Article

Tracing the Evolution of the SEPALLATA Subfamily across Angiosperms Associated with Neo- and Sub-Functionalization for Reproductive and Agronomically Relevant Traits

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Abstract: SEPALLATA transcription factors (SEP TFs) have been extensively studied in angiosperms as pivotal components of virtually all the MADS-box tetrameric complex master regulators of floral organ identities. However, there are published reports that suggest that some SEP members also regulate earlier reproductive events, such as inflorescence meristem determinacy and inflorescence architecture, with potential for application in breeding programs in crops. The SEP subfamily underwent a quite complex pattern of duplications during the radiation of the angiosperms. Taking advantage of the many whole genomic sequences now available, we present a revised and expanded SEP phylogeny and link it to the known functions of previously characterized genes. This snapshot supports the evidence that the major SEP3 clade is highly specialized for the specification of the three innermost floral whorls, while its sister LOFSEP clade is functionally more versatile and has been recruited for diverse roles, such as the regulation of extra-floral bract formation and inflorescence determinacy and shape. This larger pool of angiosperm SEP genes confirms previous evidence that their evolution was driven by whole-genome duplications rather than small-scale duplication events. Our work may help to identify those SEP lineages that are the best candidates for the improvement of inflorescence traits, even in far distantly related crops.

Keywords: MADS-box; SEPALLATA; phylogeny; core eudicots; monocots; angiosperms; inflorescence architecture; meristem determinacy; floral organ development; crops

1. Introduction

Several classes of MIKC-type MADS-box TFs are essential for the specification of all floral organs, as described by the ABC model [1]. They function by forming homo- or heterodimers that, based on the quartet model, further combine into tetramers [2,3]. Unique tetrameric combinations of MADS-box TFs specify the identities of each of the floral organs (sepal, petal, stamen, carpel) and ovules, as well as floral meristem determinacy. Although there are plenty of in vitro experiments supporting the quartet model, conclusive proof is still lacking in vivo, where, although most interactions have been confirmed [4], it has not been exactly confirmed that tetramers must form, such that it cannot be excluded that simple dimers might be functional at least in some of the target genes [5,6].

Among these classes of MADS-box TFs, the so-called SEPALLATA (SEP) is the only common component of all the known functional complexes and is thus essential for the identities of all floral organs [7–9]. Most plant genomes encode several SEP TFs, which are often functionally redundant; hence, single mutants may display no or only a slight phenotype. However, in the absence of SEP function, flowers lose determinacy and all their organs are reverted to leaf-like structures [10], thus suggesting that all floral organs are, indeed, modified leaves, as proposed by Goethe in 1790 [11]. Although the ABC model was derived from the observation of loss-of-function mutants in Arabidopsis thaliana and Antirrhinum majus and although the possible homology of perianth organs between core...
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Most angiosperms produce flowers arranged in diversified clusters termed inflorescences [17,18], which are orchestrated by the inflorescence meristem (IM) and, eventually, by a subsequent hierarchical order of specialized reproductive meristems, such as the branch meristems (BMs) [19–21]. The relevant products of most crops and ornamental plants are their fruits and seeds or flowers, respectively. Therefore, the modification of inflorescence architecture is a major goal of breeding programs in crops and ornamental plants [22–25].

A few works conducted on distantly related angiosperms have shown that some SEP TFs have important roles not only in floral development but also in the regulation of IM function and/or of the other reproductive meristems that derive from it. For example, the SEP genes of tomato (Solanum lycopersicum) JOINTLESS-2 and ENHANCER-OF-JOINTLESS-2 (J2 and EJ2) are two important domestication loci for jointless pedicel and large calyx traits, respectively, but are also important regulators of inflorescence complexity and productivity [26,27]. The loss of OsMADS34/PANICLE PHYTOMER2 (PAP2) function profoundly alters inflorescence development and architecture in rice (Oryza sativa) [28–30]. Similar SEP genes have been shown to regulate IM function and determinacy even in the highly modified and specialized capitulum inflorescence of Asteraceae [31].

Within MIKC-type MADS-box genes, SEP forms a well-defined subfamily specific to and ubiquitous in angiosperm plants. It is divided into two major sister clades, SEP3 (AGL9) and LOFSEP (AGL2/3/4) [32,33], whose split coincided with the whole-genome duplication ‘Epsilon’ (WGD-ε) that predated the most recent common ancestor (MRCA) of angiosperms [34,35].

2. Results and Discussion
2.1. Evolution of the SEP ALLATA Subfamily in Core Monocots

To better understand the evolution and complexity of the SEP subfamily in core monocots, we took advantage of high-quality genome assemblies currently available from Poales, other commelinids and a few Asparagales (orchids, Asparagus officinalis and Allium cepa), the remaining taxonomic orders being still poorly or not represented. All the SEP gene models that we retrieved from these monocots, as well as those from core eudicots
and Amborella trichopoda, had eight exons and seven introns. The few exceptions were clearly due to incomplete or incorrect annotations, showing that the SEP gene structure is highly conserved across angiosperms. By comparison with the protein structure of Arabidopsis SEP3 TF [3], we determined that, in all the SEP genes that we studied, the MADS-box domain is encoded by exon 1, the I (intervening) domain by exon 2, the K (keratin-like) domain by exons 3 to 6, and the less conserved C-terminal region by exons 7 and 8 (data not shown).

A phylogenetic analysis revealed that LOFSEP formed two large subgroups in commelinid monocots, which we refer to as LOFSEP-A and LOFSEP-B hereafter (Figure 1). Grasses, Joinvillea ascendens (sister to grasses) and palms possessed genes from both clades. The result was supported by microsynteny analysis of representative species (Figure 2), which also compensated for the low bootstrap values in the phylogenetic tree for the palm species Elaeis guineensis and Phoenix dactylifera. As shown in Figure 2, a strong microsynteny is common to each group, A and B, of LOFSEP genes. Interestingly, a lower degree of microsynteny is also shared between A and B, indicating that they originated by an ancient large-scale or whole-genome duplication. Such an event was most likely the ancient WGD-τ that took place before the MRCA of core monocots [41,42]. Although the positions of sequences from Asparagales were unresolved in the phylogenetic tree (Figure 1), the analysis of microsynteny allowed us to assign the LOFSEP sequences of orchids to group A and an orphan gene of Asparagus officinalis (06.1985; Figure 1) to group B (Figure 2).

A very similar picture emerged from the analysis of the SEP3 clade, which was also separated into two large ‘A’ and ‘B’ groups (Figure 1). In this case, all the genes from grasses fell in the SEP3-A group, suggesting that grasses lost SEP3-B after their divergence from Joinvillea ascendens, while all orchid genes clustered strongly with several other genes of commelinids to form the SEP3-B group. Pineapple (Ananas comosus), Joinvillea ascendens and banana (Musa acuminata) possessed genes from both clades (Figure 1). Microsynteny results further supported the existence of the two groups (Figure 3). The small unresolved clade of five Allium cepa and Asparagus officinalis genes (Figure 1) likely belong to SEP3-A, based on microsynteny scores (Figure 3a). As exceptions to the evidence that single-gene duplications of MADS-box genes are rapidly lost, we found tandem duplications of SEP3 genes in Asparagus and Elaeis (Figure 1).

In conclusion, core monocots are characterized by four main groups of SEP genes: LOFSEP-A, LOFSEP-B, SEP3-A and SEP3-B, which, however, have been differentially retained throughout their radiation. Among the species that we analyzed, only Joinvillea ascendens (Poales, Joinvilleaceae) possessed member genes from all four clades (Figure 1).

Deciphering the Evolution of SEPALLATA Genes along the Lineage That Led to Grasses

Since the phylogenomic data suggested that an early duplication of both LOFSEP and SEP3 occurred in monocots, we sought to reconstruct the subsequent evolutionary path of these genes in Poaceae, the family of true grasses and cereals.
Figure 1. ML phylogenetic analysis of the SEPALLATA (SEP) subfamily genes from the core monocots commelinids and Asparagales. Dichotomies unequivocally linked to the angiosperm WGD-ε and the grass WGD-ρ events are marked with a red star. The three LOFSEP subclades of grasses, OsMADS1, OsMADS5 and OsMADS34, are marked with different shades of green. The two SEP3 subclades of grasses, OsMADS7/45 and OsMADS8/24, are marked with different shades of blue. Two tandem duplication events of SEP3 genes were detected in Asparagus officinalis and in Elaeis guineensis, which are marked with green and blue connected circles, respectively.
Figure 1. ML phylogenetic analysis of the SEPALLATA (SEP) subfamily genes from the core monocots commelinids and Asparagales. Dichotomies unequivocally linked to the angiosperm WGD-ε and the grass WGD-ρ events are marked with a red star. The three LOFSEP subclades of grasses, OsMADS1, OsMADS5 and OsMADS34, are marked with different shades of green. The two SEP3 subclades of grasses, OsMADS7/45 and OsMADS8/24, are marked with different shades of blue. Two tandem duplication events of SEP3 genes were detected in Asparagus officinalis and in Elaeis guineensis, which are marked with green and blue connected circles, respectively.

Figure 2. Microsynteny analysis of LOFSEP genes from representative species of commelinids and Asparagales. Conserved loci are connected by lines of the same color. For simplicity, the non-conserved loci were omitted. In each chromosomal region, the LOFSEP locus is marked with a red asterisk. The LOFSEP-A gene is lost in the conserved region of Ananas comosus, in agreement with the phylogenetic analysis shown in Figure 1. The linked SQUA locus, when present, is marked with a black asterisk.

Two more rounds of WGD occurred in the MRCA of Poales (WGD-σ) and then in the MRCA of Poaceae (WGD-ρ) [43], which would predict up to eight SEP3 and eight LOFSEP genes in extant diploid grasses, such as rice, barley (Hordeum vulgare), Brachypodium distachyon, Sorghum bicolor and Pharbitis latifolia. Instead, only two SEP3 (OsMADS7/45 and OsMADS8/24) and three LOFSEP (OsMADS1, OsMADS5 and OsMADS34) paralogous lineages have been maintained in grasses, respectively (Figure 1, Table 1), which we named after their corresponding genes in rice [44]. These five lineages are highly conserved in diploid grasses (Figure 1, Table 1). Comparison of the relatively ancient allotetraploid maize (Zea mays; [45–47]) versus the recent allohexaploid bread wheat (Triticum aestivum; [48]) gives clues as to the speed of the process of selection of SEP homeologous genes after a polyploidization event: while only two out of five duplicated copies have been retained in maize, three homeologs for each gene still exist in bread wheat (Table 1). In addition, atypical local duplications of the OsMADS1- and OsMADS5-like genes were found in
the Aegilops–Triticum complex (Table 1), whose existence and functionality are mostly supported by transcriptome assemblies publicly available in NCBI GenBank (data not shown). Therefore, these two well-studied polyploid genomes suggest that the elimination of excessive SEP homeologous genes is quite a long process. In recent polyploids, processes of pseudogenization and epigenetic silencing are likely to take place beforehand [49].

Figure 3. Evolutionary analysis of the SEPALLATA (SEP) subfamily in core monocots. (a) Microsyn-teny analysis of SEP3 genes from representative species of commelinids and Asparagales. Conserved loci are connected by lines of the same color. For simplicity, the non-conserved loci were omitted. In each chromosomal region, the SEP3 locus is marked with a red asterisk. The linked FLC locus, when present, is marked with a black asterisk. (b) Representation of the most likely pattern that drove the evolution of the SEP subfamily in extant grasses (Poaceae), based on our analysis and previous works. Based on the phylogeny results shown in Figure 1, the grass lineage lost SEP3-B after its divergence from the sister family Joinvilleaceae.
Table 1. Accessions of all the LOFSEP and SEP3 loci found in the diploid genomes of *Oryza sativa* (rice), *Pharus latifolius*, *Brachypodium distachyon*, *Hordeum vulgare* (barley), *Aegilops tauschii* and *Sorghum bicolor*, in the ancient allotetraploid *Zea mays* (corn) and in the recent allohexaploid *Triticum aestivum* (bread wheat).

| LOFSEP          | SEP3           |
|-----------------|----------------|
| **Oryza sativa**| **Pharus latifolius** | **Brachypodium distachyon** | **Hordeum vulgare** | **Aegilops tauschii** | **Triticum aestivum** | **Sorghum bicolor** | **Zea mays** |
| OsMADS1         | LOC_Os03g11614 | Phala.01G312100 | Brad1g68980        | HORVU4Hr1G067680 | AET4Gv20607600 | AET4Gv20611300 | AET4Gv20678000 | TraesCS4A02G028100 | TraesCS4A02G057800 | TraesCS4B02G245700 | TraesCS4B02G245800 | Zm00001d028217 | Zm00001d048082 |
| OsMADS5         | LOC_Os06g06750 | Phala.04G039300 | Brad1g48520        | HORVU7Hr1G025700 | AET7Gv20313600 | AET7Gv20313900 |                      | TraesCS7A02G122100 | TraesCS7B02G020800 | TraesCS7B02G020900 | TraesCS7D02G120500 | Sobic.010G050500 | Zm00001d045231 |
| OsMADS34        | LOC_Os03g54170 | Phala.01G078100 | Brad1g08326        | HORVU5Hr1G095710 | AET5Gv20911100 |                      |                      | TraesCS5A02G391800 | TraesCS5B02G396700 | TraesCS5D02G401700 |                      | Sobic.001G086400 | Zm00001d034047 |
| OsMADS7/45      | LOC_Os08g41950 | Phala.08G027600 | Brad1g41260        | HORVU7Hr1G054220 | AET7Gv20638300 |                      |                      | TraesCS7A02G261600 | TraesCS7B02G186600 | TraesCS7D02G261600 |                      | Sobic.007G193300 | Zm00001d031620 |
| OsMADS8/24      | LOC_Os09g32948 | Phala.12G134000 | Brad1g34680        | HORVU5Hr1G076400 | AET5Gv20667800 |                      |                      | TraesCS5A02G286800 | TraesCS5B02G286100 | TraesCS5D02G294500 |                      | Sobic.002G258000 | Zm00001d021057 | Zm00001d006094 |
Grasses are devoid of SEP3-B genes, while their highly homologous OsMADS7/45 and OsMADS8/24 paralogous lineages seem to have emerged by duplication of SEP3-A after their divergence from Joinvilleaceae (Figure 1), suggesting that such duplication coincided with the grass-specific WGD-ρ. This is strongly supported by the observation that the OsMADS7/45 and OsMADS8/24 lineages reside in highly syntenic chromosomes, as can be seen, for example, in synteny maps of rice and barley [50]. Based on our phylogeny results (Figure 1), the origins of the OsMADS1 and OsMADS5 paralogous lineages were likely the same; however, they are located on unrelated chromosomes, and OsMADS5 even lost the microsynteny shared by the other monocot LOFSEP genes (data not shown). This suggests that either OsMADS5 transposed or that major rearrangements of its genomic position occurred in the grass MRCA.

The third and more functionally diverged LOFSEP clade of grasses is OsMADS34, which was believed to exist only in grasses up to now. However, our analysis clarified that it belongs to the LOFSEP-B lineage (Figures 1 and 2), meaning that it very likely diverged from its out-paralogues OsMADS1 and OsMADS5 at the time of the ancient WGD-τ, which occurred before the MRCA of core monocots [41,42]. Since rice OsMADS34/PAP2 is an important regulator of inflorescence architecture [28–30,51], this interesting and non-canonical SEP function might exist also in its orthologues within and outside grasses—a matter that requires further research.

Taken together, our data support a precise pattern of SEP subfamily evolution and expansion in grasses, which is summarized in Figure 3b, where the Poales-specific WGD-σ made no contribution.

In general, the rate of sequence divergence seems to be much higher in LOFSEP than in SEP3 TFs, which could already be noticed by comparing the homeologous peptides encoded by bread wheat A, B and D sub-genomes. The SEP3-like homeologous peptides accumulated just 0–4 aminoacidic changes only in the C-terminus (Figures S1–S5), suggesting that SEP3 is under stronger selective constraints.

2.2. Three LOFSEP Sister Clades and a Single SEP3 Clade Evolved in Core Eudicots

In core eudicots, our analysis confirmed with strong support the existence of three highly conserved LOFSEP clades (Figure 4), which we named SEP1/2, FBP9/23 and SEP4, in agreement with a previous work [32]. Since all extant core eudicots are descendants of a hexaploid MRCA, such expansion of the LOFSEP lineage is likely related to the ancestral whole-genome triplication event known as gamma (WGT-γ) [52–56]. Indeed, our analysis of grape (Vitis vinifera), a model for the study of genome evolution in core eudicots [34,55], revealed that the genomic regions of SEP1/2, FBP9/23 and SEP4 share significant collinearity with each other (Figure 5), in agreement with previous models of the origin of angiosperm-specific MADS-box subfamilies [35]. The FBP9/23 clade was lost in Brassicaceae after the divergence from Carica papaya and, probably, also in coffee (Coffea arabica) and Lamiales (which are represented by Erythranthe guttata, Antirrhinum majus and Olea europaea in our analysis) (Figure 4). Three MADS-box genes involved in inflorescence complexity in tomato, J2, EJ2 and LONG INFLORESCENCE (LIN), have been reported as SEP4 homologues [26]. However, our phylogenetic analysis unambiguously placed J2 and EJ2 in the FBP9/23 clade, while only LIN and its close homolog RIPENING INHIBITOR (RIN/LeMADS-RIN; [57]) belonged to the SEP4 clade (Figure 4).
Figure 4. ML phylogenetic analysis of the SEPALATA (SEP) subfamily genes in core eudicots. Dichotomies unequivocally linked to the angiosperm WGD-ε and the core eudicot WGT-γ events are marked with a red star. The three LOFSEP subclades of core eudicots, SEP1/2, FBP9/23 and SEP4, are marked with different shades of green. The main SEP3 subclade and the Asteraceae-specific SEP3 clade are marked with different shades of blue.
Figure 4. ML phylogenetic analysis of the SEPALLATA (SEP) subfamily, including relevant subclades of core eudicots, in grape (Vitis vinifera L.). The main chromosomal region, the linked LOFSEP and SQUA loci are marked with red and black asterisks, respectively. They form the three tandems SEP1/2–FL, SEP4–EuAP1 and FBP9/23–EuFUL [35].

In striking contrast, we only found one conserved monophyletic SEP3 group in core eudicots, except for an Asteraceae-specific clade (Figure 4) that was already reported by Malcomber and Kellogg [32]. In the Vitis vinifera genome, the only SEP3 locus resides on chromosome 1, orthologous to the whole core eudicot clade. As expected, we have identified two other microsyntenic regions in grape that derived from WGT-γ, on chromosomes 14 and 17 (data not shown), but these have lost their ancestral SEP3 copies. Intriguingly, the genomic location of the Asteraceae-specific SEP3 clade corresponds to the microsyntenic region of grape chromosome 17 (data not shown), revealing that this lineage has ancient origins related to WGT-γ. Considering the evolutionary position of Asteraceae, this implies that recurrent independent losses of this paralogous clade occurred a surprising number of times throughout the radiation of extant core eudicots.

In conclusion, the LOFSEP clade is significantly more expanded than SEP3 in core eudicots, and those genomes that experienced only WGT-γ are predicted to possess a 3:1 ratio of LOFSEP to SEP3 genes (Figures 4 and 6), while further cycles of polyploidizations and gene losses occurred repeatedly and independently in the majority of core eudicot lineages. Our analysis supports the model of SEP subfamily expansion in core eudicots represented in Figure 6.

Figure 5. Conserved microsynteny between the three LOFSEP subclades of core eudicots, visualized in grape (Vitis vinifera L.). In each chromosomal region, the linked LOFSEP and SQUA loci are marked with red and black asterisks, respectively. They form the three tandems SEP1/2–FL, SEP4–EuAP1 and FBP9/23–EuFUL [35].

Figure 6. Representation of the most likely pattern that drove the evolution of the SEPALLATA (SEP) subfamily in extant core eudicots, based on our analysis and previous works.
2.3. Conserved Genetic Linkage between SEPALATA, SQUAMOSA and FLOWERING LOCUS C Subfamilies

Phylogenomic reconstructions showed that, in the angiosperm MRCA, the ancestral LOFSEP and SQUAMOSA (SQUA) genes formed a close tandem, while the ancestral SEP3 was in tandem with FLOWERING LOCUS C (FLC), and that this configuration has been maintained in many extant angiosperms [35]. In core eudicots, SQUA underwent a process of triplication just as LOFSEP did, which led to three paralogous LOFSEP–SQUA tandems, as clearly shown in the grape genome (Figure 5). All these LOFSEP–SQUA and SEP3–FLC linkage relationships were lost in the lineage of Arabidopsis and other Brassicaceae.

While noticing several such tandems during our analyses of monocots (Figures 2 and 3a and additional data not shown), we found that only one LOFSEP–SQUA tandem, i.e., OsMADS34–OsMADS14, is still conserved in rice (Figure 2), with an intergenic space of just 6 kb, and in other grasses. These two loci act synergistically in floral induction in rice [58], and the latter regulates vernalization-induced flowering in winter cereal crops [59]. An SEP3–FLC tandem is also conserved in rice genomes: OsMADS7/45–OsMADS37 [35,44].

More generally, both LOFSEP and SQUA genes play pivotal and diversified roles in agro-nomically relevant traits, such as floral induction, vernalization, inflorescence architecture and flower and fruit development. Targeted gene modifications, selection of natural or mutagenesis-induced variants and functional characterizations must be carried out with awareness of these conserved genetic linkage groups, which also hint at possible coregulation mechanisms. In tomato, the misinterpretation of the classic rin (ripening inhibitor; [60]) mutant led to models depicting the SEP4 ortholog RIN (Figure 4) as indispensable to the induction of fruit ripening. Unexpectedly, however, rin is not a knock-out but a gain-of-function mutant encoding a chimeric protein from RIN and from the downstream SQUA gene Macrocalyx (MC), whose new properties as a transcriptional repressor actively repress ripening: RIN, indeed, is not indispensable to the induction of fruit ripening, being only required for the completion of normal ripening [61].

2.4. Patterns of Sub- and neo-Functionalization Associated with Diverged SEPALATA Lineages

Our analysis provides new insights into the evolutionary history of the SEP subfamily in core monocots and core eudicots. Inferred polyploidization events at the base of both lineages caused a first round of independent amplifications of LOFSEP and SEP3 genes, followed by many others throughout the radiation of these angiosperms. The resulting duplicated genes followed different paths of retention and loss in different taxa. In addition, SEP genes seem to have diverged significantly between commelinids and Asparagales, and even within Asparagales. Here, we were able to bypass the limits of phylogenetic analysis by analyzing microsynteny.

An increasing number of functional studies are clarifying that the concept of full redundancy is misleading and that the several LOFSEP and SEP3 subclades that we have defined are instead specialized to regulate specific functions. Arabidopsis has only one SEP3 gene (Figure 4), which is highly redundant, along with the LOFSEP genes SEP1 and SEP2, in conferring FM determinacy and the identities of the three inner floral whorls. The Arabidopsis sep1 sep2 sep3 triple mutant produces indeterminate flowers made only of sepals [7]. SEP3 in not expressed at early developmental stages in the first whorl domain [62], where the last LOFSEP member of Arabidopsis, SEP4, is expressed instead [10]. In the Arabidopsis sep1 sep2 sep3 sep4 quadruple mutant all the floral organs are converted to leaves, showing that SEP4 alone is sufficient to specify sepal identity in the sep1 sep2 sep3 triple-mutant background [10]. Despite the significant degree of redundancy shown under experimental conditions, mass spectrometry analysis of in vivo formed complexes showed that SEP3 is far more abundant than SEP1 and SEP2 in the petal, stamen and carpel identity MADS-box complexes of Arabidopsis, while SEP4 is absent [4]. Moreover, the transcriptional activation potential of SEP3 exceeds those of SEP1 and SEP2 [63]. Altogether, these data point to SEP3 as the most important SEP TF for floral identity in Arabidopsis. Unfortunately, only partial gene titration experiments on sep mutants have been reported.
with respect to J2 with contributions from SEP4. In Asteraceae, one OsMADS34 exists in other core monocots, which opens new perspectives for future functional studies, especially in monocot crops with complex inflorescences, such as pineapple and palms.

It is intriguing that LOFSEP genes have been recruited to regulate inflorescence development in several species, mostly by limiting branching and promoting the switch to FM identity, which are functions that temporally precede their well-known and essential functions in flower development. In Solanaceae, the FBP9/23 subclade is the main player, with contributions from SEPI [26,27,72], while SEPI/2 genes are the main regulators of IM determinacy in the capitulum of Gerbera hybrida [31,80]. Rice OsMADS34 and SQUA-like genes synergistically act to specify IM identity, downstream of the florigen signal [58]. Subsequently, OsMADS34 limits inflorescence primary branching by repressing IM activity [28–30]. In addition, OsMADS34 shares functions with OsMADS5 in repressing secondary branching by promoting the maturation of meristems toward the spikelet meristem stage and in promoting the elongation of the inflorescence rachis and branches [30]. As a consequence, osmads34 and osmads5 osmads34 knock-out mutants produce much more branched inflorescence primordia, but several meristems subsequently fail to develop into mature, fertile spikelets [30], similarly to what has been observed in tomato plants defective with respect to J2, EJ2 and LIN functionality [26]. Unfortunately, mild OsMADS34 alleles able to trigger more productive inflorescences, which could be beneficial for breeding programs, have not emerged so far. The function of OsMADS34 in inflorescence architecture is likely conserved in other grasses [81,82]. Our analysis reveals that genes similar to OsMADS34 exist in other core monocots, which opens new perspectives for future functional studies, especially in monocot crops with complex inflorescences, such as pineapple and palms.
Given the fact that different LOFSEP subclades have been recruited for similar inflorescence functions in rice, Solanaceae and Asteraceae reveal their ancestral potential in regulating inflorescence development, which then was lost or retained during evolution.

3. Conclusions

We found both LOFSEP and SEP3 genes ubiquitously in core eudicot and monocot species, which suggests that each clade has specific essential functions besides their shared roles in FM and floral organ identity. The strong conservation of SEP3 genes, in terms of sequences and expression patterns, suggests that their major role in petal, stamen and carpel identity complexes was established and fixed before the MRCA of monocots + eudicots, while LOFSEP genes appear to have enjoyed more functional flexibility to allow their neo-functionalization, acquiring diversified roles in different angiosperm families, such as the regulation of bract identity, pedicel abscission zone, calyx size and inflorescence architecture. Therefore, besides their relevance for understanding angiosperm evolution, some SEPALLATA genes are major players in agronomically relevant traits. While SEP3 or other MADS-box homeotic mutants are potentially useful in the creation of ornamental floral oddities and flowers less attractive to insect pests [71,83], the biotechnological manipulation of LOFSEP genes or their network shows promise with respect to the improvement of inflorescence characters, such as numbers of flowers and fruits. To this aim, genes from the FBP9/23 and SEP4 clades are promising candidates for further studies in asterid species with branched inflorescences, while homologues of rice OsMADS34 are likely the main players in grasses and, perhaps, even in other core monocots.

4. Materials and Methods

All the SEPALLATA genes used in this study were identified through BLAST analysis of the following databases: NCBI Genome (Tarenaya hassleriana, Gerbera hybrida, Petunia x hybrida, Zingiber officinale, Elaeis guineensis, Phoenix dactylifera, Apostasia shenzhenica, Dendrobium catenatum, Phalaenopsis equestris), Gramene (Aegilops tauschii and Triticum aestivum), www.oniongenome.wur.nl (Allium cepa), the Snapdragon Genome Database (http://bioinfo.sibs.ac.cn/Am/index.php; Antirrhinum majus) and Phytozome 13 (all the other species). Genes from Asparagus officinalis and Ananas comosus were identified from both the NCBI and Phytozome 13 databases, and incomplete or incorrect annotations were eventually corrected by searching the NCBI Transcriptome Shotgun Assembly (TSA) database. Accession numbers are available in Table 1 and Table S1. Protein sequences were aligned using MAFFT (https://mafft.cbrc.jp/alignment/server/), checked manually and then back-translated to nucleotide alignments with PAL2NAL (http://www.bork.embl.de/pal2nal/).

Phylogenetic trees were calculated with MEGA 11 [84]. Evolutionary history was inferred using the Maximum Likelihood (ML) method and the Tamura–Nei model [85]. The model was accepted based on the high consistency of the resulting topologies with respect to previously published clades and genes. The trees with the highest log likelihoods were shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura–Nei model and then selecting the topology with a superior log likelihood value. The trees were drawn to scale, with branch lengths measured as the number of substitutions per site. Codon positions included were 1st + 2nd + 3rd.

Microsynteny was calculated and scored using SynFind [86] on the CoGe platform (https://genomevolution.org/coge/). Then, selected genomic regions and genes were downloaded from Phytozome 13 Phytomine and NCBI Genomes. Gene homology was confirmed manually with BLAST analysis. The final images shown in this work were generated with Simple Synteny online (https://www.dveltri.com/simplesynteny/; [87]). All the databases and online tools were accessed between November 2021 and July 2022.
Images were edited with InkScape 0.92 (https://inkscape.org/) and GIMP 2.10.32 (https://www.gimp.org/).

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants11212934/s1, Figure S1: Alignment of all the LOFSEP, OsMADS1-like proteins of Triticum aestivum (bread wheat), Figure S2: Alignment of all the LOFSEP, OsMADS5-like proteins of Triticum aestivum (bread wheat), Figure S3: Alignment of the three LOFSEP, OsMADS34-like homeolog proteins of Triticum aestivum (bread wheat), Figure S4: Alignment of the three SEP3, OsMADS7/45-like homeolog proteins of Triticum aestivum (bread wheat), Figure S5: Alignment of the three SEP3, OsMADS8/24-like homeolog proteins of Triticum aestivum (bread wheat), Table S1: Accession codes for all the genes reported in this study.

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