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RESEARCH ARTICLE
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Genome-Wide Variation Analysis and *ARF19B* Marker Development for Parthenocarpy in Tomato Cultivar 'CH 154'

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ABSTRACT

Parthenocarpy, fruit development without fertilization, is a valuable trait in tomato breeding, especially under high-temperature conditions that impair pollination. In this study, the tomato cultivar 'CH 154' was confirmed to exhibit facultative parthenocarpy, producing seedless fruit under heat stress. The whole genome resequencing of 'CH 154' and a seeded cultivar ('CH 267') led to the identification of 71 SNP loci associated with parthenocarpy. These loci span eight hormone-related gene groups, including *ARF19B*, *ARF2A*, *ARF3*, *ARF5*, *TIR1*, *PAD1*, *MET1*, and *AP3*, while only *ARF19B* contained polymorphic SNPs differentiating the two cultivars. Two SNP markers within *ARF19B*, SL-SNP59758072 and SL-SNP59761199, were developed using tetra-primer ARMS-PCR, demonstrating a hundred percent correlation in genotype discrimination associated with parthenocarpy. This marker system offers a novel, reliable tool for marker-assisted selection (MAS) targeting parthenocarpy, providing a foundation for developing heat-resilient, seedless tomato varieties.

Keywords: Facultative, Number of seeds, Heat tolerance, Whole genome sequencing

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INTRODUCTION

Tomato (*Solanum lycopersicum*) is a globally important fruit crop consumed as a vegetable, particularly in Asia (Sekara et al., 2019). A diploid species (2n = 2x = (24with a genome size of approximately 950megabase (Mb) exhibits a self-pollination rate exceeding .%95Based on use, tomatoes are categorized into processing and fresh-market types; cherry tomatoes are particularly favored for their sweetness and mild aroma (Gill & Kaur, 2019) .Key quality traits include fruit size, color, firmness, flavor, and seedlessness, driving breeding efforts toward premium cherry cultivars to meet market demands (Sinesio et al., 2021). One desirable trait is parthenocarpy, the ability to produce seedless fruits without fertilization. This trait offers multiple advantages, including improved texture, ease of consumption, reduced processing costs and more stable yields under adverse conditions, such as high temperatures that can impair pollination. Understanding the genetic and hormonal mechanisms underlying parthenocarpy is crucial for developing effective breeding strategies that target stress-tolerant, seedless cherry tomato cultivars. Parthenocarpy in tomatoes can be classified as obligate or facultative. Facultative parthenocarpy allows seed formation under favorable conditions and seedlessness under stress, making it more suitable as a sexually reproducing species. In contrast, obligate parthenocarpy is often linked to vegetatively propagated plants (Gorguet et al., 2005). In tomatoes, this trait reduces flower drop and fruit loss under suboptimal pollination conditions, enhancing yield and lowering labor costs (Varoquaux et al., 2000).

Several key genes regulate parthenocarpy. *Pat* genes, such as *pat-2* and *pat-k*, promote fruit development without fertilization by modulating hormonal pathways involving auxins and gibberellins (Takisawa et al., 2020). Additionally, the *DefH9-RI-iaaM* gene enhances auxin biosynthesis, encouraging parthenocarpic fruit set (Rotino et al., 1997). Mutations in *ARF* (Auxin Response Factors) and *GA20ox* (Gibberellin Biosynthetic Genes) further underscore the role of hormonal regulation in this process (Tang et al., 2015). The number of days with heat stress (HS) and elevated maximum temperature (EMT) was also examined according

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to the procedure described by Damayanti et al. (2019). A comprehensive review Molesini et al. (2020) conducted confirmed that auxin and gibberellin are pivotal in regulating fruit initiation and parthenocarpy, primarily through the activity of ARF proteins such as *SlARF7* and *SlARF8*, and the auxin signaling repressor *SlIAA9*. These components suppress ovary development before fertilization; disrupting their regulatory balance leads to seedless fruit formation. In addition, MADS-box transcription factors, including *TAG1*, *TAGL1*, *TM29*, and *AGL6,* are implicated in floral organ identity and fruit development, modulating their function by hormone-dependent mechanisms.

Environmental factors, particularly temperature, significantly impact tomato growth, seed set, flowering, Optimal fruit set occurs at 28-30°C during the day and 17-20°C at night. Elevated temperatures, particularly above 35°C during the day, can lead to flower drop and reduced fruit set (Sitathanee, 2000). The cherry tomato cultivar 'CH 154' demonstrates moderate heat tolerance and can set fruit at nighttime temperatures exceeding 22°C. High temperature also affects floral morphology and physiological processes, such as carbohydrate metabolism and the production of polyamines and proline (Pressman et al., 2002; Song et al., 2002; Sato et al., 2006; Alsamir et al., 2017). Studies have shown that parthenocarpy can be induced under heat stress or by emasculating flowers to prevent self-pollination. Both genetic and environmental factors influence this phenomenon. The present study focuses on seed development in the cherry tomato cultivar 'CH 154', a commercially valuable cultivar widely favored by consumers for its pleasing fruit quality traits. Notably, it exhibits facultative parthenocarpy, making it a strategic genetic resource and a standard parental line in tomato breeding programs to improve fruit set under adverse environmental conditions. The whole genome sequence of 'CH 154' was investigated to reveal genes associated with parthenocarpy through Single Nucleotide Polymorphism (SNP) analysis. The genetic information obtained was then employed to develop SNP-based DNA markers using the tetra-primer ARMS-PCR to detect and select alleles associated with parthenocarpy. These markers have the potential to enhance the accuracy and efficiency of selecting seedless phenotypes, thereby supporting the development of better-quality cultivars in future breeding programs. Furthermore, this study evaluated whether the parthenocarpy observed in 'CH 154' represents a facultative type by analyzing the impact of environmental conditions on trait expression. This investigation fulfills the underlying mechanisms that regulate parthenocarpy under environmental stress.

MATERIALS & METHODS

Plant Materials

To evaluate the effect of genetics on fruit parthenocarpy, the tomatoes were planted from January to March 2023. The seeds of tomato varieties 'CH 154' (parthenocarpic tomato) and 'CH 267' (non-parthenocarpic tomato) were sown in plastic trays containing peat moss as the growing medium. The plants were grown inside a netted greenhouse with drip fertigation systems at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand.

Evaluation of Parthenocarpy

Parthenocarpic traits in tomato cultivars 'CH 154' and 'CH 267' were evaluated by recording fruit set and seedlessness in flowers subjected to mechanical emasculation. The experiment involved four plants per cultivar, with three flower buds randomly selected from each plant. Emasculation, involving the removal of anthers, was performed two days before anthesis to prevent self-pollination, following the standard method described by Carrera et al. (2012). The emasculated flowers were covered with thin cotton wool to avoid natural cross-pollination. Fruit development was subsequently monitored to assess the potential for parthenocarpy in each cultivar. The experiment involved three plants per cultivar, with four flowers per plant, resulting in 12 flowers per cultivar.

Temperature Effects on Facultative Parthenocarpy in Tomato

To evaluate the effect of temperature on fruit parthenocarpy from January to February 2023, the 'CH 154' tomato seeds were planted under two temperature treatments: (1) low temperature (LT) and (2) high temperature (HT). The plants were subsequently transferred to 30cm pots. Fifteen plants of each cultivar were used for the experiment. The LT treatment was grown inside a semi-closed greenhouse with an active mechanical HVAC (heating, ventilation and air conditioning) system, at the Concrete Products and Aggregate Co., Ltd. facility in Bang Sue, Bangkok, Thailand. The plants in the HT treatment were grown inside a netted greenhouse at the Tropical Vegetable Research and Development Center (TVRC), at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. The microclimate inside the greenhouse was monitored throughout the experimental period by using the WatchDog 3250 wireless weather station (Spectrum Technologies Inc., USA). The number of days with heat stress (HS) and elevated maximum temperature (EMT) was also examined according to the procedure described by Damayanti et al. (2019).

Data was collected from Naturally Pollinated Tomato Plants

Thirty mature fruits were sampled from all flower trusses on each plant across all individuals for each planting. Seed numbers per fruit and the percentage of parthenocarpic fruits were recorded and averaged across 15 plants per planting. Measurements included total fruit weight, length, width, number of seeds per fruit, and the expression of parthenocarpy. The experiment was conducted with three replications per location, with each replication consisting of measurements from tomato fruits. Statistical analysis was performed using a t-test to compare means, conducted in RStudio version 4.2.2 (Horton & Kleinman, 2015).

Isolation of total Plant DNA

Genomic DNA from 'CH '154and 'CH '267was extracted according to the Fulton et al. (1995) method with some modifications .Approximately 3-2leaves were ground in a microprep buffer and incubated at 65°C for 60min-30. The DNA was purified using chloroform: isoamyl alcohol ((24:1, precipitated with cold isopropanol, and washed using %70ethanol. The DNA pellet was resuspended in TE buffer and incubated at 65°C for 15min. DNA quality and concentration were assessed using a NanoDrop spectrophotometer and 0.5X TBE agarose gel electrophoresis. Samples were stored at -20°C for downstream applications.

Genome-wide Variation Analysis Using Whole Genome Sequencing

The extracted DNA was sent to Novogene Co., Ltd. for whole-genome sequencing using the standard MGISEQ with paired-end 150 bp by DNBSEQ-T7 platform, with an output of 30 GB for each cultivar. The analysis began with quality control using using FastQC (Andrew, 2010) and Trimmomatic (Bolger et al., 2014) to remove adapter sequences, low-quality reads (quality score < (20, and short reads (< 50 bp). Using BWA MEM (Li & Durbin, 2009), high-quality reads were aligned to the tomato reference genome (GCF_000188115.5, NCBI) (Li, 2013) .Alignment files were processed with SAMtools (Danecek et al., 2021), sorted into BAM format, and manipulated by Picard. Then the duplicate reads were removed using GATK's MarkDuplicatesSpark (McKenna et al., 2010) to improve variant calling accuracy. Variant calling was performed using FreeBayes v 1.3.6(Garrison & Marth, 2012). Low-confidence variants were filtered out using GATK VariantFiltration based on quality metrics (QD < 2.0, QUAL < .(30Gene region overlap was determined using Bedtools (Quinlan & Hall, 2010) intersect.

Design of Primers for SNP Markers Using the Tetra-Primer Amplification Refractory Mutation System Polymerase Chain Reaction (Tetra-Primer ARMS-PCR)

Tetra-primer ARMS-PCR was employed to detect specific SNPs, using two outer and two allele-specific inner primers designed via Primer1 (Ye et al., 2001; Collins & Ke, 2012; Zhang et al., 2015). Primer design parameters included a Tm of 50-65°C, a length of 26-30 bp, and a GC content of 40-60%. PCR optimization involved gradient PCR to determine optimal annealing temperature and primer concentrations (Table 1). and optimal PCR conditions were established in a 20µL reaction volume consisting of the following components: (1) 3µL of template DNA at 100ng/µL, (2) four primers at 10µM each FI and RI at 0.75µL, FO and RO at 0.3µL, (3) 0.2µL of Taq DNA polymerase (5 U, Thermo Scientific™), (4) 3µL of dNTPs (1 mM, Thermo Scientific™), (5) 1.6µL of $MgCl_2$ (25 mM, Thermo Scientific™), (6) 1.6µL of 10X PCR buffer (Thermo Scientific™), and (7) nuclease-free water to reach a final volume of 20µL. Amplification products were evaluated by agarose gel electrophoresis using a 2.0% gel, run for 40min at 100 volts.

RESULTS & DISCUSSION

Preliminary Evaluation of Parthenocarpy in Tomato Cultivars 'CH 154' and 'CH 267'

The 'CH 154' produced eight seedless fruits from 12 flowers, resulting in a 75% parthenocarpic fruit set. In contrast, 'CH 267' did not make any fruit (0% parthenocarpic fruit set) as demonstrated in Fig. 1. This preliminary evaluation suggests that 'CH 154' possesses parthenocarpic potential, while 'CH 267' does not. This finding aligns with the study Mapelli et al. (1979), which indicated that parthenocarpy is influenced by temperature and pollination methods. The emasculation technique, performed two days before anthesis to prevent self-pollination, results in parthenocarpic flowers, which develop into seedless fruits (Carrera et al., 2012).

Table 1: Optimized PCR reaction

PCR	Temperature (°C)	Time	Cycle
Pre-denaturation	95	5 min	1
Denaturation	95	30 second	
Annealing	*	30 second	35
Extension	72	30 second	
Final extension	72	5 min	1
Hold	4	∞	

*Annealing; SL-SNP59758072 = 53°C, SL-SNP59761199 = 56°C

Fig. 1: Comparison of seedless (parthenocarpy) in 'CH 154' (a, c) and seeded in 'CH 267' (b, d) tomato fruits and their respective plants grown under controlled greenhouse conditions.

We validated a facultative parthenocarpy in 'CH 154' that was influenced by temperature on development and growth from seedless fruit, as the hourly air temperatures inside the HT greenhouse were consistently higher than those inside the LT greenhouse. The average daytime and nighttime air temperatures in the HT greenhouse were 32.8 and 27.0°C, respectively. The LT greenhouse average daytime and nighttime temperatures were 28.8 and 24.8°C, respectively (Table 2). Several studies have found that a day/night temperature cycle of 32/26°C significantly affects fruit setting and yield (Peet et al., 1998; Sato et al., 2001; Pressman et al., 2002; MJ Paupière, 2017). Days with an average daily air temperature of 29°C or higher were defined as heat stress (HS) days, and days with a maximum air temperature of 32°C or higher were defined as elevated maximum temperature (EMT) days. In the LT condition, 1 HS and 50 EMT days were recorded, and no parthenocarpic fruits were formed in all tomato plants. Under HT

conditions, air temperatures were high, with 43 HS and 59 EMT days. Parthenocarpy was observed in the HT condition, with 100% of the fruits being parthenocarpic (Table 2 and 3). The temperature does not affect the fruit weight, fruit width, or fruit length of cherry tomato (Table 3). This finding aligns with the study by Grange and Andrews (1993). It can be concluded that parthenocarpy in the tomato cultivar 'CH 154' is facultative in nature, with high temperatures playing a noteworthy role in inducing this trait.

Table 2: Recorded temperatures during daytime and nighttime

	n	Temperature (°C)			HS, day	EMT, day
		Daytime[1]	Nighttime[2]	Daily		
LT	59	28.8	24.8	26.8	1	50
HT	59	32.8	27.0	30.0	43	59
p-value		**	**	**	**	**

[1] day = Average time between 06.00-17.59 (h), [2] nights = Average time between 18.00-05.59 (h). ** indicated the difference between the observed data and the expected ratio at P<0.01.

Table 3: Fruit formation and related parameters of tomato under different temperature conditions

Conditions	Fruit formation	Number of seeds, seed fruit^{-1}	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)
LT	0/30 (0%)	12.8±1.1	7.3±0.3	20.9±0.3	27.0±0.4
HT	30/30 (100%)	0.0±0.0	6.8±0.3	20.4±0.4	26.7±0.8
p-value		**	Ns	ns	ns

** indicated the difference between the observed data and the expected ratio at P<0.01, ns = non-significant at P>0.05.

Table 4: The number of SNPs distributed in the candidate parthenocarpic-associated genes

Gene	Number of SNPs	Chromosome	Reference ID
AP3	4	4	(Kramer et al., 1998)
PAD1	4	1	(Matsuo et al., 2020)
MET1	7	11	(Yang et al., 2019)
TIR1	15	9	(Ren et al., 2011)
ARF2A	22	3	(Ren et al., 2017)
ARF3	1	2	(Zhang et al., 2015)
ARF5	3	9	(Liu et al., 2018)
ARF19B	15	5	(Wu et al., 2011)

Genome-wide Variation Analysis using Whole-genome Sequencing

The whole-genome sequencing (WGS) data generated using the MGISEQ platform demonstrates high quality and reliability. A total of 30.4 GB (33.78x genome coverage) and 32.1 GB (35.67x genome coverage) of raw data with a GC content of 36.76 and 36.66% were generated for 'CH 154' and 'CH 267', respectively. Key quality metrics indicate high sequencing accuracy, including Q30 scores exceeding 92% across all samples and the average error rate of 0.02-0.03%. As expected on the MGISEQ platform, a slight increase in error rate is observed with longer read lengths due to reagent depletion, a phenomenon previously described by Erlich et al. (2008) and Jiang et al. (2011). The nucleotide distribution remains stable, with minor fluctuations in the first 6-7 bases attributed to primer bias. Quality control also involved filtering out low-quality reads, including those with adapter contamination, those with over 10% undefined bases, or those with over 50% low-quality bases ($Q \leq 5$). These findings confirm the dataset's suitability for accurate downstream analyses.

More than 95% of 'CH 154' and 'CH 267' reads were correctly mapped to a reference genome with a high-quality mapping. A total of 3,439,470 SNP loci were identified in both genomes. We further investigated SNPs identified through genome-wide variation analysis by focusing on 27 genes associated with parthenocarpy in tomato, as reviewed by Baranov et al. (2024), resulting in 157 SNPs sitting in the candidate genes. Interestingly, 71 SNPs in eight candidate genes *AP3, PAD1, MET1, TIR1, ARF2A, ARF3, ARF5*, and *ARF19B* as shown in Table 4, were polymorphic and potentially associated with parthenocarpy.

The tomato's *APETALA3* (*AP3*) gene in tomato regulates flower development, particularly in forming petals and stamens (Kramer et al., 1998). *PAD1* regulates auxin homeostasis and seed development; mutations in this gene can stimulate ovary growth without fertilization (Matsuo et al., 2020). The *SlMET1* gene plays a crucial role in normal leaf morphology, inflorescence development, and the regulation of fruit set in tomato plants (Yang et al., 2019). The *SlTIR1* gene in tomato functions as an auxin receptor, which plays a critical role in plant development, particularly during the transition from flower to fruit and leaf formation (Ren et al., 2011). The genes *ARF2A*, *ARF3*, *ARF5*, and *ARF19B* are members of the *ARF* gene family, which regulates the expression of auxin-responsive genes. According to Ren et al. (2017) *SlARF2A* functions as an essential regulator of lateral root formation and floral organ senescence, potentially influencing fruit development during the late stages of flowering. In the case of *SlARF3*, Zhang et al. (2015) has been demonstrated that *SlARF3* plays a significant role in regulating the development of epidermal cells and trichomes in plants. However, its role in the parthenocarpy process has not been conclusively established. *SlARF3* may exert an indirect effect by modulating auxin-responsive signaling pathways at the molecular level, which could influence fruit set induction independent of fertilization.

Furthermore, Liu et al. (2018) reported that downregulation of *SlARF5* induces parthenocarpic fruit set by modifying hormone signaling pathways within the flower, supporting its function in fruit development independent of fertilization. Among the ARF gene family members investigated, only *ARF19B* yielded polymorphic SNPs suitable for primer design using the tetra-primer ARMS-PCR method. While other ARFs, such as ARF3 and ARF2A, have been implicated in auxin-regulated developmental processes, including those potentially linked to parthenocarpy, no reliable SNPs were found in these genes among the studied cultivars. This suggests that the associated alleles may be population-specific and less conserved across genotypes. Conversely, *ARF19B* has been reported to regulate auxin signaling during fruit set and is expressed in floral organs. Its functional relevance to parthenocarpy has been previously demonstrated through RNAi-mediated suppression, resulting in seedless fruit formation. Therefore, *SlARF19B* was prioritized for marker development due to biological significance and technical feasibility, and the resulting SNP markers are promising tools for marker-assisted selection in breeding programs targeting parthenocarpy (De Jong et al., 2009; Wu et al., 2011; Ezura et al., 2023).

Design of Primers for SNP Markers Using Tetra-Primer ARMS-PCR

The tetra-primer ARMS-PCR technique effectively

converts SNP loci into molecular markers that accurately distinguish different genotypes. It is a low-cost, simple, and compatible method for routine genotyping, suitable for standard molecular genetics laboratories, without requiring specialized equipment, making it a practical and efficient approach. Due to these advantages, it is widely used in various fields, including plant breeding (Chiapparino et al., 2004; Yupayao et al., 2018), animal genetics improvement (Singh et al., 2014; Liu et al., 2015) and human disease diagnostics (Shariati et al., 2008; Hajihoseini et al., 2015). Therefore, this technique is a promising tool for developing robust DNA markers to support marker-assisted selection (MAS) programs, particularly for traits such as parthenocarpy governed by specific genetic variations. Therefore, the tetra-primer ARMS-PCR technique was designed to target all 71 polymorphic SNP positions. Resulting in the primer sets SL-SNP59758072 and SL-SNP59761199, which are in genes *ARF19B*, provided clear genotype discrimination based on allele-specific band patterns, as shown in Fig. 2. The genomic DNA was extracted from the leaf tissues of tomato cultivars 'CH 154' and 'CH 267', as well as the F_1 population. In panel (a), SL-SNP59758072 identified the AA genotype in 'CH 154', the GG genotype in 'CH 267', and the heterozygous AG genotype in the F_1 hybrid. Panel (b), SL-SNP59761199 successfully differentiated the TT genotype in 'CH 154', the GG genotype in 'CH 267', and the heterozygous TG genotype in the F_1 hybrid. No amplification was observed in the negative controls (n^-), confirming the specificity and reliability of the PCR reactions. The genotyping result shows a 100% correlation between the allele and the parthenocarpic trait, calculated from 10 plants of each cultivar for both co-segregation SNP markers.

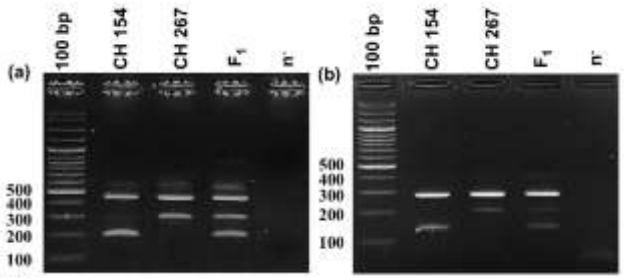

Fig. 2: Genotyping by tetra-primer ARMS-PCR of two SNPs in ARF19B genes. (a) Genotyping with the primer set SL-SNP59758072 distinguishes between GG and AA genotypes. (b) Genotyping with the primer set SL-SNP59761199, which distinguishes between TT and GG genotypes.

The ARF19B locus harbored two high-quality SNPs that segregated with the facultative parthenocarpy phenotype and could be readily amplified with ARMS-PCR. The absence of functional variation in *Pat-k* and *DefH9-iaaM,* yet robust polymorphism in *ARF19B,* highlights the population-specific nature of parthenocarpy-associated alleles. Hence, while *Pat-k* and *DefH9-iaaM* remain valuable for other germplasm, *ARF19B*-based markers provide a cultivar-relevant, low-cost tool for marker-assisted selection in breeding programs that utilize 'CH 154' or related cherry tomato lines. These results underscore the importance of tailoring marker development to the genetic background of the target population and demonstrate that even within a conserved gene family such as ARF, only certain members may contain exploitable variation in each breeding pool. The details of the two primer sets were described in Table 5. It is noted that empirical optimization is crucial in developing SNP markers with tetra-primer ARMS-PCR, as even small changes in primer concentrations can significantly affect amplification efficiency and genotype discrimination. Ye et al. (2001), recommended a 1:10 ratio, whereas Suhda et al. (2016), found a 1:2 ratio optimal for detecting SNP rs3813865 in the *CYP2E1* gene. The optimal ratio for our developed markers was 1:2.5. These findings highlight the potential of SL-SNP59758072 and SL-SNP59761199 as molecular markers for SNP genotyping in tomato.

Identifying *ARF19B*-associated SNP markers has broader implications for tomato breeding under rising temperatures, where successful fertilization is frequently compromised. The ability to select facultative parthenocarpy using genotype-based markers provides breeders with a practical tool to develop seedless cultivars suited for heat-stressed environments, potentially stabilizing yields under suboptimal pollination conditions (Bashir, 2025). However, this study also acknowledges certain limitations. The expression and penetrance of the parthenocarpy trait may be influenced by environmental cues such as temperature and humidity, as well as the genetic background of the cultivar. The current marker validation was conducted in a limited population derived from specific parental lines (CH 154' and 'CH 267'), which may restrict the generalizability of these findings. Therefore, further validation across diverse genetic backgrounds and multiple environments is essential before large-scale deployment in breeding programs. In addition, to confirm the role of *ARF19B* in regulating parthenocarpic fruit set, future research employing CRISPR/Cas-based gene editing will be essential to validate its functional contribution. Additionally, transcriptome profiling of floral tissues under controlled heat stress conditions could provide deeper insights into temperature-responsive regulatory networks and identify key genes or pathways mediating facultative parthenocarpy. Together, these approaches will enhance the molecular understanding of parthenocarpy and support the development of climate-resilient tomato cultivars (Baranov & Timerbaev, 2024).

Table 5: Sequence of primers of SNP markers associated with Parthenocarpy used in Tetra-Primer ARMS-PCR technique

SNPs		Sequence (5'-> 3')	Tm (°C)	Ta (°C)	Amplicon size (bp)
SL-SNP59758072	FI:	GCAAGCATGAGCTTATCATAA	60	53	A = 195
	RI:	TGTGGTCATTGAATAGTGAATTC	60		G = 298
	FO:	AGAAGGATTCTTCTGCATGTT	61		Outer primer = 449
	RO:	GAGTCTCTATTTGGGAGATTGA	60		
SL-SNP59761199	FI:	GGAAAATCTTAGATAAGAAAATCCG	59	56	G = 133
	RI:	GTTCTTTGTTTTTTATTCCCTTTCTA	60		T = 199
	FO:	GCATCATATTTTCCTGGAAAAT	58		Outer primer = 281
	RO:	AATGTTAATCCAGCTGAAGGTC	62		

FI = forward inner primer, RI = reverse inner primer, FO = forward outer primer, RO = reverse outer primer, Tm = melting temperature.

Conclusion

This study highlights the environmental and genetic influence on parthenocarpy in tomatoes. The parthenocarpic trait in the tomato 'CH 154' was confirmed as facultative, indicating that temperature influences the expression of this trait, with higher temperatures leading to increased expression of seedless but fruit-bearing characteristics. Based on genome-wide variation analysis using whole-genome sequencing, primer sets SL-SNP59758072 and SL-SNP59761199, which target the *ARF19B* gene, were successfully developed. It can clearly distinguish genotypes associated with parthenocarpy in tomatoes. These genes play crucial roles in hormonal signaling pathways, particularly those involving auxin and gibberellin, which are essential for fruit development independent of fertilization.

DECLARATIONS

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Author's Contribution: PT, PS - Research concept and design; PS - Collection and assembly of data; PS, WU - Data analysis; PT, PS, PD, WU - Interpretation; PT, PS - Writing the article; All authors have read the manuscript and agreed with its content.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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