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Caladium Genetics and Breeding: Recent Advances

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ABSTRACT

Caladiums are important ornamental aroids; they are valued for their colourful and variably-shaped leaves. Numerous advances have been made in recent decades in caladium breeding and genetic studies. Techniques have been developed to increase flower production, store pollen, and maintain seed viability. Sources of genetic resistance have been identified for important diseases and pests (such as Fusarium tuber rot, Pythium root rot, bacterial blight, and root-knot nematodes) and abiotic stress factors including chilling injury. Mode of inheritance for important foliar traits has been elucidated through analysis of trait segregation in progeny populations. Caladiums have evolved three alleles at one locus that control colour of leaf main veins (red, white or green) and two co-dominant alleles at an independent locus that determine leaf shapes (fancy, lance, or strap). Gene loci for leaf spotting and blotching are both simply inherited but tightly linked to green veins. *In vitro* culture and plant regeneration were successful with several types of tissues/organs through somatic embryogenesis and/or organogenesis. Shoot-tip culture has been used to eliminate viral and fungal pathogens and invigorate planting stock; protoplasts isolated from leaf callus regenerated into whole plants; foreign genes from maize or humans have been introduced into caladium through *Agrobacterium* co-cultivation. Molecular markers, including highly specific and informative SSRs, have been developed and applied to caladium to distinguish cultivars, assess genetic diversity, and analyze genetic relationships. The availability of these improved techniques, sources of desirable traits, and cellular or molecular tools will be very valuable for enhancing caladium breeding efficiency, achieving specific breeding objectives, and developing valuable new cultivars.

Keywords: breeding, caladium, disease resistance, genetic transformation, inheritance, molecular marker, ornamental aroid, tissue culture
Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; **2,4,5-T**, 2,4,5-trichlorophenoxyacetic acid; **AFLP**, amplified fragment length polymorphism; **BA**, 6-benzyladenine; **CaMV**, *Cauliflower mosaic virus*; **GA_3**, gibberellic acid; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butyric acid; **MS**, Murashige and Skoog; **NAA**, naphthaleneacetic acid; **PCR**, polymerase chain reaction; **SSR**, simple sequence repeat; **TRAP**, target-region amplification polymorphism

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INTRODUCTION

Caladium is a member of the Araceae family, belonging to the tribe Caladieae in the subfamily Aroideae (Mayo *et al*. 1997). The genus is indigenous to tropical America (Mayo *et al*. 1997). The exact number of species in this genus is still a matter of discussion, varying from seven (Madison 1981) to 17 (Croat 1994). The debate mainly centers around the classification of three species, *Caladium bicolor* Vent., *Caladium marmoratum* Mathieu, and *Caladium picturatum*

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Invited Review

Fig. 1 Caladiums forced in containers (**A**) or used in the landscape (**B**); an open bloom of caladiums (**C**); caladium tubers resistant (left) or susceptible (right) to Fusarium tuber rot (**D**); caladium roots resistant (left) or susceptible (right) to Pythium root rot (**E**); typical caladium leaf shapes and vein colors (**F**) (top row from left to right: fancy leaves with white, red, or green main veins; bottom row from left to right: lance leaves with white, red, or green main veins; rightmost column: strap leaf with red main vein); a spotted lance leaf of 'Gingerland' caladium (left) and a non-spotted fancy leaf of 'Candidum' caladium (right) (**G**); blotched fancy leaves of 'Carolyn Whorton' caladium (**H**); and non-spotted, non-blotched fancy leaves of 'Freida Hemple' caladium (**I**).

C. Koch. Madison (1981) merged these three species into one species, *C. bicolor* (*sensu* Madison). However, this treatment was considered by some taxonomists to be too broad. Croat (1994) maintained the species status for each of the three species and considered that the genus was comprised of 17 species. More recently, Mayo *et al*. (1997) reclassified the genus into *ca*. 12 species. Birdsey (1951) proposed to refer to the cultivated caladiums as *Caladium*×*hortulanum*. It is generally believed that cultivated caladiums resulted from hybridization among *C. bicolor* (*sensu stricto*), *C. marmoratum*, *C. picturatum*, and/or *Caladium schomburgkii* Schott (Hayward 1950; Birdsey 1951; Wilfret 1993).

In the floriculture trade, caladiums are valued for their colorful and variably-shaped leaves, and they are often grown as pot or hanging basket plants or planted in the landscape as accent or border plants (**Figs. 1A, 1B**). Seed propagation is possible but often results in considerable variability among progeny (Hartman *et al*. 1972). Thus, commercial caladium plants generally are forced from tubers. Most of the caladium tubers used in the world are produced in central Florida (Bell *et al*. 1998; Deng *et al*. 2008c). Caladium tuber production is a year-long process: planting of seed stock (tuber pieces) beginning in March each year and harvesting of new crops (new tubers) from December to February. Each year Florida caladium growers ship as many as 50 to 70 million tubers throughout the U.S. and to some 40 countries in the world.

The great majority of caladium cultivars commercially produced result from hybridization between breeding lines, existing cultivars, and/or species. Caladium breeding was pioneered in France in the mid 1800's (Hayward 1950); it was active in Florida in the 1920's, but it was not until 1976 that a public research institution initiated a caladium breeding program (Wilfret 1993). Over the past three and half decades, the University of Florida caladium breeding program has released nearly 30 new cultivars and has made considerable efforts to improve breeding techniques, identify sources of resistance to diseases and pests, understand the mode of inheritance of important traits, and develop molecular tools for caladium breeding. Meanwhile, a number of researchers in China, India, Japan, Singapore, Thailand, and other countries have devoted their efforts to develop and apply tissue culture, protoplast culture, DNA fingerprinting, and genetic transformation to caladium improvement. This paper provides an overview of the recent advances that have been made in caladium breeding and genetics.

IMPROVING BREEDING TECHNIQUES

Caladium breeding has primarily relied upon sexual hybridization between breeding lines, existing cultivars, and/or species (Wilfret 1993). Controlled pollination, seed production and population development are the fundamental activities of caladium hybridization breeding. These activities can be performed readily provided that receptive female flowers and viable pollen are available. Pollinated caladium female flowers can produce juicy fruits (berries) within 5 to 6 weeks. Fruit (or seed) set can vary considerably from inflorescence to inflorescence. Self incompatibility seems to be not a problem. Ploidy level barriers do not exist among existing cultivars, as they all are diploids with $2n = 2x = 30$ (Darlington and Wylie 1955; Marchant 1971). Well developed berries each can contains as many as 14 seeds, and well pollinated inflorescences each can produce as many as over 1,000 seeds (Z. Deng, personal observation). Extracted, cleaned seeds can germinate within 2 to 3 weeks after sowing.

However, a number of biological factors can hinder seed production and population development in caladium. Unlike some of the related aroids, such as anthurium (*Anthurium* Schott), *Spathiphyllum* Schott, and calla lily (*Zantedeschia aethiopica* Spreng), caladiums produce few

blooms under natural conditions. For example, 'Freida Hemple', 'Carolyn Whorton' and 'Candidum' produced only 0.2 to 1.9 blooms per plant per season. Additionally, flowering is often sporadic and unpredictable. Harbaugh and Wilfret (1979) found that GA_3 treatment increased bloom production to 2.4-3.5 per plant per season (**Fig. 1C**). Their flower induction technique involved selecting large caladium tubers (6.4-8.9 cm in diameter), curing and storing them at 21°C for over 2 months, and soaking the tubers in GA_3 (250 mg/L) for 8-16 h at 23°C. The treated plants flowered more uniformly. Recently Deng and Harbaugh (2004) noted that caladium cultivars responded to this treatment differently and took different amounts of time to produce blooms.

Like many aroid species, caladium produces pollen that is very short-lived and difficult to store. When kept under an ambient temperature and humidity for 1 day, only ~5% of pollen grains germinated *in vitro*. This short pollen longevity makes it very difficult to complete desired pollinations in many instances. Deng and Harbaugh (2004) stored freshly collected pollen under different temperatures and found that storing at 4°C slowed down the decline of pollen germinability to some extent: approximately 4% of the pollen grains germinated and produced normal pollen tubes *in vitro* after 4 days in storage. This technique has been useful in increasing the number of crosses.

To facilitate evaluating the effects of storage conditions on caladium pollen viability, Deng and Harbaugh (2004) reported an *in vitro* pollen germination procedure. They found that sucrose concentration in the medium had significant effects on pollen germination. Concentrations above 10% seemed to inhibit germination. The optimal sucrose concentration for *in vitro* germination was estimated to be 6.8%.

Caladium seeds can be readily produced by hand pollination, but they lose germination ability rapidly. To extend seed storage, Carpenter (1990) examined the effect of temperature and relative humidity on seed viability. He demonstrated that by maintaining temperature at 15°C and relative humidity between 22% and 52%, it was possible to store caladium seeds for 6 months without significant reduction in germination. Light is also essential for caladium seed to germinate. Carpenter (1990) showed that 20 lighting periods with 4- to 12-h duration resulted in the highest germination in the shortest time. The optimal temperature for seed germination was between 25 and 30°C. At these temperatures, 85% of caladium seeds germinated in 2 weeks.

IDENTIFYING SOURCES OF RESISTANCE TO MAJOR DISEASES AND PESTS

Disease and pest resistance has become one of the most important traits for caladium tuber producers, as revealed by recent surveys of grower needs (Deng *et al*. 2008c). Disease and pest resistance has also become a highly desired trait for greenhouses or nurseries and consumers. Over the past several years, several studies have responded to these needs and screened commercial cultivars for resistance to two fungal and one bacterial pathogen and one nematode species. These studies have resulted in the identification of a number of resistant cultivars (**Table 1**). These cultivars are providing valuable sources of resistance for new cultivar development.

Fusarium tuber rot

Fusarium tuber rot has been the most important disease impacting caladium tuber quality and quantity. During the past three decades, it has caused a steady decline in tuber yield of many commercial cultivars and led to the elimination of a number of cultivars from commercial production. The causal agent is *Fusarium solani* (Mart.) Saa. Goketpe *et al*. (2007) showed that temperature was an important factor influencing the fungus' mycelial growth and ability to cause tuber rot. On artificial media, fungal colonies expanded most rapidly between 25 and 31°C. However, lower temperatures (13 or 18°C) were more favorable for the fungus to cause tuber rot. To avoid potential cold damage to inoculated tubers, 18°C was selected as the appropriate temperature for screening cultivars for resistance to this disease. Goktepe *et al*. (2007) observed a considerable level of variation among *F. solani* isolates in aggressiveness or ability to cause tuber rot. Using three highly aggressive isolates as the inoculum, they identified five cultivars ('Aaron', 'Candidum', 'Florida Sweetheart', 'Rosebud', and 'White Christmas') with resistance to Fusarium tuber rot and two cultivars ('Red Flash' and 'White Wing') with moderate resistance to the disease (**Fig. 1D; Table 1**).

Pythium root rot

The causal agent was identified as *Pythium myriotylum* Drechs. Studies have shown that when grown in a *Pythium*-infected substrate, caladium tubers sprouted 3-5 weeks later, plant growth was reduced by up to 70%, and crop yield decreased by up to 40%. Deng *et al*. (2005a) observed that when young caladium plants were directly inoculated with *Pythium*, as much as 90% of the plants' roots could be rotted and as much as 85% of the plants' leaves could be lost in 2-3 weeks after inoculation. *Pythium* infection of roots can result in discolouration and necrotic blotches on leaf blades, epinasty and wilting on petioles, and whole leaf collapse. These leaf symptoms could appear as early as 3 days after *Pythium* inoculation. A linear relationship was observed between leaf loss and root rot severity (Deng *et al*. 2005a, 2005b).

To screen caladiums for resistance to Pythium root rot, Deng *et al*. (2005a) collected three *P. myriotylum* isolates, and they all were aggressive and able to cause rapid root rotting on caladium plants. Deng *et al*. (2005a) described an artificial *Pythium* inoculation and disease evaluation protocol. The protocol involved propagating young plants from tissue culture liners, growing the plants in small plastic cells filled with coarse vermiculite for approximately 8 weeks, applying *Pythium* oospore suspension directly to the root balls, maintaining the inoculated plants under 26-37°C with root spheres continuously saturated with water, and assessing root rot and leaf loss severity 2-3 weeks after inoculation. Using this protocol, Deng *et al*. (2005a, 2005b) assessed the resistance of 42 major commercial caladium cultivars to Pythium root rot. Most (35) of the cultivars evaluated were susceptible or highly susceptible to Pythium infection, but seven cultivars ('Apple Blossom', 'Candidum', 'Candidum Junior', 'Florida Blizzard', 'Freida Hemple', and 'White Christmas') showed moderate levels of resistance to Pythium root rot (**Fig. 1E; Table 1**).

Complete resistance or immunity would be most ideal, but such a level of resistance may not exist for Pythium root rot. Like many soil-borne plant pathogens, *Pythium* is generally facultative and possess little parasitic specialization. Plant resistance to nonspecific pathogens is seldom high (Bruehl 1983). Partial resistance or field resistance is more common to this type of pathogen. Considering the continuous distribution of root rot severity scores among the 42 cultivars, Deng *et al*. (2005b) suggested that the resistance would likely be a quantitative trait with additive gene action. Four of the seven resistant cultivars are fancy-leaved and white-veined. It is not clear whether this distribution is caused by genetic linkage among leaf vein colour, leaf shape, and Pythium resistance. Answering this question will require segregation analysis and genetic mapping.

Bacterial blight

This disease is also known as *Xanthomonas* leaf spot. It is caused by *Xanthomonas axonopodis* pv *dieffenbachiae* (*Xad*; formerly *X. campestris* pv. *dieffenbachiae*). Little information is available about the bacterial infection process on caladium leaves. It is reported that most bacterial

Table 1 Sources of resistance to two fungal diseases, one bacterial disease, one nematode species, and chilling injury recently identified in caladium cultivars (McSorley *et al*. 2004; Deng *et al*. 2005a, 2005b; Dover *et al*. 2005; Deng and Harbaugh 2006a; Goktepe *et al*. 2007; Seijo *et al*. 2010).

Cultivars	Fusarium tuber rot	Pythium root rot	Bacterial blight	Root-knot nematodes	Chilling injury
Aaron	R	S	MS		
Apple Blossom		MR			
Candidum	R	MR	HS	R	S
Candidum Junior	S	MR	MS	R	S
Carolyn Whorton		S	MS		S
Etta Moore		MR			
Fannie Munson	HS	VS	MS	R	
Florida Blizzard		MR	HS		
Florida Red Ruffles		S	R		R
Florida Sweetheart	R	S	MS		S
Freida Hemple	HS	MR		R	S
Marie Moir		VS			R
Miss Muffet	S	S		S	R
Mrs. Arno Nehrling		S	R		
Pink Beauty		S		R	S
Pink Gem		S	MS	R	
Pink Symphony		VS	R	R	
Postman Joyner	S	VS	HS	R	
Red Flash	MR	VS	HS		S
Rosebud	R	VS	HS	R	
White Christmas	R	MR	HS	R	S
White Queen	S	VS	R	S	S
White Wing	MR	VS	MS	S	

Note: HS = highly susceptible, MR = moderately resistant, MS = moderately susceptible, R = resistant, S = susceptible, and VS = very susceptible.

blight infections of anthurium (*Anthurium* Schott), a close relative of caladium, commence through entrance at hydathodes on leaf margins or wounds (Norman *et al*. 1999). Guttation droplets form at hydathode openings at night when planting media is warm and saturated with water, and humidity is high (Norman *et al*. 1999). Amino acids in the guttation fluid provide nutrients for invading bacteria (Sakai 1990).

Symptoms of bacterial blight begin as small, angular, water-soaked lesions on the lower leaf surface of caladium (Seijo *et al*. 2010). The lesions can expand and cause necrotic areas between veins (often V-shaped) or on veins and adjacent areas. Development of the disease often leads to severe tissue necrosis and premature defoliation. It could be an annual problem in tropical regions where the hot, humid and rainy weather conditions routinely exist for disease development and the spread of *Xad*. Leaf necrosis and defoliation during severe epidemics, especially those occurring early in the production season, can decrease tuber size and yield in commercial tuber production. Spot symptoms can also decrease the marketability of potted caladium plants in nurseries and greenhouses.

Seijo *et al*. (2010) screened 17 cultivars by spraying cultured bacterial cells onto young mature plants and incubating the plants under high humidity and a constant temperature. While most of the cultivars (12) were moderately or highly susceptible, several cultivars ('Candidum Junior', 'Carolyn Whorton', 'Florida Red Ruffles', 'Florida Sweetheart', 'Mrs. Arno Nehrling', 'Pink Symphony', and 'White Queen') were resistant to the inoculated bacteria (**Table 1**). 'Candidum Junior' was the direct parent for both 'Florida Red Ruffles' and 'Florida Sweetheart'. It is likely that 'Candidum Junior' had contributed the resistance to the two progeny cultivars. 'White Queen' is resistant to bacterial blight but has also been observed to exude bacteria from the lower surface of apparently healthy leaves. The exuded bacteria are *Xad* and pathogenic as well. Thus 'White Queen' should not be used a breeding parent in breeding for resistance to bacterial blight, as plants of such kind of resistance may carry pathogenic *Xad* that can infect adjacent susceptible plants.

Root-knot nematodes

They are plant-parasitic nematodes from the genus *Meloidogyne*. The species *M. incognita* can infect caladium roots and tubers. Severe infection can stunt plants and reduce tuber yield. Control of root-knot nematodes in caladium production has relied on hot water treatment of planting stock and fumigation of soil with methyl bromide. However, the use of methyl bromide in agriculture contributes to the ozone depletion in the atmosphere, and thus has been banned in recent years. Therefore, host plant resistance has been sought as an additional tool to manage root-knot nematodes. McSorley *et al*. (2004) evaluated eight caladium cultivars for resistance to root-knot nematodes, followed by Dover *et al*. (2005), who assessed a wider range of cultivars. In these evaluations, nematode eggs were extracted from greenhouse-grown pepper plants, incubated in the laboratory to produce juveniles, and the juveniles were injected to the soil around each plant grown in the pot. Five months after inoculation, root galling on the inoculated plants was assessed; nematode eggs in the soil and roots were extracted and counted. McSorley *et al*. (2004) and Dover *et al*. (2005) found that a number of cultivars supported high nematode populations, but a number of other cultivars were relatively resistant, with none or only a few eggs in the roots and soil of these cultivars (**Table 1**).

In many plant-nematode pathosystems, the severity of root galling is closely correlated with susceptibility to nematodes; the lack of root galling is commonly used as an indicator of nematode resistance. In caladium, however, galling has been observed on roots of resistant and highly susceptible cultivars. The latter did not have significantly more root galls (McSorley *et al*. 2004; Dover *et al*. 2005). Thus root galling alone is not a reliable indicator for nematode resistance, and breeding for nematode resistance in caladium will rely upon development of new screening methodologies, probably molecular markers.

IDENTIFYING SOURCES OF RESISTANCE TO CHILLING INJURY

Caladium is very sensitive to low temperatures and chilling injury. Temperatures below 15.5°C can delay tuber sprouting and damage leaves. This low temperature sensitivity not

only restricts the use of caladium in the landscape, but also affects caladium plant performance. To prevent chilling injury, higher temperatures have to be maintained in the greenhouse, which increases fuel costs for production of pot caladium plants. Identifying sources of chilling resistance and developing new resistant cultivars has become important in caladium breeding.

Deng and Harbaugh (2006a) excised mature caladium leaves from pot-grown plants, subjected them to low temperatures, and examined severity of chilling injury on leaves. Their study showed that exposing leaves to 7.2°C for 3 days could differentiate cultivars in chilling sensitivity. As expected, most of the cultivars (13 out of 16) evaluated so far were very sensitive to low temperatures, with 48-65% of leaf area injured. Surprisingly three cultivars ('Florida Red Ruffles', 'Marie Moir' and 'Miss Muffet') showed much less leaf injury (**Table 1**), and both parents of 'Florida Red Ruffles' ('Candidum Junior' and 'Red Frill') were chilling-sensitive.

The selection of a resistant cultivar out of the progeny of cold-sensitive parents may suggest the possibility of identifying transgressive chilling-resistant segregants in caladium breeding populations. Using 'Florida Red Ruffles' as a breeding parent, a new chilling-resistant cultivar ('Dr. Brent') has been recently developed (Deng *et al*. 2008b).

INHERITANCE OF IMPORTANT FOLIAR CHARACTERS

The ornamental value of caladiums depends largely on their leaf characters. Commercial caladium cultivars display a wide diversity of leaf shapes, colours, and colouration patterns that are rarely found in other cultivated plants. Several recent studies have been directed toward understanding the inheritance of some of these characters.

Leaf shape

Caladium leaves can be categorized into three shapes: fancy, lance, and strap (Wilfret 1993) (**Fig. 1F**). Fancy-leaved caladiums have heart-shaped leaves with three main veins on each leaf, a petiole attached to the back of the leaf (peltate), and two basal lobes joined for > 1/5 of their length and separated by a short narrow sinus. Strap-leaved caladiums have narrow linear leaves with one main vein and no obvious basal lobes. Lance leaves are an intermediate between fancy and strap types; leaf blades are broad sagitate to cordate-lanceolate and basal lobes are not obvious or broadly separated by a sinus, if present.

In caladium, leaf shapes seem to be closely associated with a number of other important characteristics, such as plant growth habit, stress tolerance, and tuber yield. Generally strap- or lance-leaved plants are shorter and smaller, but they develop more leaves and are more tolerant of sunburn and wind damage. Thus leaf shape is one of the most important characteristics of caladium species and cultivars.

Wilfret (1986) proposed that caladium leaf shape is controlled by a single gene, with one homozygous genotype producing the fancy leaf, the other homozygous genotype showing the strap leaf, and the heterozygous genotype resulting in the lance leaf.

This inheritance model was supported by a subsequent analysis by Deng and Harbaugh (2006b). They examined the segregation of leaf shapes in more than 2,600 progeny from 38 crosses among 10 cultivars and two breeding lines. All 859 progeny from 16 fancy × fancy crosses exhibited fancy leaves. Two types of leaves (fancy and lance) appeared in the 1,332 progeny of seven fancy × lance crosses and the segregated in a ratio of 1:1. The same segregation pattern was evident in the progeny of 10 lance × fancy crosses. In the progeny of two lance × lance crosses, three types of leaves (fancy, lance and strap) appeared in the ratio of 1:2:1.

The data confirmed that leaf shape is controlled by two co-dominant alleles at one locus. The two alleles have been designated as *F* and *f* for fancy and strap leaves, respectively. The genotypes for fancy-, lance-, and strap-leaved caladiums are therefore *FF*, *Ff*, and *ff*, respectively. Skewed segregation patterns have been observed in several fancy × lance crosses, which may suggest the existence of other genes, gene interactions, or non-random assortment of genes involved in the development of leaf shape in caladium.

Main vein colour

Leaf colour and colouration pattern are diverse and intriguing in caladium. The primary determinants of leaf colour and colouration are the colour of veins, interveinal areas, spots, and/or blotches. The colour of leaf main veins can be classified into green, red and white (**Fig. 1F**). Main vein colours are stable under different environments, on different plants, or at different developmental stages, and thus they have been commonly used in cultivar description and identification. Segregation analyses consistently indicate a recessive allele for green veins (Wilfret 1983, 1986; Deng and Harbaugh 2006b). Representatives of cultivars with green veins are 'Candidum' and 'White Christmas'. Red main veins are present in many caladium cultivars. Wilfret (1983, 1986) showed that a single dominant allele is responsible for red main vein and red vein is dominant over green vein. This relationship has been confirmed by a subsequent analysis of vein colour segregation in the progeny of 11 crosses involving four red-veined cultivars (Deng and Harbaugh 2006b). White leaf veins, although rare in most plants, are quite common in caladium. Once, white veins were thought to be dominant over both red and green veins (Wilfret 1986), but this model has since been rejected. Deng and Harbaugh (2006b) observed the dominance of white over green, but red dominated over white.

Overall, caladium has evolved a single locus with three alleles to control its main vein colours. The locus has been designated as *V*, and the order of dominance is V^r (red) > V^w (white) > V^g (green). Four major red-veined cultivars, 'Florida Fantasy', 'Florida Red Ruffles', 'Florida Sweetheart', and 'Red Flash' all have a heterozygous genotype containing both the red and green vein alleles (**Table 2**). White-veined cultivar 'Aaron' is homozygous with a V^wV^w genotype, and 'Florida Moonlight' is heterozygous with a V^wV^g genotype (**Table 2**).

Leaf spotting

Leaf spots are the most important colouration pattern in a number of important commercial cultivars, including 'Gingerland' that expresses numerous brick-red spots on leaf blades (**Fig. 1G**). Progeny of 'Gingerland' selfing did not segregate obviously in spot colour, but did segregate in the presence or absence of spots (3 spotted: 1 non-spotted) (Deng *et al*. 2008a). The possibility of a single dominant nuclear gene controlling leaf spots in 'Gingerland' was confirmed by a 1:1 (spotted: non-spotted) segregation in the progeny of eight testcrosses with five non-spotted cultivars. The non-spotted cultivars were used as seed or pollen parents in the testcrosses, and no obvious deviation from the 1:1 ratio was observed. Accordingly, Deng *et al*. (2008a) concluded that no maternal factors are involved in the inheritance of leaf spotting. The leaf spotting locus in 'Gingerland' has been designated as *S*, with alleles *S* and *s* for spotting and non-spotting, respectively.

A different leaf spotting pattern has been observed in 'Painter's Palette'. Plants of this cultivar express both red and white spots on the same leaves. Zettler and Abo El-Nil (1979) proposed one locus with alleles *R* and *W* for the red and white spots. Gager (1991) then analyzed the segregation of leaf spots and spot colours in the progeny of 'Painter's Palette' with non-spotted 'Aaron' or 'Florida Cardinal' and sib-mated progeny. The observed segregations led Gager (1991) to speculate that a single locus controls the expression of spotting, with two co-dominant alleles S_R for

Table 2 Phenotype of major caladium cultivars in leaf shape, main vein colour, spotting and blotching and genotype inferred from recent studies (Wilfret 1983, 1986; Deng and Harbaugh 2006b; Deng *et al*. 2008a; Deng and Harbaugh 2009).

Cultivars	Leaf shape		Main vein color		Leaf spots		Leaf blotches	
	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype
Aaron	Fancy	*FF*	White	V^wV^w	No		No	
Candidum	Fancy	*FF*	Green	V^gV^g	No	*ss*	No	*bb*
Candidum Junior	Fancy	*FF*	Green	V^gV^g	No	*ss*	No	*bb*
Carolyn Whorton	Fancy	*FF*	Red	V^rV^g	No	*ss*	Yes	*Bb*
Fannie Munson	Fancy	*FF*	Red		No	*ss*	No	*bb*
Florida Blizzard	Fancy	*FF*	White	V^wV^g	No	*ss*	Yes	*Bb*
Florida Fantasy	Fancy	*FF*	Red	V^rV^g	No		No	
Florida Irish Lace	Lance	*Ff*	Green	V^gV^g	No		No	
Florida Moonlight	Fancy	*FF*	White	V^wV^g	No		No	
Florida Red Ruffles	Lance	*Ff*	Red	V^rV^g	No		No	
Florida Sweetheart	Lance	*Ff*	Red	V^rV^g	No		No	
Freida Hemple	Fancy	*FF*	Red	V^rV^g	No	*ss*	No	*bb*
Gingerland	Lance	*Ff*	White	V^wV^g	Yes	*Ss*	No	*bb*
Miss Muffet	Fancy	*FF*	White	V^wV^g	Yes		No	*bb*
Red Flash	Fancy	*FF*	Red	V^rV^g	Yes		No	
Rosebud	Fancy	*FF*	Red	V^rV^w	No	*ss*	No	*bb*
White Christmas	Fancy	*FF*	Green	V^gV^g	No	*ss*	Yes	*Bb*

Table 3 Segregation ratios for leaf shape and vein colour in progeny of caladium crosses (Deng and Harbaugh 2006b).

Crosses (♀ × ♂)	Fancy			Lance			Strap		
	Red	White	Green	Red	White	Green	Red	White	Green
Aaron × Florida Sweetheart	1	1		1	1				
Florida Sweetheart × Aaron	1	1		1	1				
Florida Sweetheart × White Christmas	1		1	1		1			
White Christmas × Florida Sweetheart	1		1	1		1			
Florida Irish Lace × Florida Fantasy	1		1	1		1			
Candidum × Florida Sweetheart	1		1	1		1			
Red Flash × Florida Sweetheart	3		1	3		1			
Florida Red Ruffles × Florida Fantasy	3		1	3		1			
Florida Red Ruffles × Florida Moonlight	2	1	1	2	1	1			
Florida Sweetheart × Florida Moonlight	2	1	1	2	1	1			
Florida Irish Lace × Florida Red Ruffles	1		1	2		2	1		1

red spots, S_W for white spots, and a recessive allele *s* for no spots. Furthermore, the genotype S_R/S_W could result in two phenotypes, pink spots or co-existence of red spots and white spots, depending on whether the expression of red spots and white spots overlaps at the same locations or are in different locations. A separate locus was proposed for controlling the location of spots (isolated, border touching, or overlapping). However, the observed segregation deviated from the expected ratios in 21 out of 45 crosses. This was thought to be caused by the presence of a factor with lethal effects on genotype S_R/s, especially on genotypes S_R/S_R, S_W/S_W, and S_R/S_W. The relationship between this locus in 'Painter's Palette' and the *S* locus in 'Gingerland' remains to be elucidated.

Leaf blotching

Leaf blotches refer to irregularly-shaped coloured areas between main veins. In caladium blotches may appear singly or coalesce to form larger blotches. This pattern of colouration in combination with bright colours has resulted in several popular cultivars, such as 'Carolyn Whorton' (**Fig. 1H**) and 'White Christmas'. Progeny from selfing these cultivars were either blotched or non-blotched, in a ratio of 3:1, and progeny from selfing non-blotched cultivars (**Fig. 1I**) were all non-blotched (Deng and Harbaugh 2009). Such segregation patterns suggest that the presence or absence of leaf blotches is controlled by a single nuclear locus with two alleles. Symbols *B* and *b* have been suggested for the alleles conferring blotching and non-blotching, respectively. The blotched cultivars tested so far are all heterozygous at this locus. Interestingly, in some of the crosses between blotched cultivars and 'Gingerland' (a spotted cultivar as mentioned above), the segregation of leaf blotching was skewed toward more non-blotched individuals. This skewed segregation may indicate the existence of other factors and/or modes of gene interaction influencing leaf blotching.

Relationships among foliar traits

Leaf shape and main vein colour have been found to assort independently in the progeny of caladium crosses (Deng and Harbaugh 2006b). Because of this, as well as the multi-allelic system for main vein colour and co-dominance between leaf shape alleles, several patterns of segregation can appear in caladium progeny (**Table 3**). For example, four types of individuals (fancy white, fancy red, lance white, and lance red) appeared in a ratio of 1:1:1:1 in the progeny of 'Aaron' (fancy white, $FF\ V^wV^w$) × 'Florida Sweetheart' (lance red, $Ff\ V^rV^g$). Another four types of individuals (fancy red, fancy green, lance red, and lance green) was observed in a ratio of 1:1:1:1 in the progeny of crosses 'Florida Sweetheart' (lance red, $Ff\ V^rV^g$) × 'White Christmas' ($FF\ V^gV^g$), 'Florida Irish Lace' (lance green, $ff\ V^gV^g$) × 'Florida Fantasy' ($FF\ V^rV^g$), and 'Candidum' ($FF\ V^gV^g$) × 'Florida Sweetheart'. The same four types (fancy red, fancy green, lance red, and lance green) segregated in the ratio of 3:1:3:1 in the progeny of 'Red Flash' (fancy red, $FF\ V^rV^g$) × 'Florida Sweetheart' and 'Florida Red Ruffles' (lance red, $Ff\ V^rV^g$) × 'Florida Fantasy'. Six types of individuals (fancy white, fancy red, fancy green, lance white, lance red, and lance green) appeared in a ratio of 1:2:1:1:2:1 in the progeny of 'Florida Red Ruffles' or 'Florida Sweetheart' × 'Florida Moonlight' ($FF\ V^wV^g$). The ratio of 1:1:2:2:1:1 (fancy red, fancy green, lance red, lance green, strap red, and strap green) were observed in the progeny of 'Florida

Irish Lace' × 'Florida Red Ruffles'. These different patterns of segregation between leaf shape and main vein color provide an excellent source of genetic variation for caladium breeding.

Leaf spotting and blotching have also been found to be independent from leaf shape, but both seem to be tightly linked to main vein colour (Deng *et al.* 2008a; Deng and Harbaugh 2009). Using two double recessive cultivars ('Candidum' and 'White Christmas') with the genotype V^g *s*//V^g *s* as parents in testcrosses, the average recombination frequency between leaf spotting and green vein in 'Gingerland' was approximately 4.4% (Deng *et al.* 2008a). The linkage between leaf blotching and green vein seems to be even tighter, as no double recessive recombinants were observed in 357 progeny of crosses between blotched 'White Christmas', 'Carolyn Whorton', and 'Florida Blizzard' (Deng and Harbaugh 2009).

Based on these genetic relationships, the genotypes of 'Carolyn Whorton', 'Florida Blizzard', 'Gingerland', and 'White Christmas' for leaf shape, vein colour, blotching and spotting have been inferred: *FF* V^r *b s*//V^g *B s*, *FF* V^g *b s*//V^g *B s*, *Ff* V^w *b s*//V^g *b S*, and *FF* V^w *b s*//V^g *B s*, respectively.

CALADIUM TISSUE CULTURE AND GENETIC TRANSFORMATION

The advent and application of plant tissue culture and genetic transformation techniques has revolutionized the breeding and/or propagation processes in a number of crops. Substantial efforts have been made to apply these techniques to accelerate caladium breeding and genetic studies.

Several types of caladium tissue and organ such as unopened leaf rolls, young leaf blades and petioles, mature leaves, shoot tips, young inflorescences, and roots, have been used as explants for *in vitro* culture. These types of explant have demonstrated totipotency to regenerate into new plants, either through somatic embryogenesis or organogenesis.

Zhu *et al*. (1984) observed that caladium callus induced from leaf explants produced somatic embryoids at high frequencies and the callus maintained this regenerative capacity even after 4 years of subculture. Embryoids originated from the surface of callus masses. When transferred onto differentiation media, the callus first turned from light yellow to green or light green and then developed white globular embryos with a smooth surface, which matured into a shape typical of the sexual embryos of monocotyledon plants (Zhu *et al*. 1984). Hartman (1974) noted that shoot tip-derived callus maintained its regeneration capacity for at least 1.5 years without apparent adverse effects on regenerated plantlets. Every 3 months, each culture produced 10-20 plantlets suitable for transplanting as well as enough callus for making 10-20 subcultures. A temporary immersion culture technique has been developed for mass propagation of caladium plants (Daquinta *et al*. 2007). When this technique was used, caladium multiplication rate was increased more than 12 fold. Overall, tissue culture techniques have been used in caladium for various purposes, as shown below.

Pathogen elimination

Pathogen elimination has been the most common application of tissue culture techniques in caladium. Generally shoot tips with one or two leaf primordia are isolated from inside the main buds on the tuber with aid of a dissecting microscope; they are then cultured on solid media containing desired plant growth regulators. Hartman (1974) used a 'revised' MS medium containing myo-inositol, IAA (15.0 mg/L) and kinetin (1.0 mg/L) to culture the shoot tips of 'Candidum' and 'Freida Hemple'. Shoot tips turned from cream-coloured to green within 2 weeks after excision, developed callus masses, and differentiated into numerous shoots. All regenerated plants (>1000) were free of dasheen mosaic virus, a virus common among caladium and several other aroids. Mature plants were true to type, except that they were more vigorous and produced larger tubers than their virus-infected counterparts. Similar procedures have been used to invigorate other commercial cultivars or produce pathogen-free planting stock. The availability of these procedures will be very valuable for developing and implementing a pathogen indexing and seed stock certification program in caladium.

In vitro tuberization

Sugaram *et al*. (2007) induced *in vitro* tuber formation in *Caladium humboldtii* 'Phraya Savet'. Their technique involved several steps: (1) inducing callus from leaf pieces, (2) regenerating shoots, and (3) culturing micropropagated shoots (each with 2-3 leaflets) on appropriate media. Sucrose and activated charcoal were found to be the most important factors for successful tuber induction. High concentrations of sucrose (6-10%) promoted the growth of tubers. Using MS medium containing 8% sucrose and 0.5% activated charcoal resulted in the largest and heaviest tubers. Tubers are the most commonly used propagules in caladium. The availability of this technique may help understand the physiology associated with the formation of these propagules.

Somaclonal variation

Compared to other plants, somaclonal variation is common in caladium, especially in certain cultivars. Chu and Yazawa (2001) reported that 65.6-79.8% of regenerated plants had a vein colour change from white, red or pink to green in 'Fire Chief', 'Miss Muffet' and 'Tropicana'. High frequencies of variation have been observed also in 'Pink Cloud', 'Rosalie', 'White Queen', and 'White Wing'. Variation can involve leaf pattern characteristics (shape, apex, margin and base), petiole characteristics (attachment and colour), and leaf colour characteristics (colour of lamina, patch, leaf margin, primary vein, secondary vein, and anastomosis end). Each individual variant may express changes in one or more of these characteristics (Thongpukdee *et al*. 2010). Overall, leaf colour variants occurred at the highest frequency, followed by leaf pattern variants. Leaf shape variants were much less frequent. The type and concentration of plant growth regulators used in the culture medium have had remarkable influences on the occurrence of somaclonal variation. Ahmed *et al*. (2004) cultured leaf explants of 'Pink Cloud' on MS medium containing various auxins (2,4-D, 2,4,5-T, IAA, IBA, and NAA) in combination with BA and observed that 100% of the regenerated plants had leaf colour variation when the medium contained 0.1-1.0 mg/L 2,4-D, while only 15% of plants had the variation when the medium contained 0.1 mg/L NAA. Thongpukdee *et al*. (2010) noticed that the highest frequency (87.3%) of variation occurred on the MS medium containing 2.0 mg/L BA and 1.0 mg/L NAA and the lowest frequency (22.4%) on the medium containing 2.0 mg/L BA and 2.0 mg/L NAA.

Somaclonal variation can serve as a new source of variation for cultivar development. A good example of this application may be the selection of *C. humboldtii* cultivar 'Marcel'. It resulted from a mutation found during tissue culture of this species in the laboratory of Marcel Lecoufle (Lecoufle 1981). However, for the most part, the high frequencies of variation occurring in caladium tissue culture have become the limiting factor for applying micropropagation to caladium. A number of studies have aimed to find ways to minimize the occurrence of undesirable variants. As mentioned above, selection of cultivar, type of explant and medium is critical for such a goal. Few vein colour variants have been observed when 'Candidum Junior', 'Aaron', 'Freida Hemple' and 'Kathleen' were cultured (Chu and Yazawa 2001). Compared to leaf explants, shoot-tips seem to produce fewer variants in tissue culture and are desirable explants for micropropagation of caladium (Ahmed *et al*. 2002). As for plant growth regulators, use of 2,4-D in the

medium should be avoided unless absolutely necessary, and the concentration of NAA may need to be optimized for specific cultivars. In addition, certain pretreatments prior to explanting seem to be important as well. Ahmed *et al*. (2002, 2007) have demonstrated that using younger tissue, especially young green leaves from axillary buds as explants, could reduce the frequency of variation to 6-8%. They excised axillary buds from tubers and grew them under heavy shading (75-100%) for several weeks before collecting the newly developed young green leaves as explants.

Protoplast culture

Jing and Wang (1991) reported isolation and culturing of caladium protoplasts and successful regeneration of plantlets. They used leaf callus as the starting material for protoplast isolation and cultured protoplasts in liquid or solid K3 medium (Nagy and Maliga 1976) supplemented with 0.1 mg/L BA, 0.5 mg/L 2,4-D and 0.5 M sucrose. Protoplasts regenerated cell wall and resumed cell division after 2-3 days in culture and formed callus after 1 month in culture. When transferred onto MS medium containing 2.0 mg/L BA and 0.5 mg/L NAA, callus initiated organogenesis and embryogenesis and further regenerated into plantlets. This protoplast isolation and culture system may be very useful for caladium protoplast fusion and somatic hybridization.

Protoplast fusion has been used successfully in a number of fruit, vegetable and agronomic crops to combine disease and pest resistance traits from different cultivars, even different species or genera, and to create new germplasm for breeding (Johnson and Veilleux 2001). This technique may help combine resistance to Fusarium tuber rot, Pythium root, and root-knot nematodes from different caladium cultivars into somatic hybrids.

Genetic transformation

Genetic transformation has been achieved through cocultivating *Agrobacterium* cells with caladium leaf discs or petiole segments. The *Agrobacterium* used was strain LBA4404; transformed cells were first induced to form callus and then to regenerate plantlets. By optimizing culture, cocultivation and selection conditions, up to 10% of inoculated explants produced transgenic callus (Li *et al*. 2005). The maize anthocyanin synthesis regulatory gene *Lc* (leaf colour) was introduced into 'Jackie Suthers', a white-veined cultivar. Constitutive expression of the foreign gene under the CaMV promoter resulted in transgenic plants accumulating anthocyanin in leaves, petioles, tubers and roots and turning these organs red. Besides the colour change, the transgenic plants did not show any other phenotypic changes (Li *et al*. 2005). One of the plants regenerated was half transgenic and half non-transgenic and developed a symmetrical leaf with red veins on one half of the blade and white veins on the other. It was likely that only one of the two precursor meristematic cells forming the plant was transformed. If this kind of sectorial chimera is stable during successive propagation, it may be of ornamental value. Prior to this report, a human growth hormone gene was introduced into caladium using a similar procedure (Li *et al*. 1994). These studies indicate that *Agrobacterium*-based systems are applicable and efficient for genetic transformation of caladium.

MOLECULAR MARKER ANALYSIS OF *CALADIUM* CULTIVARS AND SPECIES

Several molecular marker systems have been applied to caladium. Loh *et al*. (1999, 2000) reported that AFLP markers produced reliable and reproducible DNA fingerprints in caladium; each AFLP primer combination amplified, on average, as many as 110 fragments detectable by silver staining. TRAP markers, another type of PCR-based marker, have been used on caladium (Deng *et al*. 2007). Eight TRAP primer combinations generated 297 dominant markers. Although this system amplified fewer DNA bands than AFLP, it was reproducible and reliable.

Recently caladium-specific SSR markers have become available (Gong and Deng 2011). They were developed from 'Florida Sweetheart' genomic sequences enriched with the oligo $(GA)_{15}$. Out of 114 primer pairs designed from the genomic sequences, 99 pairs amplified clear PCR products of expected sizes. When tested on a panel of nine caladium cultivars, 46.5% of the markers revealed polymorphism; each marker amplified 2 to 7 alleles (average of 3.8). 74.0% of the alleles amplified by these markers were polymorphic between 'Candidum' and 'Gingerland', parents of two segregating populations that had been constructed for caladium genetic mapping. In contrast, only about 16.0% of TRAP markers were polymorphic between the two parents. Additionally, the reported SSR markers showed high levels (50-100%) of transferability to *C. bicolor*, *C. schomburgkii*, *C. steudneriifolium*, *C. picturatum*, *C. humboldtii*, and *C. marmoratum*.

The availability of these markers is providing a powerful tool for distinguishing caladium cultivars, assessing genetic diversity, and understanding genetic relationships among caladium cultivars and species. Since they are mostly co-dominant and highly informative, the developed SSR markers will be particularly useful in these applications and genome or gene mapping.

Equipped with these marker technologies, researchers have begun to gain new insights into caladium. For example, marker analyses have helped elucidated the relationship between *C. humboldtii* and *C. bicolor*. Plants of the former are dwarf and have miniature leaves yet are similar to the latter in leaf colouration pattern. This similarity has led Madison (1981) to speculate that *C. humboldtii* is a chromosomal race of *C. bicolor*. AFLP profiling has revealed a vast difference between them and suggested that *C. humboldtii* is a distinct and separate species rather than a chromosomal race of *C. bicolor* (Loh *et al*. 2000). Subsequent marker analysis performed by Deng *et al*. (2007) using the TRAP marker system supported Loh's finding. *C humboldtii* accessions had high similarity within its accessions but low similarity with other caladium species.

Molecular marker analysis has raised an interesting question about the status of *C. lindenii* Madison. This species was a member of the genus *Xanthosoma*, but it was transferred to *Caladium* based on their similarity in pollen shedding pattern (Madison 1981). *Xanthosoma* sheds pollen in tetrads, while *Caladium* sheds pollen as single grains. Loh *et al* (2000) noticed that *C. lindenii* did not carry any of the *Xanthosoma*-specific AFLP markers, so was not a *Xanthosoma* species. In a subsequent marker analysis by Deng *et al*. (2007), *C. lindenii* had extremely low Jaccard similarity coefficients (0.060-0.148) with and were clustered very distantly from other caladium species. Recently, Gong and Deng (2011) reported that only a low percentage (25%) of the SSR markers developed in cultivated caladium could be transferred to *C. lindenii* and some of the amplified alleles were outside the size range observed in other caladium cultivars and species. This low level of genetic as well as morphological similarity between *C. lindenii* and other caladium species may suggest a separate genus for *C. lindenii*.

CONCLUSION

There has been a strong resurged interest among growers and gardeners in growing caladiums. As described above, significant advances have been made recently in many aspects of caladium genetic research and breeding. It is expected that these advances will accelerate the improvement of important ornamental and horticultural traits and create novel traits in caladium. Thus these advances have brought excellent opportunities to develop a new generation of caladium cultivars for consumers in the 21st century.

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