40 | 0.16 |
| Widest length ratio | 0.26 | -0.04 | 0.85 | 0.41 |
| Eigenvalue | 8.77 | 4.23 | 1.76 | 1.17 |
| Contribution (%) | 48.71 | 23.51 | 9.78 | 6.51 |
| Cumulative contribution (%) | 48.71 | 72.23 | 82.01 | 88.52 |

Table 9. Results of principal component analysis performed on 19 vegetative and 22 reproductive morphological characteristics among *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca* and *F.* ×*ananassa* 'Taoyuan No. 3'.

表 9. 以 19 個營養形態性狀及 22 個生殖形態性狀對台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓'桃園三號'進行主成分分析之結果

| Characteristics | Principal component eigenvector | | | |
|---|---|---|---|---|
| | PC1 | PC2 | PC3 | PC4 |
| Terminal leaflet length | 0.99 | 0.14 | 0.08 | 0.00 |
| Terminal leaflet Width | 0.99 | 0.02 | -0.10 | 0.01 |
| Terminal leaflet length to the widest point | 0.99 | 0.16 | 0.02 | 0.01 |
| Terminal leaflet Length to the first tooth | 0.79 | 0.56 | 0.24 | 0.05 |
| Petiole length | 0.94 | 0.31 | -0.14 | 0.05 |
| Petiolet length | 0.91 | 0.26 | 0.18 | -0.26 |
| Longest petiole length | 0.95 | 0.26 | -0.14 | 0.08 |
| Petiole diameter | 0.88 | -0.39 | -0.23 | -0.09 |
| Angle at base | 0.63 | -0.63 | -0.44 | -0.06 |
| Shape of terminal leaflet | 0.50 | -0.64 | -0.58 | -0.03 |
| Shape of terminal leaflet base | 0.39 | -0.91 | 0.14 | -0.02 |
| Shape of terminal leaflet apex | -0.62 | -0.71 | -0.29 | -0.16 |
| Shape of incisions of margin | 0.00 | 0.00 | 0.00 | 0.00 |
| Number of serrate | -0.36 | -0.86 | 0.14 | -0.33 |
| Shape in leaflet cross section | -0.64 | 0.57 | -0.50 | 0.05 |
| Leaf area | 0.99 | 0.02 | -0.12 | 0.03 |
| Leaflet length/ width Ratio | -0.01 | 0.40 | 0.91 | -0.10 |
| Serrate ratio | 0.07 | -0.96 | -0.29 | -0.04 |
| Widest length ratio | 0.30 | -0.30 | 0.90 | -0.11 |
| Petal length | 0.72 | -0.61 | 0.28 | 0.16 |
| Petal width | 0.86 | -0.49 | 0.08 | 0.12 |
| Corolla diameter | 0.76 | -0.61 | 0.21 | 0.11 |
| Receptacle diameter | 0.31 | -0.95 | -0.02 | 0.05 |
| Calyx diameter | 0.50 | -0.84 | -0.02 | -0.19 |
| Number of petals | 0.63 | -0.21 | 0.63 | 0.40 |
| Number of flowers per inflorescence | 0.88 | 0.02 | -0.24 | -0.41 |
| Total number of flowers | 0.61 | -0.31 | 0.34 | -0.64 |
| Relative position of inflorescence to foliage | -0.62 | -0.64 | 0.45 | -0.04 |
| Arrangement of petals | 0.94 | -0.26 | -0.20 | -0.03 |
| Size of calyx in relation to corolla | -0.76 | -0.36 | -0.17 | -0.51 |

Table 9. Results of principal component analysis performed on 19 vegetative and 22 reproductive morphological characteristics among *Fragaria hayatae*, white-fruited strawberry, *F. nilgerrensis*, *F. vesca* and *F.* ×*ananassa* 'Taoyuan No. 3'. (continued)

表 9. 以 19 個營養形態性狀及 22 個生殖形態性狀對台灣草莓、白果草莓、黃毛草莓、森林草莓及栽培種草莓'桃園三號'進行主成分分析之結果(續)

| Characters | Principal component eigenvector | | | |
|---|---|---|---|---|
| | PC1 | PC2 | PC3 | PC4 |
| Petal color | -0.43 | 0.10 | -0.62 | 0.65 |
| Petal length/ width ratio | -0.96 | -0.04 | 0.26 | -0.11 |
| Berry height | 0.95 | 0.27 | -0.13 | -0.03 |
| Berry diameter | 0.88 | -0.42 | -0.13 | -0.14 |
| Width of band without achenes | 0.70 | 0.69 | 0.09 | -0.15 |
| Berry weight | 0.93 | -0.26 | -0.25 | -0.06 |
| Berry color | 0.52 | 0.55 | -0.53 | 0.39 |
| Evenness of color | 0.63 | 0.67 | -0.33 | 0.22 |
| Berry ratio | 0.43 | 0.90 | 0.00 | 0.12 |
| Position of calyx attachment | 0.18 | 0.93 | 0.29 | -0.13 |
| Berry shape | 0.49 | 0.86 | 0.13 | -0.10 |
| Eigenvalue | 20.63 | 12.56 | 4.69 | 2.12 |
| Contribution (%) | 51.58 | 31.40 | 11.72 | 5.30 |
| Cumulative contribution (%) | 51.58 | 82.98 | 94.70 | 100.00 |

Table 10. Primers and their polymorphism in the RAPD analysis.

表 10. RAPD 分析使用之逢機引子及多形性條帶分佈資料

| Primer No. | Sequence (5’ to 3’) | No. of polymorphic bands | No. of monomorphic bands | No. of scorable bands |
|---|---|---|---|---|
| 2 | CCT GGG CTT G | 18 | 0 | 18 |
| 3 | CCT GGG CTT A | 14 | 0 | 14 |
| 4 | CCT GGG CTG G | 17 | 0 | 17 |
| 6 | CCT GGG CCT A | 2 | 1 | 3 |
| 13 | CCT GGG TGG A | 16 | 1 | 17 |
| 15 | CCT GGG TTT G | 13 | 0 | 13 |
| 16 | GGT GGC GGG A | 17 | 0 | 17 |
| 18 | GGG CCG TTT A | 11 | 0 | 11 |
| 23 | CCC GCC TTC C | 15 | 0 | 15 |
| 25 | ACA GGG CTC A | 17 | 1 | 18 |
| 29 | CCG GCC TTA C | 17 | 0 | 17 |
| 30 | CCG GCC TTA G | 15 | 0 | 15 |
| 33 | CCG GCT GGA A | 8 | 1 | 9 |
| 34 | CCG GCC CCA A | 14 | 0 | 14 |
| 41 | TTA ACC GGG G | 7 | 0 | 7 |
| 43 | AAA ACC GGG C | 15 | 0 | 15 |
| 44 | TTA CCC CGG C | 11 | 0 | 11 |
| 50 | TTC CCC GCG C | 11 | 0 | 11 |
| 61 | TTC CCC GAC C | 12 | 0 | 12 |
| 63 | TTC CCC GCC C | 15 | 0 | 15 |
| 64 | GAG GGC GGG A | 10 | 0 | 10 |
| 65 | AGG GGC GGG A | 10 | 0 | 10 |
| 66 | GAG GGC GTG A | 10 | 0 | 10 |
| 67 | GAG GGC GAG C | 1 | 1 | 2 |
| 70 | GGG CAC GCG A | 14 | 0 | 14 |
| 72 | GAG CAC GGG A | 16 | 0 | 16 |
| 77 | GAG CAC CAG G | 6 | 0 | 6 |
| 81 | GAG CAC GGG G | 8 | 0 | 8 |
| 82 | GGG CCC GAG G | 6 | 1 | 7 |
| 83 | GGG CTC GTG G | 13 | 0 | 13 |
| 84 | GGG CGC GAG T | 15 | 0 | 15 |
| 88 | CGG GGG ATG G | 7 | 0 | 7 |
| 91 | GGG TGG TTG C | 8 | 1 | 9 |
| 92 | CCT GGG CTT T | 4 | 1 | 5 |
| 96 | GGC GGC ATG G | 8 | 0 | 8 |

Table 11. Effects of temperature and its duration on petiole length, leaf area, leaf formation rate, and runner formation rate in *Fragaria hayatae*.

表 11. 溫度與日長對台灣草莓之葉片數、葉面積、葉柄長以及走莖數的影響

| | Petiole length (cm) | | Leaf area ($cm^2$) | | Leaf formation rate | | Runner formation rate | |
|---|---|---|---|---|---|---|---|---|
| | end of treatment | 17th week | end of treatment | 17th week | end of treatment | 17th week | end of treatment | 17th week |
| Temperature | | | | | | | | |
| 15/10℃ (12 hr/ 12 hr) | 3.98 a[z] | 7.29 b | 13.76 a | 14.05 b | 0.64 a | 0.99 a | 0.23 a | 0.68 a |
| 10/5℃ (12 hr/ 12 hr) | 3.93 a | 8.56 a | 11.08 b | 17.13 a | 0.35 b | 0.88 b | 0.13 b | 0.63 a |
| Duration of treatment | | | | | | | | |
| 2 weeks | 5.36 a | 7.13 b | 11.94 a | 14.84 b | 0.66 a | 0.88 b | 0.28 a | 0.63 bc |
| 4 weeks | 4.95 a | 8.41 a | 12.50 a | 18.19 a | 0.39 b | 1.03 a | 0.16 a | 0.83 a |
| 6 weeks | 3.49 b | 8.17 ab | 12.23 a | 13.80 b | 0.42 b | 1.02 a | 0.17 a | 0.69 b |
| 8 weeks | 2.98 b | 7.14 b | 12.64 a | 13.17 b | 0.45 b | 0.94 ab | 0.12 a | 0.64 bc |
| 10 weeks | 2.96 b | 8.93 a | 12.37 a | 18.41 a | 0.49 b | 0.82 b | 0.15 a | 0.53 c |
| Source of variation | | | | | | | | |
| Temperature (A) | 0.9186 | 0.0003 | <0.0001 | 0.0001 | <0.0001 | 0.0032 | 0.0375 | 0.1723 |
| Duration of treatment (B) | <0.0001 | 0.0013 | 0.9128 | <0.0001 | 0.0002 | 0.0021 | 0.3369 | 0.0002 |
| A*B | 0.0426 | 0.7663 | 0.1774 | 0.0057 | 0.1949 | 0.5114 | 0.6052 | 0.0012 |

[z]Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 12. Effects of temperature and its duration on flowering in *Fragaria hayatae*.

表 12. 溫度與處理週期對台灣草莓開花的影響

| Treatment duration (week) | % of plants flowering | | No. of flowers per plant | | No. of weeks to first bloom | |
|---|---|---|---|---|---|---|
| | 15/10℃ | 10/5℃ | 15/10℃ | 10/5℃ | 15/10℃ | 10/5℃ |
| 2 | 0.0 | 0.0 | 0.0 | 0.0 | - | - |
| 4 | 0.0 | 0.0 | 0.0 | 0.0 | - | - |
| 6 | 25.0 | 25.0 | 7.5 | 1.0 | 10.5 | 12.0 |
| 8 | 25.0 | 25.0 | 3.0 | 1.5 | 9.5 | 9.5 |
| 10 | 0.0 | 50.0 | 0.0 | 1.8 | - | 9.3 |

Table 13. Effects of day/night temperature and day length on number of leaves, leaf area, petiole length, and number of runners in *Fragaria hayatae*.

表 13. 溫度與日長對台灣草莓之葉片數、葉面積、葉柄長以及走莖數的影響

| Treatment | No. of leaves | | Leaf area ($cm^2$) | | Petiole length (cm) | | No. of runners | |
|---|---|---|---|---|---|---|---|---|
| | end of treatment | 16th week | end of treatment | 16th week | end of treatment | 16th week | end of treatment | 16th week |
| Control | 9.3 a[z] | 11.8 a | 10.60 a | 8.33 b | 5.21 a | 3.94 a | 5.0 a | 1.8 a |
| 15/10℃ 14hr | 8.6 a | 15.1 a | 7.42 c | 9.30 b | 2.26 b | 2.84 b | 0.6 b | 1.5 a |
| 15/10℃ 10hr | 7.4 b | 14.7 a | 9.03 b | 8.53 b | 2.23 b | 2.70 b | 0.7 b | 1.3 a |
| 15/5℃ 10hr | 6.9 b | 11.9 a | 7.08 c | 11.19 a | 2.11 b | 3.16 b | 0.1 b | 1.0 a |
| *P* value | 0.0002 | 0.0700 | <0.0001 | 0.0045 | <0.0001 | <0.0001 | <0.0001 | 0.1945 |

[z]Means in each column not followed by the same letter are significantly different at $P < 0.05$ according to Duncan's multiple range test.

Table 14. Effects of day/night temperature and day length on flowering in *Fragaria hayatae*.

表 14. 溫度與日長對台灣草莓開花的影響

| Treatment | % of plants flowering | No. of flowers per plant | No. of weeks to first bloom |
|---|---|---|---|
| Control | 0.0 | - | - |
| 15/10℃ 14hr | 8.3 | 10.0 | 7.0 |
| 15/10℃ 10hr | 8.3 | 4.0 | 10.0 |
| 15/5℃ 10hr | 8.3 | 6.0 | 9.0 |

Table 15. Results of interspecific hybridization in *Fragaria hayatae* and *F.* ×*ananassa* ‘Taoyuan No. 3’.

表 15. 台灣草莓與栽培種草莓‘桃園三號’之雜交結果

| | No. of pollinated flowers | No. of fruit set | Total no. of seeds | No. of germinated seeds | No. of survived seedlings | No. of seedlings that flowered |
|---|---|---|---|---|---|---|
| *F.* ×*ananassa* ‘Taoyuan No. 3’ × *F. hayatae* | 29 | 12 | 130 | 44 | 21 | 7 |
| *F. hayatae* × *F.* ×*ananassa* ‘Taoyuan No. 3’ | 15 | 11 | 144 | 0 | - | - |

# Appendix

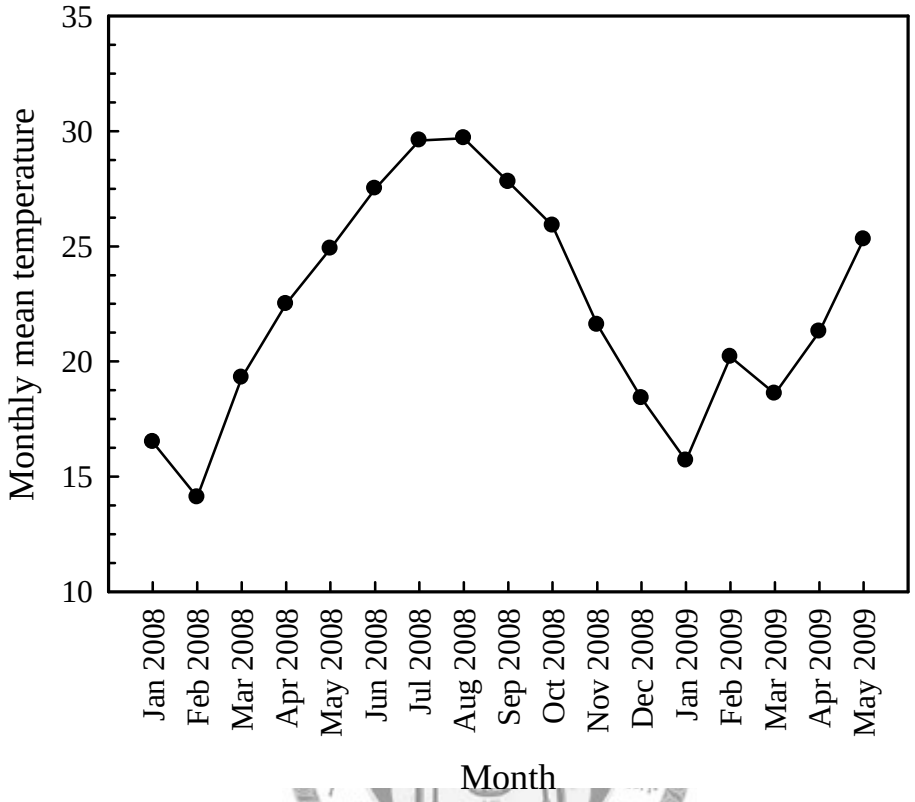


Fig. Monthly mean temperature during experiment in Taipei.

圖 試驗期間台北地區之月平均溫

(Data from: Central Weather Bureau)