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Review

The Biological and Genetic Mechanisms of Yield Self-Regulation in apple Tree (*Malus × domestica* Borkh.)

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Abstract: Apple tree (*Malus × domestica* Borkh.) belongs to the Rosaceae. Due to its adaptability and tolerance to different soil and climatic conditions, it is cultivated worldwide for fresh consumption. The priorities of apple growers are high-quality fruits and stable yield for high production. About 90 to 95 percent of fruits should fall or be eliminated from apple trees to avoid overcropping and poor-quality fruits. Apple trees engage in a complex biological process known as yield self-regulation, which is influenced by several internal and external factors. Apple buds develop in different stages along the branches they can potentially give rise to new shoots, leaves, flowers, or fruit clusters. Apple genotype determines how many buds will develop into fruit-bearing structures and the capacity for yield self-regulation. Plant hormones such as ethylene, cytokinins, auxins, and gibberellins play a crucial role in regulating fruit set, growth, and development, and the balance of these hormones influences the flowering intensity, fruit size, and fruit number on the apple tree. Apple growers often interfere in the self-regulation process by manually thinning fruit clusters. Different thinning methods, such as by hands, mechanical thinning or applying chemical substances, are used for flowers and fruit thinning. The most profitable in commercial orchards is the use of chemicals for elimination, but more environmentally sustainable solutions are required due to the European Green Deal. This review focuses on the biological factors and genetic mechanisms in apple yield self-regulation for a better understanding of the regulatory mechanism of fruitlet abscission for future breeding programs targeted at self-regulating yield apple varieties.

Keywords: *Malus × domestica*; abscission; fruit - drop; yield self- regulation; phytohormones; genetic mechanisms

1. Introduction

Apple (*Malus × domestica* Borkh.) is one of the most popular fruit trees for growing and consumption in the world [1]. Over the past two decades, global apple-fruit production has grown rapidly and increased by 54 % [2]. The focus of the production is fresh fruits with high quality, which depends on different internal and external fruit parameters, like size, colour, acidity, sweetness, and maturity of fruits [1,3-4].

Yield self-regulation in apple trees is a complex biological process which is influenced by environmental and genetic factors. The main factors involved in this mechanism are bud development, hormonal regulation, genetic control, and thinning practices. Apple trees naturally tend to form a large number of flowers and fruitlets. However, only 5-10 % of blooms are capable of developing into marketable-quality fruits [5-6]. Heavy blooming and overcropping are major problems for apple growers, as apple trees are not capable of growing a large amount of fruits. In case of overcropping many small and low-quality fruits are produced, and the weight of them brakes branches, this increased risk of diseases. Fruit quality depends on competition between fruits for nutrients, water, and sunlight [7-8] . Additionally, the overcropping can cause biennial bearing, where trees alternate between heavy fruit production one year and minimal or no fruit production the following year [9-10]. This irregular fruit-bearing cycle can disrupt orchard yields and profitability [11]. The stability of apple fruit yield depends on many factors, including physiological traits and genotype. Even after a high-yield season, different apple genotypes differ in flowering intensity, the number of eliminated fruitlets, and the absence of biennial bearing [12]. There are well-known apple cultivars like Gala, Ruby Jon, and Pink Lady that are permanent annually bearing [13-14], and cultivars Honeycrisp, Fuji, Braeburn, Jonagold, Golden Delicious, and Elstar that are biennial bearing varieties [9,13-14].

Apple trees have a unique growth habit in which buds develop in different stages along the branches. These buds can potentially give rise to new shoots, leaves, flowers, or fruit clusters. Additionally, at different stages of life, plants have the ability to eliminate old, abnormally developing, redundant, or immature parts [15-16]. This is a very crucial event as it determines the quality of the fruit and the size of the yield. Apples and other pome fruits, such as pear [17] or stone fruits, avocado [18], cherry, plum, peach and apricot [19], have a natural system of fruitlet self-elimination. The natural optimal self-elimination of apple fruitlets is rare, and only a few apple-tree varieties naturally thin fruitlets up to one fruit per inflorescence [12,20]. In most cases, apple trees do not eliminate enough fruitlets to produce a qualitative fruits and productive yield [21-22]. The genetic programming within the tree determines how many of these buds will develop into fruit-bearing structures and how many immature fruitlets will be dropped.

In apples-tree, three periods of fruit drop are known. The first occurs soon after flowering (1-2 weeks) and is usually caused by incomplete pollination. Depending on genotype, 46 – 71 % of flowers are eliminated during this period [12,23]. The second fruit drop occurs 4-6 weeks post-blooming and experiences the most severe fruit shedding, commonly known as the "June drop". Fruits removed in June vary in their degree of development. The intensity of the drop depends on the variety and the weather conditions of the year. In drought conditions, genotypes with a lower number of developing seeds tend to eliminate more fruitlets than genotypes with a higher number of viable seeds [12]. Fruits with more developing seeds are more likely to survive the June drop than those with fewer seeds. The seeds in the fallen fruit are at different levels of development, but most are wrinkled and have stopped developing with germs at different stages of development. Fruit growth is arrested due to impaired endosperm development. The pedicels of fruit drops during the "June drop" are underdeveloped. A lack of nutrients can explain the disorders in seed development. This may be due to too low concentration of phytohormones in seeds [24-25]. During the third period of fruit-drop part of almost ripe fruits are eliminated. Several factors, such as mineral nutrition, climatic conditions, insect or disease severity, and growing season temperature, affect the severity of fruit drop [23,26].

Regulation of fruitlets number on the tree is an essential technological aspect in horticulture as flowers or fruitlets thinning is used to optimize the yield. In apple orchards, growers often intervene in self-regulation by manually thinning fruitlets. This helps to optimize fruit size, quality, and overall yield by reducing competition among developing fruits and promoting better resource allocation. This could be done by hands, mechanically or by using chemical substances [27-28]. Hand thinning is the most effective way to regulate tree loads. However, it is a labour-intensive and high-cost solution [29]. Due to automatization and poorly controlled thinning prosses, mechanical thinning does not guarantee the persistence of fruitlets with high quality and generative organs like leaves, flowers, petals, fruitlets and mature fruits potential [30]. Chemical thinning is the most used method to reduce the number of fruitlets. However, it is highly dependent on the variety and environmental conditions during application and some chemical thinners, like ethephone and terbacil, could have a slightly harmful effect [31-32]. Chemical thinners like benzyladenine (BA) and *naphthaleneacetic acid* (NAA) may threaten the environment, and the use of these agents is banned [22]. In addition, the increasing demands of the European Green Deal require more environmentally sustainable solutions [33].

Overall, the biological and genetic mechanisms of yield self-regulation in apple trees are a sophisticated interplay of internal factors like plants physiology, genetics and hormones and external influences like environmental cues and management practices. The internal factors are the focus of this review.

2. Physiological Processes during Fruit Drop

2.1. Physiology of Abscission

Abscission is the process of removing unnecessary organs. This phenomenon occurs in different phases of a plant's life cycle. In the northern hemisphere, the most common abscission event is the removal of the leaves prior to winter [34-35]]. Abscission allows plants to discard infected, damaged, or senescent vegetative and generative organs like leaves, buds, flowers, petals, fruitlets and mature fruits [35]. Abscission is an essential process of plant reproduction that enables the dropping of mature fruits and the dispersal of seeds. From an evolutionary perspective, fruit and seed abscission processes have significant advantages [36]. In certain situations, fruit abscission may occur from pollination to ripening [23]. Abscission can be triggered by factors like fertilization and ripening. The process of flower petals dropping post-pollination is well understood in *Arabidopsis*. The environmental factors could lead to abscission. A shorter photoperiod and lower temperature trigger the abscission of leaves. In autumn, leaves fall to to save energy for survival in cold periods [37-38]. Damage to tree organs caused by insects and bacterial disease can also lead to abscission [26,39].

The abscission process occurs at a particular area defined as the abscission zone (AZ) [35,40]. From a physiological point of view, abscission consists of two crucial processes: detaching and forming a protective layer, which typically happen simultaneously [41]. For the fruit to detach, a particular layer must first develop. An AZ contains smaller cells than surrounding tissues and has a dense cytoplasm. After initiation of abscission, cells in AZ increase in size and middle lamella break down via hydrolytic enzymes, leading to cell detachment. Following the abscission process, a newly developed epidermal layer is formed to cover the abscission site and to protect it from infection. Differentiation of AZ occurs long before the separation process has started [41].

According to various studies, there are four main phases in organ separation:

1. Early development of cells that will form the AZ in the future. In an early stage of development AZ cells are described as relatively small, localized regions, lacking large vacuoles and any differentiation. They are arranged transversely to the axis of the pedicel, petiole, or long axis of floral organs [42-43].

2. AZ layer cells gain competence to receive an abscission signal. The abscission process is often associated with stress and senescence. In general, it is thought that the distal organ perceives stress signals (drought, salinity, extreme temperature, low irradiance or pathogen attack) and passes into senescence. This abscission signal is transmitted to the AZ, causing abscission. Phytohormones mediate the signal, primarily changes in auxin and ethylene concentration involved in the activation of AZ cells [42-44].

3. The separation, where the degradation of the cell wall in the AZ, is a result of hydrolytic enzymes' activity. In this process hydrolytic enzymes causing changes in cells and resulting in shedding have the major role, namely endo-ß-1,4-glucanase (EG) and polygalacturonase (PG) [40, 45-47]. In the past years, several genes whose expression is increased during abscission have been discovered [48-50], some of which are also responsible for the synthesising hydrolytic enzymes. A multigene family controls PG. For *Arabidopsis thaliana* there are at least 66 genes linked to PG enzyme [35,51].

4. The protection layer is formed on the wound surface. Organ abscission creates a situation whereby formerly internal tissues are exposed directly to the external environment. The new exterior surface must adopt the critical functions of the epidermis, including providing a barrier to water loss and pathogen infection. The protective layer forms basal to the separation layers and is continuous with the periderm of the stem. In most cases observed, the development of the protective layer involves transverse cell division, which may produce daughter cells with epidermal identity [52-53].

2.1.1. Role of Seeds in Regulation of Abscission.

Physiologically, apple-fruit growth can be separated into four stages: cell division, cell enlargement, maturation, and ripening [54-56]. A complex of internal and external factors influences every stage of development. Seed content and their growth were more vital role in controlling the growth of apple fruits [57-58]. Seeds ensure the persistence of fruit by producing phytohormones and regulating the movement of assimilates and nutrients [59-60].

Seeds and their endosperm are where phytohormones are produced, particularly auxins. Once the endosperm develops, the fruits grow rapidly, influenced by auxin. Following that, the embryo consumes the endosperm. This coincides with a lag phase of fruit growth and is commonly associated with fruit drop. After this event, the embryo has finished its development, and secondary endosperm emerges, which still generates auxin, which contributes to preventing abscission from occurring [61-62]. Additionally, the quantity and arrangement of seeds within the core tissue influenced fruit size and shape. Fruit asymmetry in Granny Smith results from unbalanced seeds sectors growth, associated with an asymmetrical seed weight distribution [63].

In pome fruits, the number of viable seeds is essential for fruit development. It was observed that fruits with fewer seeds drop more quickly during fruit drop, and genotypes with fewer seeds are more vulnerable to environmental stress like water drawback and poor nutrition [64-65]. The number of seeds needed for fruit development depends on the genotype. Profusely flowering apple genotypes need more seeds to maintain fruitlets at the beginning of fruit development than medium-density genotypes [12]. The diploid genotypes of apple trees need a minimum of 5-6 seeds per fruit to survive, and fertilization is more critical than triploid apples. It was established that the fruits with less than three seeds drop first during abundant fruit set [66]. The number of viable seeds required to maintain fruit changes gradually during the fruit growth period. In East Europe during June-drop, apple fruits need 4,25 viable seeds for development; before ripening fruits with more than 6,25 seeds persists [24]. The importance of developing of viable seeds has been characterized by studying of apple MADS-box gene expression [67-68].

In the early stages of fruit growth, transcriptional regulation tightly controls the metabolism of both cortex and seed tissue [69-70]. The ability of the fruit to attract nutrients is closely linked to internal factors like hormones and the development stage of the seed [71]. Smaller, immature fruits were less competitive than more developed fruits since they had fewer seeds and a lower auxin concentration. This led to their weaker sink strength than more developed fruits [72].

2.1.2. Role of Leaves in Regulation of Abscission

Leaves also control the growth of apple fruits. The quantity of fruits a tree can produce is determined by the photo assimilates generated by the plant's photosynthetic apparatus, primarily from the leaves near the inflorescence [73]. The fruit tree's photosynthetic system is retrieved not just by developing fruit but also by young shoots, which have a reduced photosynthetic capacity due to the presence of young leaves [74]. The sugars produced during photosynthesis are moved from the leaves to the fruit. Without the nutrient source (leaves), the fruit will be dropped [75]. The relationship between fruit development and shoot growth changes during the growing season [24]. At least 1 – 4 leaves are required for apple fruit to maintain before petal fall. During the June drop, 10 to 15 leaves are required, for fruit development. Before maturity, 40 leaves are needed to provide nutrients to the fruit [76-77]. A decreased ratio of leaves/flowers during blooming leads to a lower fruit set percentage and encourages early fruit-drop. Correlations have been found between shoot growth and fruit drop. Apple varieties such as `Cox Orange Pipin' and `Starking' that produce few fruits in fruitlets are impacted by vigorous shoot growth. The intense growth of shoots creates a shortage of carbohydrates for fruitlets and results in abscission, even if there is a small amount [78]. Relationships have been identified between shoot development and fruit shedding. Apple varieties with vigorous shoot growth generally drop more fruit than cultivars with less vigorous vegetative growth [53,79].

Leaves can induce fruit abscission, related to abscisic acid movement from leaves to fruits [80]. According to Goren and Goldschmidt [81], mature citrus leaves have higher abscisic acid levels in the later stages of the growth season than young leaves [82]. A similar tendency was observed in the apple tree leaves since young leaves can prevent fruits from dropping, while older leaves can trigger it [80].

2.2. Phytohormones and their Role in Fruit-Drop Process

Plants can use enough light, water, minerals, and carbon dioxide to synthesize vital chemical compounds like phytohormones for survival. At extremely small amounts, phytohormones act as signalling molecules that induce plant physiological reactions and regulate various aspects of the plant's life, such as cell division, growth, tissue development, reproduction, and response to the environment. Almost every plant cell can produce phytohormones [83-84].

Phytohormones control seed germination, plant architecture, flowering, fruit abscission, maturation, and ripening. There are six main classes of phytohormones: auxin (IAA), cytokinin (CK), gibberellin (GA), abscisic acid (ABA), brassinosteroids (BRs) and ethylene (ET) [3]. Phytohormones are crucial in controlling organ abscission events. IAA and ET were confirmed to be key elements involved in the control of abscission. These two phytohormones act in an antagonistic manner towards each other [85]. Generally, plant hormones that stimulate abscission are ET and ABA, while IAA and GA suppress abscission [37,53]. Research on different plants (*Arabidopsis*, apple, tomato) supports the idea that a decrease in auxin biosynthesis and an increase in ethylene biosynthesis are key factors in the abscission signalling pathway. In the studies with *Arabidopsis*, it was found that lower auxin levels in the abscission zone led to the early fall of floral organs [51,72,85]. Conversely, suppose the pathway of auxin signalling or response in AZ is disrupted. In that case, the abscission of floral organs is delayed, indicating that a functional auxin pathway in abscission zone cells is needed for abscission initiation [86]. The auxin level decreased compared to non-abscissed fruits in the dropped fruitlets of *Areca catechu* L. (Betel nut palm) and their abscission zone [87].

Ethylene is closely related to the abscission signalling molecule that triggers cell detachment in the abscission zone [82]. In a study comparing the internal ethylene levels of persisted apple fruits were compared to abscissed fruits, higher levels were found in the detached fruit, indicating a correlation between higher ethylene levels and fruit abscission [3]. *Arabidopsis* mutants deficient in ethylene receptors such as ein2, ein3, etr1, and ers2 showed different degrees of delay in the removal of floral organs. CK and ABA also influenced the abscission process. Agustí and Estornell et al. [53,82] suggesting that the impact of ABA and CK on plant organ abscission may be influenced by auxin or ethylene rather than acting directly. It has been demonstrated that gibberellin acid and brassinolide can prevent fruit from abscission. Spraying of GA on peach (*Prunus persica*) branches stopped fruit drop [88]. Spraying of BRs prevented the shedding of fruitlets by suppressing the transcription of the genes LcACS1/4 and LcACO2/3 through LcBZR1/2 in litchi. (*Litchi chinensis*) [84]. Jasmonic acid promotes organ abscission. The external application of methyl jasmonate induced the abscission of apples and tomatoes [89-90]. Jia et al. [87] reported that abscessed fruitlets of *A. catechu* showed significantly higher levels of JA, SA, and ABA in AZ than non-abscessed fruitlets. Starkus [12] reported that phytohormones IAA, ABA, and the ratio of IAA/Z are the most important regulators of fruit abscission. Genotypes that tended to bloom abundantly had higher IAA content than genotypes that bloomed on average. As a stress signalling hormone, ABA influenced fruit abscission only when environmental conditions were unfavourable, and fruit trees were exposed to drought [12].

Eccher et al. [91] suggested a hypothetical model for apple-tree fruitlet elimination. He stated that the cortex is a primary sensor of nutrition stress in fruit trees. In the cortex molecular mechanisms linked to nutrition starvation activate hormone signalling pathways. The ratio of ethylene in the cortex and the seeds controls abscission. The ethylene receptors act as inhibitors. Once receptors are complete, they trigger the ethylene signalling pathway, leading to seed death and fruitlet abscission. Due to competition between fruits, gene expression levels vary depending on the stage of fruit development. Lateral fruit is less developed compared to central fruit. The ratio of ethylene production to receptors is greater in lateral fruitlets than in central ones [91]. The model shows that the inverse relationship leads to a higher sensitivity to ethylene, as the receptors need less ethylene to reach saturation [91-92]. This leads to the conclusion that apple fruitlets are abscissed after reaching the required threshold of ethylene level [91-92].

During the early stage of abscission, auxin deficiency genes like PK7, ERF1, and WRKY could be upregulated or downregulated. This causes an increase or decrease in the expression of auxin-responsive genes [93]. These genes trigger the activation of transcription factors or post-translational regulators specific to abscission. After that, the AZ became sensitive to ethylene and gained the ability to undergo abscission. During the final stage of abscission, genes activated in the abscission zone, like cell wall-degrading enzymes, carry out the abscission process [93].

2.3. Competition for Carbohydrates in Generative Organs

Bangerth stated that ethylene is not the only factor causing fruit to abscise [72]. The "correlative dominance effect" of adjacent fruit or nearby shoot tips could have an effect. Regarding this theory, determining which fruit will fall depends on the level of its auxin transport. When more substantial polar auxins transport meets weaker auxin, the weaker is abscised [94]. The dominance of one fruit over another depends on: (1) the gap in time (hours or days) for fruit set between dominant and suppressed fruit; (2) the number of seeds per fruit; (3) the strength of vegetative shoots' growth and distance from fruitlet; (4) the number of fruits within the inflorescence [72].

The combination of the dominant effect may be related to the export of auxin to a particular fruit. The centre flower (CF) is known to bloom earlier than the lateral flower (LF), giving it an advantage in development over LF, resulting in dominant polar auxin transport. The strength of the auxin pathway is also influenced by the quantity of developing seeds in the fruit. The distance and growth strength of adjacent vegetative shoot tips are other factors that could affect the transportation of polar auxin in fruits [72].

The final effect of the correlative dominance on auxin transport in dominant fruits causes a scenario where the auxin content drops below a specific threshold value. This causes the cessation of abscission inhibition, sensitization and activation of the AZ by ethylene, ultimately leading to the abscission of the fruits [72,95]. In addition to these factors mentioned previously, the amount of sunlight and temperature and their interaction also play a role in fruit abscission. Several studies have shown that a reduction in photosynthetic active radiation (PAR) affects fruit abscission [96].

Besides polar auxin flow to dominant fruits or vegetative shoots, the imbalance of organic substances is the second reason causing the abscission of fruitlets [24]. Abundant blooming rate typically leads to a low fruit set percentage [97]. Fruits that begin growth earlier dominate over fruits that develop later. Because of an inadequate nutrient supply, fruits that are set later are smaller than earlier-set fruits. Eventually, earlier-set fruits dominate, and other fruits are abscissed due to a lack of nutrition supply [21,98].

In fruit trees carbohydrate reserves are vital for sustaining tree function (respiration, cell differentiation, bud development) and for supporting early spring growth before trees can produce photoassimilates independently [25,99]. Apple trees store carbohydrates in the form of starch. In apple and pear trees, sorbitol is the main product of photosynthesis. It is the primary form of transported carbohydrates and a crucial soluble reserve. Sorbitol can be converted into sugars like sucrose, glucose, fructose, and starch Apple trees do not have specialized carbohydrate storage structures, so the fluctuation of carbohydrate content is present during growth season can be identified [99-101].

After blooming, a large amount of carbohydrates supplied from primary leaves is utilized by growing fruits, leading to a positive carbon balance in the tree [102-103]. The shoots have enough sorbitol content to provide early fruit development [104]. During growth season, demands for carbohydrates grow from other active meristems and rapidly growing shoots, resulting in a deficit of carbohydrates [105].

Two weeks after blooming, the amount of sorbitol is insufficient for fruit growth [104]. Carbohydrate deficiency results in a slower growth of fruitlets and elimination of those left behind [105-106]. As soon as the first leaves expand carbohydrate supply begins and continues throughout all season, but several shortages periods may occur [99].

3. Genetic Regulation of Fruit Drop

Regulation of separation of cells in the abscission zone is a complicated physiological process precisely regulated by gene expression. It is known that the hormones ethylene and auxin play an important role. However, the abscission cannot be explained just by the action of these hormones. Neither the sole increase in ethylene nor the decrease in auxin is 100 % responsible for the abscission [108-109]. Besides the genes of hormone synthesis, critical pathways are the receptors for the hormones and the transport of them. Hormone gene expression is regulated by transcription factors belonging to the MADS group. Kinases regulate the interplay of all participants in the abscission [110].

The actual abscission process must be performed by cell wall alteration and separation enzymes and protection layer-forming substances. To elucidate the genetic mechanism of abscission, researchers utilized apple cultivars that exhibited contrasting abscission behaviours or artificially induced fruit drop and identified DEG [94,111-113].

3.1 Ethylene Pathway Genes Participating in Abscission

The ethylene biosynthesis pathway was established in higher plants in the last century and later became even more specified. This begins with the production of S-adenosyl methionine (SAM) from the amino acid methionine. Subsequent The reactions of aminocyclopropane-1-carboxylic (ACC) acid synthase (ACS) and aminocyclopropanecarboxylate oxidase (ACO) convert the SAM to ethylene [114]. Ethylene production-related gene MdACS1 is a marker associated with fruit drop. To investigate genes related to fruit drop, the preharvest fruit drop (PFD)-susceptible cultivar 'Golden Delicious' was compared with the PFD-resistant cultivar 'Fuji' [94]. Ethylene gene expression was evaluated. Genes MdACS5A and MdACO1 in the abscission zone of the cultivar 'Golden Delicious' were upregulated, and the ethylene level increased. On the other hand, 'Fuji' did not show any changes in the same genes [94]. Varieties vary in susceptibility to PFD due to the transcription of the precursor to ethylene production, known as MdACS1 [115]. Ethylene can trigger fruit abscission by activating genes that stimulate the production of hydrolytic enzymes such as polygalacturonase and cellulase. In the abscission-prone apple variety 'Golden Delicious', genes MdPG2 and MdEG1 are activated during high ethylene production. These genes are related to enzymes that break down the abscission zone cell wall [51,94,111]. Differences between individuals bearing ACS1-1 and ACS1-2 alleles had median fruit drop rates of 35.4% and 8.6%, respectively. The early ripening and increased fruit drop could be attributed to ethylene production by the ACS1-1 allele [116]. Li et al. [94] found that the transcript levels of ACC synthase genes MdACS1, MdACS3, and MdACS5A increased rapidly in the fruit cortex of ‘Golden Delicious’ apples, which typically perform PFD and have a higher ethylene level and rapid softening during ripening. Only MdACO1 transcripts increased in ‘Fuji’ apples, which appeared to have low ethylene levels, slow softening, and no PFD [94]. Sun et al. [117] studied *Malus* accessions of wild species, domestic cultivars, and hybrids. They found that the majority had wild-type ACS, “the *ACS1-1/1* allelotype was identified in >70% of accessions, mostly in wild species, whereas only ~9% of accessions exhibited the *ACS1-2/2* allelotype. They found that each allelic group (*MdACS1-1/1, -1/2,* or *-2/2)* contained abscising and non-abscising accessions. The *MdACS1-1/1* allelotype was overrepresented among accessions, showing the earliest natural abscission, whereas the *MdACS1-2/2* allelotype was overrepresented among non-abscising accessions. The correlation between internal ethylene and allelotype was not very strong [117]. Since abscising and non-abscising cultivars are present in each of the categories of the wild-type allele, the low-transcription allele, and the heterozygous allele (MdACS1- 1/2), this suggests the importance of other factors [4]. The association of MdACS1- 1/2 alleles with fruit drop was still confirmed to be high. [116]. Wang et al. [118] observed only moderate change in ACS in fruit-abscission and fruit-retention cultivars. However, ACO varied significantly across cultivars with different fruit abscission types [118]. ACS expression was downregulated in apples treated with ethylene inhibitor, while other ethylene synthesis and signalling genes had relatively similar expression levels [119]. The increased ethylene production signal is received by ethylene receptors (ETR, ERS). When bound to ethylene these receptors (ETR, ERS) reduce a protein kinase, called constitutive triple response 1(CTR1) activity, which leads to lower inhibition of membrane protein ethylene-insensitive 2 (EIN2) and further transduction of the ethylene signal to ethylene response factors (ERFs) [120]. The ethylene receptor genes MdETR1, MdETR2, MdERS1, and MdERS2 increased in the fruit cortex of cultivars known for PFD and non-PFD, but only MdETR2 and MdERS2 increased in the abscission zone of 'Golden Delicious' apples [94]. Ferrero et al. [121] noticed higher initial expression with a subsequent drop of MdETR2 and MdETR102 in lateral fruitlet seeds, suggesting that receptor increase shows fruitlet's attempt to maintain homeostasis and prevent abscission. The application of chemicals reducing pre-harvest fruit drop results in lower ethylene synthesis related to reduced MdACS1, MdACO1, MdETR1, and MdERS1 expression but unchanged levels of MdCTR1 [122]. Eccher et al. suggest that differential expression of ethylene receptors in seeds and synthesis genes of ethylene in the cortex may result in a critical ratio for the onset of abscission [123]. Upregulated receptor genes ETR2 and ERS1 and downregulated ethylene biosynthesis genes (ACO3, ACO4, and ACO4-like, SAM2) correlate with reduced abscission [112].

3.2. Abscission Hormone Abscisic Acid

As the mane of hormone states, it was found to be related to abscission. High levels of abscisic acid (ABA) were found in abscised fruitlets; the mechanism of action is not very clear, but there is a proposal that higher levels of ABA in abscission-prone fruitlets compared to resistant is related to differentially expressed genes of ABA metabolism. Eccher et al. found that ABA synthesis final sept β-glucosidase is increased in lateral fruitlets, whereas ABA 8′-hydroxylase that leads to degradation of abscisic acid is increased in central fruitlets [123]. Zhu et al. [124] also found an increase in ABA synthesis genes in artificial abscission-induced fruitlets. However, genes and the degree of expression differed depending on the method used.

3.3 Auxin Pathway Genes in Abscission

A high auxin concentration inhibits abscission by decreasing sensitivity to ethylene [53]. Changes in genes related to auxin biosynthesis, signal transduction, transport, and homeostasis modulate auxin flow. Devoghalaere et al. [125] studied the effect of exogenous auxin application on fruit drop and auxin-related genes and emphasized the importance of auxin response factor (ARF) in the auxin effect. ARF3 expression was upregulated in the chemical abscission induction [126]. Wang et al. [118] did not observe the change in the auxin synthesis pathway in abscission-prone and resistant apples, whereas chemically induced abscission resulted in auxin biosynthesis-related gene downregulation [112]. There was a difference in auxin-related gene expression in chemically and shade-induced apple abscission, particularly related to the auxin transport gene (PIN1 and auxin efflux carrier) [124]. Auxin, produced by the developing seed of the fruitlet, is translocated down the pedicel through the abscission zone, preventing abscission. Celton et al. observed down-regulation of auxin response factors and the PIN1 transporter gene in abscission-prone fruitlets, while genes encoding NAC domain transcription factors were up-regulated in the pedicel of non-abscising fruitlets [127].

3.4. Transcription Factors in Abscission Regulation

Ethylene and auxin have been identified as the main abscission regulators; therefore, TFs involved in ethylene and auxin signalling pathways, EIN3, ARFs, and Aux/IAAs, are also important in abscission [84]. Abscission-inducing conditions resulted in ten differentially expressed TFs, including ERF/AP2 transcription factors, bZIP proteins, MADS-box, and MYB domain proteins co-regulated by abscission-inducing conditions [124]. Independently of ethylene signalling, differentiation of the abscission zone is regulated by MADS-box genes. The tomato gene JOINTLESS is considered as one of the master regulators for the specification of abscission zone cells [128]. Complex formation with other MADS-box proteins belonging to SEPALLATA and MACROCAYLYX is required in AZ formation in tomatoes [129]. In the SEPALLATA class gene MADS8/9-suppressed apples, the GH3 auxin-conjugating enzymes had less expression [130], highlighting the importance

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of lowering auxin concentration in the abscission process. A lack of this complex results in changes in many gene expression patterns. One of these genes, the transcription factor SlERF52, is underexpressed and is essential for the upregulation of genes associated with abscission, particularly those encoding cell wall hydrolytic enzymes such as PG and Cel. As a result, TAPG1, TAPG2, TAPG4, and Cel5 are induced at significantly lower levels, with PG being more severely affected than Cel5 and Cel1 remaining unchanged [131]. Nakano et al. identified two apple JOINTLESS homologues, but their interaction with SEPALLATA and MACROCAYLYX was different from that of tomato JOINTLESS [131]. In apple abscission studies JOINTLESS gene was upregulated when abscission was artificially induced [124]. Heo et al. suggest that MdJOINTLESS may not be related to apple abscission since in lateral pedicels that are prone to abscission, the expression of MdJOINTLESS was not increasing even though it was much higher than in abscission-resistant fruitlets [132]. Also, Lee et al. [133] did not observe an increase in MdJb expression. In the group of WRKY TFs known to be activated and engaged in fruit abscission, there were expression differences between fruit-abscission cultivars, and fruit-retention cultivars as most were upregulated in fruit-abscission cultivars. However, McWRKY19 was considerably down-regulated in fruit-abscission cultivars [118]. Zhu et al. also pointed out distinct sets of WRKY TFs that were differentially expressed depending on the abscission induction method [124]. Lee et al. found an increased expression of MdWRKY57, which activates the ABA biosynthesis related to abscission [133].

3.5 Abscission Zone Cell Remodelling Genetics

Treatments that cause abscission correlate with the downregulation of a more considerable number of photosynthesis-related genes, sorbitol/sucrose transporters, cyclin and cyclin-dependent kinase (CDK), also cellulose synthase genes were repressed. Zhu et al. [124] reported that abscission-causing treatments led to membrane and cytoskeleton function genes, including microtubule, vesicle-mediated membrane transporter and cell adhesion genes downregulations. However, these treatments lead to the upregulation of genes related to glycolysis and starch degradation and genes related to cell wall loosening and hydrolysis, including β-1,3-glucanase (Cel), polygalacturonase (PG) and expansin (EXPA) [124]. The cell wall loosening expansion (MdEXPA10) expression was upregulated in the pedicel [133]. Fruitlets that are more likely to undergo abscission had under-expressed sugar transport genes and, fruitlets that are less likely to undergo abscission had overexpression of genes associated with vascular tissues, mainly phloem may be related to a better supply of nutrients [127].

4. Interplay of Phytohormones and Gene Expression in Abscission

Since abscission is a complex process, huge physical changes occur in the abscission zone. In addition to cell wall-altering genes, there are changes in cell structure and transport-related genes [124]. The abscission zone had expressed genes related to vesicle trafficking, such as the Rab family of small GTPases, dynamins, and syntaxins [113]. Button et al. noticed upregulation of gene participating in vesicle trafficking [126]. Abscission-prone cultivar GD with higher levers of ethylene had increased levels of *MdPG2*, a polygalacturonase gene, but only in the abscission zone. The MdPG1 gene was expressed in the cortex of fruit or in non-PFD cultivars and is not related to abscission. The gene responsible for fruit abscission, Cel1 (*MdEG1)*, was also overexpressed in the abscission zone but not in the cortex of PFD-prone cultivar [94]. After treatment with abscission inhibitor, downregulation of genes related to cell wall degrading enzymes (PG, Cel, and EXPA) was reported [112].

The abscission control in central and lateral apple fruitlets differs (Figure 1). The central fruitlet has better-developed seeds than the lateral. Therefore, production of auxin (ARF) is higher, there are more polar auxin transporters (PIN) and the expression of ethylene receptors (ETR) and sensors (ETS) is higher, which prevents ethylene (Et) signalling, thereby reducing abscission zone (AZ) cell competence for abscission. The expression of 8′-hydroxylase leads to abscisic acid (ABA) degradation, preventing it from closing stomata, allowing normal transpiration, photosynthesis and release of

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ethylene from the cortex. In lateral fruitlets, the number of seeds is lower, and they are less developed than seeds of central fruitlets. Therefore, the reduced carbohydrate availability induces key enzymes ACS (ACC synthase; ACC-1-aminocyclopropane-1-carboxylic acid) and ACO (ACC oxidase) responsible for ethylene biosynthesis in the cortex. Additionally, higher expression of β-glucosidase – the ABA synthesis enzyme in lateral fruitlets leads to higher concentration of ABA and closure of stomata, which cause reduced transpiration and photosynthesis. Due to the lower number of ethylene receptors (ETR and ETS) in the lateral fruitlets seeds leads to faster saturation with ethylene and ethylene signal transduction, as saturated ETR and ETS prevent inhibition of ETHYLENE INSENSITIVE 2 (EIN2) so that the signal is transduced to ETHYLENE RESPONSIVE FACTOR (ERF). ERF induces programmed cell death in seed cells and synthesises of cell wall degrading enzymes in the AZ. However, ERF expression is regulated by MADS-box genes JOINTLESS, MACROCALYX and SEPALLATA complex, which overexpressed in abscising tomato fruitlets but still not confirmed in apple fruitlets (Figure 1).

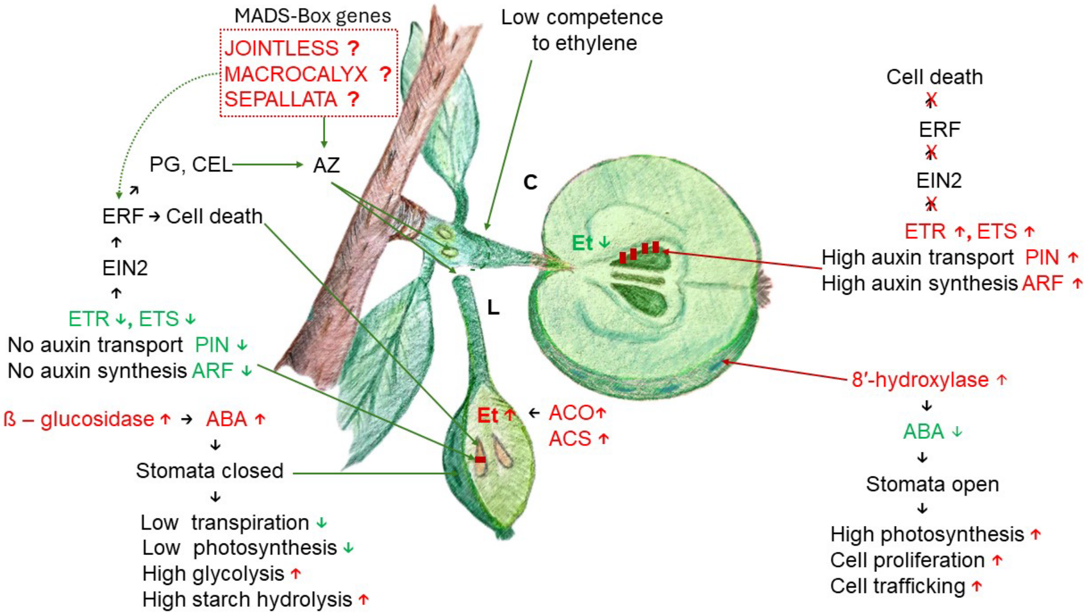

Figure 1. The abscission control is based on phytohormone and gene expression in central (C) and lateral (L) apple fruitlets. Red arrows indicate the increase, while green - the decrease of processes. ABA - abscisic acid, AZ - abscission zone, AFR – auxin, ACS - ACC synthase; ACC-1-aminocyclopropane-1-carboxylic acid, ACO - ACC oxidase), EIN2 - ETHYLEN INSENSITIVE 2, ERF -ETHYLENE RESPONSIVE FACTOR, ethylene (Et), ethylene receptors (ETR), ethylene sensors (ETS), polar auxin transporters (PIN). .

5. Conclusions

Abscission is the process of removing unnecessary organs and is essential in reproduction. The process occurs in a specific area called the abscission zone. It has four stages: 1 development of small AZ cells in localized regions, 2 gain of the competence of AZ cells usually mediated by phytohormones, 3 the separation caused by cell wall degrading enzymes, and 4 the formation of the protecting layer. The main emphasis in this review is on stages 2 and 3. The ability of apple cultivars to regulate fruit load, especially during “June drop”, is a desirable characteristic for commercial use. The breeding focusing on optimal fruit load needs a better understanding of genetic mechanisms for fruitlets self-elimination. Lack of seed development causes the production of auxin, which prevents fruit drop. Another important signal for the abscission is reduced availability of assimilates as there is a higher number of fruitlets than the tree can support. The number of leaves is critical in determining how many fruitlets can be supported. There is competition between central and lateral fruitlets, and the control of the abscission differs at phytohormone and gene expression levels (Figure 1). Increased vasculature in central fruitlets ensures sufficient transport of nutrients. The signal for abscission is believed to be first detected by the cortex. Reduced carbohydrate availability induces increased ethylene synthesis, confirmed by higher transcription-related genes (SAM, ASC and ACO). The MdASC1 could be a relatively good marker for abscission prediction. Ethylene signal transduction should pass through ethylene receptors and sensors (ETR and ETS). Lower expression of these receptors in lateral fruitlets leads to faster saturation with ethylene. The signal is then passed to ETHYLENE INSENSITIVE 2 (EIN2) since CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) no longer has inhibitory activity. Ethylene response factor (ERF) participates in programmed cell death signalling that leads to the abortion of embryos' lateral fruitlets. Seeds are the primary source of auxin production, so abortion leads to lower auxin production. This is a positive feedback loop since less auxin leads to higher sensitivity to ethylene. The lateral fruitlets have lower expression of auxin polar transport gene (PIN) and increased expression of GH3 auxin-conjugating enzymes that leads to increased competence of abscission zone cells for the abscission. Central fruitlets have more and better-developed seeds that produce more auxin, PIN and higher expression of ethylene receptors, which prevents ethylene signalling, thereby reducing abscission zone cell competence for abscission. The abscission process is related to the loosening of the cell wall and the transcription enzymes, including β-1,3-glucanase (CEL), polygalacturonase (PG) and expansin (EXPA). The expression of CEL and PG are related to the ERF transcription factor, which expression in tomato is regulated by JOINTLESS, MACROCALYX, and SEPALLATA complex. The studies of apple homologue of MADS-box JOINTLESS do not report consistently increased transcription in *Malus domestica* JOINTLESS (MdJ) genes. Therefore, further research is needed to evaluate the MADS-box genes on abscission regulation in apples.

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