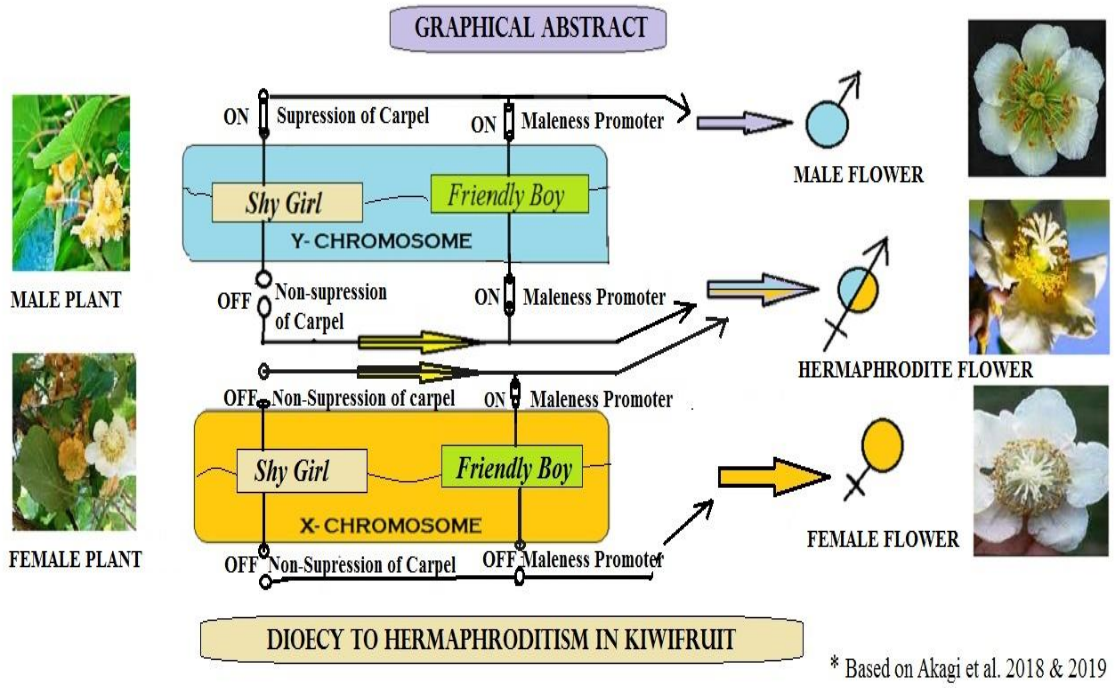
Review Article

Hermaphroditism in kiwifruit (*Actinidia deliciosa*): A Review

Abstract:*Actinidia*, the kiwifruit is approximately 20 to 26 million years old. It is known widely for its dioecious nature. Recently, hermaphroditism has also been found to exist in its male inconstant type. Its floral development follows the common (A)BCE floral model and is completed in seven distinct stages. Male parts of the flower develop early during the third stage whereas programmed cell death leads to pollen degeneration in female flowers at the later stages. After exploring the sex-linked segment of the genome, it was determined that the Y-encoded sex-determinant genes *Shy Girl* and *Friendly Boy* act independently as the suppressors of feminization and promoters of male factors, respectively. Non-expression of *Shy Girl* in males results in the formation of hermaphrodites; however, the incorporation of *Friendly Boy* in female plants can also result in hermaphroditism in this fruit crop. These findings have opened new possibilities for generating self-pollinating hermaphrodite vines of high horticultural importance.

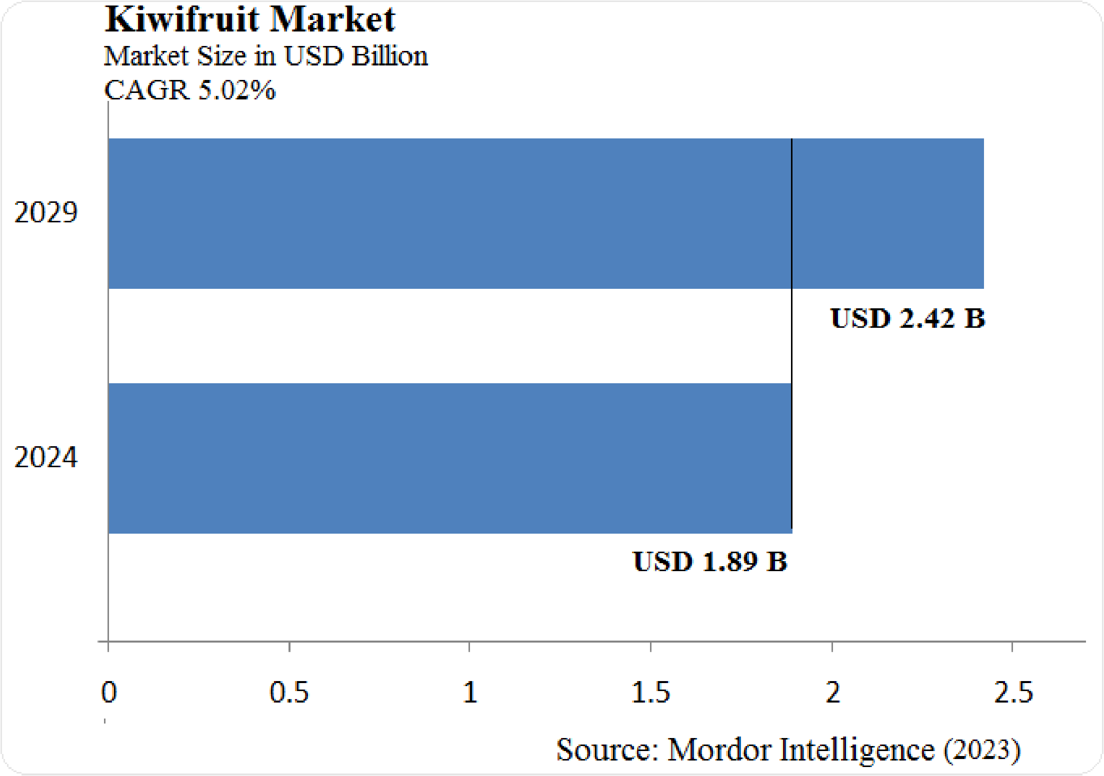
Keywords: Dioecy, Inconstant male, (A)BCE Floral Model, *Shy girl*, *Friendly boy*



Introduction

Kiwifruit (*Actinidia deliciosa*) is a rapidly developing fruit crop from one side of the planet to the other. Currently, it is grown across more than 270457hectares worldwide. (UNDATA, 2020). In 2021, the global production of kiwifruit reached 4.47 million tonnes,with China, New Zealand, Italy, Greece, Iran and Chileleading the production

(Shahbandeh, 2023).Over the previous decade, its exports has seen an average increase 20.1% among all exporting nations. Inferable from its nutritional importance and demand, the market share of this fruit is expected to increase to USD 2.42 billion by 2029, at a CAGR of 5.02% (Mordor Inteligence, 2023). Moreover, its inherent taste, nutritional content, and storage capabilities are contributing to its escalating popularity. The increasing awareness among consumers regarding its health advantages is persuading a broader population globally to regularly consume kiwifruit as part of a balanced diet.Recent figures indicate that as kiwifruit consumption grows in regions such as Asia, the European Union, and the United States, its demand is projected to remain strong for several decades (Eurofruit, 2022).



Source: Mordor Intelligence (2023)

Fig. 1. Kiwifruit Market

The demand-driven production goals for kiwifruits are pushing global researchers to develop varieties that can offer higher returns per unit area of cultivation. Present-day growers are seeking types that can self-pollinate and eliminate the need for specific type pollinators and pollinizers. Thus, self-pollinating varieties are anticipated to simplify orchard management and optimize the use of land in kiwifruit vineyards. Even though there are few self-pollinating varieties (inconstant male varieties or female varieties with lower proportion of viable pollen), they too produce more reliable crops when pollinated by the pollen from the male kiwifruit plants (Bellini et al. 1991, Morton 2011 and Pinto & Vilela 2018). Therefore, building knowledge in kiwifruit flowering and the floral organs development is projected to represent a significant advancement towards creating hermaphroditic genotypes.

Hermaphroditism is a condition in which both male and female sex organs are present on each flower born on a plant of a particular species. It is the most common sexual state, and only a few species (5 to 6%) have evolved to bear sex-explicit plants (dioecious plants) (Casimiro-Soriquer, 2015). Asparagus, papaya, persimmon, cannabis, and kiwifruit are examples of dioecious flowering plants. Most of these species are distantly related, reflecting that these plants digressed from hermaphroditism several times throughout their transformative history.Most flowering plants are otherwise hermaphroditic or monoecious, with both male and female flowers occurring on the same plant; although androdioecy, gynodioecy or trioecy are also notuncommon. But in kiwifruit, dioecy is quite dominant. According to the literature, scientists around the world are making great progress inunderstanding kiwifruit flowering to elucidate the sexual differentiation process of this fruit crop and these efforts are providing a great impetus to promote the development of hermaphrodite varieties of *Actinidia* species. This mini-review therefore aims to highlight kiwifruit hermaphroditism, which is otherwise less known among many working groups of this fruit crop.

Floral biology and cytogenetics of *Actinidia*

The most notable species in kiwifruit are *Actinidia deliciosa* and *Actinidia chinensis*, which produce kiwifruits, *Actinidia arguta* (kiwi berry), *Actinidia polygama* (silver vine), and the ornamental *Actinidia kolomikta* (Ferguson, 1999; Campbell-Culver, 2001 and Marner, 2023). Studies have revealed that *A. chinensis* and *A. deliciosa* are diploid and hexaploid, respectively (Liang and Ferguson, 1984). However, Ferguson (2016) revealed that these two species are recognized as two assortments of the same species, *A. chinensis* var. chinensis and *A. chinensis* var. deliciosa, due to the presence of clines and extensive introgressive hybridization at places where they both grow. The best-known kiwifruits produced today are*Actinidia chinensis* var. Deliciosa, also known as *Actinidia deliciosa*. This species developed during thetwentieth century, and in the last fifty years it has attained the status of an economically important crop across the globe.

Paleobotanical studies indicate that the genus *Actinidia* is approximately 20-26 million years of age (Qian and Yu, 1991). All individuals from this genus (enveloping at least 76 species)are practically dioecious with male and female flowers being born on separate plants (Ferguson, 1990, Ferguson and Huang, 2007 ~~Huang and Ferguson, 2007~~). The male vines bear apparently staminate flowers with viable pollen and a rudimentary ovarythat lacks style and does not form ovules. On the other hand, pistillate flowers with functionallywell developedovaries containing numerous ovules are borne on the female vines. These flowers apparently bear fully-developed stamens, which, however, produce nonviable pollen due to impaired intine wall synthesis (Messina, 1993).

All the *Actinidia* species possess a basic chromosome number of x=29, although diploid, tetraploid, hexaploid and even octaploid species are also not uncommon (McNeilage and Considine, 1989; Ferguson and Huang, 2007). McNeilage (1991a, b) reported that a single determinant controlling sex at any ploidy level is the presence of gender inconstant males (that is, males producing occasional fruits), as the sex ratios they obtained after crossing males to females wereinvariably 1:1 and 3:1 when selfed. The male sex in this fruit crop is thus reported to be heterozygous (XY),and the femalesex must be homozygous (XX) (Testolin et al. 1995, 1999; Harvey et al. 1997).However, necessarily, this does not signify the presence of differential sex chromosomes, as was observed in some dioecious species by Charlesworth (2002).Furthermore, a single genetic determinant always controls sex with a unique set of X/Y chromosomes. The probable presence of a sex-neutral (XX)n chromosome set in *Actinidia*has been reported, where n depends on ploidy level (McNeilage, 1997; Testolin et al., 1999, 2004; Fraser et al., 2009).

Sex differentiation

The identity of floral organs in this genus is also determined mainly by the popular (A)BCE-like floral model (Coen et al. 1991,Bowman et al. 1997 and Causier et al. 2010), where the A function is not clear (Varkonyi-Gasic et al., 2011), and the B- and C-functions act after the establishment of meristem identity to determine organ identity. Only the expression domains of the B- and C-functions align with an organ identity function in two adjacent whorls and have both individual and combined roles in establishing floral organ identity exclusively (Causier et al. 2010). Caporali et al. (2019) identified 7 distinct stages of floral development in kiwifruit. They stated that the sex differences in kiwi flower development become evident from the initial stage (stage 3) of floral development around the 10th day from the initiation of stamen development when flowers are about 3.5 to 4.5 mm in length. It was reported to be distinguishable as a compacted gynoecium in female flowers; the staminate flowers lackedthe carpel meristem and ovule primordium. They further speculated that pollen degeneration in female flowers occurs due to programmed cell death at later stages, although pollen release in staminate flowers was reported to occur after that during the 7th stage of floral development. As these 7 developmental stages were linked to specific morphological differences, this provided the required information for gene expression studies related to sex differentiation and other floral developmental processes in this fruit plant.

Previously, McNeilage (1991b) described the characteristics of fruiting males along with those of female and pure male genotypes based on variations in numerical and morphometric floral characteristics. He reported that fruiting males produced both staminate and bisexual flowers in both uniform and mixed inflorescences. The proportion of hermaphrodite on such vines reportedly varies from 40 to 70%. He added that all the floral organs do not develop similarly in all the three (pistillate, staminate and hermaphrodite) sex forms; differences were reported in stylar length, ovary length, ovary diameter and the number of ovules per carpel. Staminate and bisexual flowers were reported to have the same number of flowers per inflorescence, length of filaments, pollen viability, and length of the rachis, carpel number but differed from the female flowers. Regardless of sex, all the flowers had similar number of sepals and petals. Furthermore, McNeilage and Steinhagen (1998), in a rare progeny of crosses in between Hayward and an inconstant (fruiting) male, obtained complete hermaphroditic flowers with no restrictions on selfing; however, fruits were not of commercial importance.

1. *Friendly Boy* and *Shy Girl* genes:

Flowering plants exhibit a normal tendency toward hermaphroditism, and separate male and female sex(dioecious) plants are rare. However, recent findings suggest otherwise, stating that, evolutionary dioecy is reversible when species move in and out of dioecy at a certain frequency (Käfer et al. 2017). Theories explaining the shift between hermaphroditism and dioecy predict the occurrence of at least two mutations for evolving separate sexes – the one that is responsible for male sterility and the other for female sterility. The independent evolution of dioecy in several species indicates that each transition to dioecy may involve different genes and evolutionary scenarios (Renner, 2014). Thus, in the genus *Actinidia,* plants of separate male and female sex are now considered ancestral. After exploring the sex-linked segment of the kiwifruit genome Akagi et al. (2018 & 2019) reported that dioecy in *Actinidia* appeared 20 million years ago. After analyzing the transcripts from young flowers, they explained that a Y-encoded sexdeterminant candidate gene acting as the suppressor of feminization (SuF) is a male-specific type-C cytokinin response regulator that is expressed in developing male flowers (Varkonyi-Gasic et al., 2021). These authors named this response regulator gene *Shy Girl (SyGl).* This geneis expressed at the surface of the rudimentary carpel in male flowers and suppresses carpel development. However, the gene responsible for pollen sterility in female flowers could not be identified during these studies. Later, Akagi et al. (2019) identified a second Y-encoded sexdeterminant, named *Friendly Boy (FrBy)*, which was reported to act to maintain male functionsindependently of *SyGI*. Genome re-sequencing of natural hermaphrodite flowers revealed that a hermaphrodite flower is genetically male, but partial deletions in the Y chromosome, including those of *SyGI*, have led to hermaphroditism. Furthermore, they found that the incorporation of *FrBy* in female flowers also resulted in hermaphroditism; when Akagi et al. (2019) plunked a copy of *FrBy* into the genome of a female kiwifruit plant, within months the plants produced hermaphrodite flowers. These findings clearly indicated that Y-encoded *SyGI* and *FrBy* act independently as the suppressor of feminization (SuF) and male factors (M), confirming the presence of a two-factor sexdetermination system for the evolution of gynodioecy from hermaphrodite or monoecious individuals, as explained earlier by Charlesworth and Charlesworth (1978). This elucidation of the mechanisms underlying sex determination has extended the possibilities of manipulating the *SyGI* and *FrBy* genes to generate self-pollinating hermaphrodite cultivars in Kiwifruit.

5. Horticultural significance of hermaphroditism:

Hermaphrodite plants may benefit from reproductive assurance, efficient pollinator attraction and the ability to optimize resource allocation to boost regenerative accomplishment during the blooming period. It dodges the requirement for a mating partner. In addition, the transition from dioecy to hermaphroditism in kiwifruit will dispense with the need to plant non-fruiting pollinizer cultivars. Ordinarily, pollination and fruit set are important issues under harsh weather conditions when pollinator activity is restricted; pollinator-dependent fruitset issues can be decreased impressively by planting bisexual cultivars. Moreover, effective pollination and fertilization through selfingnormally result in better seed numbers and thus better fruit size and quality through hermaphrodite vines.

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