Besides and Beyond Flowering: Other roles of euAP2 genes in plant development

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Abstract: EuAP2 genes are famous for their role in flower development. A legacy of the founding member of this subfamily of transcription factor, whose mutants lacked petals in Arabidopsis. However, studies of other euAP2 genes in several species have accumulated evidence highlighting the diverse roles of euAP2 genes in other aspects of plant development. Here, we emphasize other developmental roles of euAP2 genes in various species and suggest a shift from regarding euAP2 genes as just flowering genes to consider the global role they may be playing in plant development. We hypothesize that their almost universal expression profile and pleiotropic effects of their mutation suggest their involvement in fundamental plant development processes.

Keywords: EuAP2 genes; Flowering; Plant Development

Introduction

APETALA2 (AP2) genes are named after a series of Arabidopsis mutants characterized by homeotic transformations of their sepals to leaves and petals to staminoid petals. Analysis of the ap2 mutants along with other floral mutants gave birth to the ABC model of flower development where AP2 is classified as an A-class gene [1,2].

The forerunner AP2 protein was cloned and characterized in Arabidopsis [3]. The Arabidopsis AP2 protein comprising 432 amino acids (aa) is mainly characterized by the possession of two AP2 domains, each made up of 68-aa with an 18-aa core conserved section that forms an amphipathic α-helix. The two AP2 domains called AP2-R1 and AP2-R2 (R for Repeat) have 53% amino acid identity and 69% amino acid homology. Their 18-aa core conserved sections show 83% amino acid homology [3]. Sequence analysis of the AP2 gene showed that it has a domain that can activate RNA polymerase II transcriptions factor and another domain that is a putative nuclear localization signal. The presence of these domains served as evidence to suggest that the AP2 protein is a transcription factor [3,4].

Following the cloning and characterization of the AP2 gene, other genes encoding two AP2 domains were identified in Arabidopsis [5-7]. About the same time, ethylene-responsive element binding proteins (EREBPs) from tobacco were shown to contain a conserved DNA binding domain [8]. Sequence comparison by alignment of EREBPs (aka ethylene responsive factor (ERF)) and AP2 domains revealed they were related [4,5]. This relationship subsequently lead to the classification of genes having AP2/EREBPs domains into one superfamily of transcription factors called Apetala 2/Ethylene Response Factor (AP2/ERF)
The AP2/ERF superfamily is divided into four subfamilies based on the number of AP2 domains and sequence similarity (Figure 1).

The AP2/ERF superfamily of transcription factors is one of the largest in most plant species whose genome sequences have been analysed [11]. The superfamily is divided into subfamilies viz; AP2, ERF, RAV and Soloist. Functional analysis of proteins belonging to AP2/ERF superfamily suggests that while genes belonging to AP2 and RAV subfamily are generally involved with developmental processes, ERF subfamily genes have been largely implicated in stress response processes [12-14].

AP2-subfamily is further divided into euAP2 and ANTEGUIMENTA (ANT) lineages (Figure 1.). EuAP2 lineage genes are distinguished from ANTEGUIMENTA (ANT) lineage genes by their possession of miRNA 172 binding sequence towards their C-terminal. They also lack 10 and 1 amino acid insertions found respectively in the first and second AP2 domains of ANT lineage genes [13].

The forerunner Arabidopsis AP2 protein belong to the euAP2 lineage. Genome-wide analysis showed that the euAP2 lineage is made up of six genes in Arabidopsis [13]. These six genes have been actively studied in the context of their role in floral ontogeny. The six members of this lineage in Arabidopsis have been linked with aspects of flowering such as floral meristem identity and flowering time, [15-17]. For recent updates on the ABC floral model see [18,19] and references therein. However, functional characterization in Arabidopsis and several other plants indicate that euAP2 genes are involved in other developmental processes besides flower development. Here, we present a summary of their expression profiles in various plant species, and attempt to summarize evidence that underscore the roles of euAP2 genes in other aspects of plant development. By highlighting other roles of euAP2 genes in plant development, we aim to bring attention to their possible involvement in global and fundamental plant developmental process(es).
Figure 1. Structure of AP2/ERF Transcription factor superfamily. *EuAP2* genes belong to the AP2 subfamily. They are distinguished from ANT lineage genes by the presence of miR172 binding site. Not to scale. Adapted from Kagaya et al. (1999) [52] and Kim et al (2005)[13].
Expression of euAP2 genes

EuAP2 genes are found expressed in major tissues (Figure 2). However, there are differences in the expression profile of individual genes. Their expression profile suggest a prominent gene that is more highly expressed in all tissues compared to others. This gene is called AP2 in Arabidopsis, INDETERMINATE SPIKELET (IDS) in maize, RICE STARCH REGULATOR 1 (RSR1) in rice, Q in wheat, and SLAP2a in tomato. This gene has been functionally characterized in the species listed. From such studies we learn that mutations in this prominent euAP2 gene leads to pronounced phenotypes [3,20-23].

Mutations in other euAP2 genes that are expressed quite broadly but less highly than the prominent euAP2 gene lead to no or less pronounced phenotypes. This has prompted the suggestion that they play redundant roles [17,24]. Interestingly, one or two euAP2 gene(s) in various species are not universally expressed (Figure 2). They may be found not expressed in one or two organs. A loose consensus is that they are not expressed in mature fruits and seeds.

However, mRNA expression profile of euAP2 genes should be interpreted carefully because miR172 has been proven to regulate translation of euAP2 mRNA into protein [15-17,25,26].
Figure 2. Expression Profile of euAP2 genes in selected Monocot and dicot species. The expression profile of all the euAP2 genes in root, leaf, flower and fruit of Arabidopsis, maize, rice and tomato. Irrespective of species, some EuAP2 genes are expressed in all the tissues surveyed, while one or two are not expressed in some tissues. The expression profiles were sourced from Expression Atlas (www.ebi.ac.uk/gxa/home) from the following experiments; Arabidopsis (Liu et al. 2012) [27], maize (Stelpflug et al. 2016) [28], rice (Sakai et al. 2011)[29] and tomato (Tomato Genome Consortium).
Briefly on miR172 regulation of euAP2 genes
A careful study of literatures reporting miR172 regulation of euAP2 genes suggest a seeming pattern of partial or total tempo/spatial regulation of euAP2 genes by miR172 at critical steps in the development of reproductive plant tissues. Apparently, euAP2 genes are freely expressed in various tissues during early vegetative growth stage. However, as the plant approaches reproductive stage, miR172 is recruited to regulate expression of euAP2 genes in timely and spatially restricted manner leading to the development of normal reproductive tissues [15,16,24-27]. Hence, ectopic autologous and heterologous over-expression of miR172 interrupts the vegetative growth stage activities of euAP2 genes and leads to precocious transition to reproductive stages in plants [17,28].

MiR172 regulation of euAP2 genes is very efficient even when euAP2 genes are over-expressed [16,17]. However, the regulatory ability of miR172 on euAP2 genes is very sensitive to base mismatches on the complimentary binding sequence on euAP2 gene mRNA. One base substitution on the miR172 binding site is enough to render an euAP2 gene resistant to miR172 regulation [26].

It appears that miR172 only regulates euAP2 genes [17]. This can be exploited in future studies by over-expressing miR172 to study the developmental roles of euAP2 genes in any plant species.

Roles of euAP2 Genes in Plant Development

Fruit Development

Functional analyses have shown that AP2-like genes are involved in tomato fruit development in two similar but independent studies [22,29]. In both studies, expression of tomato AP2 gene; SIAP2a was suppressed by RNA inhibition. Tomato AP2 gene (SIAP2a) was shown to affect the colour of ripe fruits. Ripe wild type fruits were totally red in colour while SIAP2a-RNAi fruits had uneven orange colour when ripe [22,29]. In these two studies, the investigators showed that the observed differences in the pigmentation of ripe tomato fruits can, in addition to other factors, be attributed to increased β-carotene to lycopene ratio in SIAP2a-RNAi fruits compared to the wild type.

Fruit softening and disintegration was also observed to occur earlier and rapidly in SIAP2a-RNAi tomato fruits than in wild type fruits [29].

Mature green tomato fruits of AP2i-1 lines were shown to have abnormal shape with indentations and uneven surface that splits open when ripe compared to wild type fruits which were round in shape and had smooth surface. [29].

In Arabidopsis whose fruit is a silique, null ap2 mutant fruits were found to possess larger replum compared to wild type. The lignin layer of the valve margin of ap2 fruits were also found to have more and larger cells compared to wild type [30]. The authors suggested that
AP2 negatively regulate replum development thereby preventing its overgrowth. They also reported AP2 to repress valve margin lignification [30].

The $Q$ gene in wheat is an $euAP2$ gene reported to be a major domestication gene that confers various agronomic traits that gives wheat its prominence among temperate cereals [21,31]. Wheat $Q$ gene is known to be associated with grain yield and yield components such as grain shape, grain threshability, thousand grain weight (TGW), glume shape and tenacity, [21,31-33].

The effects of $Q$ in wheat processing quality was recently reported by Xu et al. (2018)[34]. They mapped a new allele of $Q$ called $Q^{c1}$ from a wheat mutant ($S$-Cp1-1) characterized by dense spike. Their results demonstrated higher significant values in four wheat grain processing parameters in the mutant compared to wild-type. Remarkably, the new allele correlated with about 60 g kg$^{-1}$ increase in grain protein content (GPC) compared to $Q$. When used to make bread, loafs from the $Q$ mutant dough were larger compared to wild-type [34].

*RICE STARCH REGULATOR 1* (RSR1) has been demonstrated to negatively regulate starch synthesis genes in developing endosperm of rice grain [23]. Overexpression of *RSR1* severely downregulate starch synthesis genes leading to abnormal amyllopectin structure and increased gelatinization temperature. On the other hand, *rsr1* mutants had larger grain size, increased grain mass and yield [23].

*Triticum aestivum* *RICE STARCH REGULATOR 1* (TaRSR1) an orthologue of *RSR1* has also been reported to negatively regulate a group of important starch synthesis related genes during endosperm development in wheat [35]. In another study, Liu et al. (2016) [36], used barley stripe mosaic virus—virus induced gene-silencing (BSMV-VIGS) to silence TaRSR1. Their results confirmed that TaRSR1 negatively regulates critical starch synthesis related genes in wheat endosperm. This manifested in significant increase in grain starch contents, one thousand kernel weights, grain length and width of BSMV-VIGS-TaRSR1-infected wheat plants compared to wild-type.

**Seed Development**

*Arabidopsis AP2* (AtAP2) gene is known to play roles in seed coat morphology. [3,37]. The seed epidermal cells of *ap2-6* null mutants are rectangular in shape contrasting hexagonal shaped epidermal cells of wild type seeds. Developmental analysis by Western *et al.*, (2001)[37], revealed that the outer integument development proceeds normally in *ap2-6* seed coats until about 4 days after pollination (DAP). At this point further differentiation is terminated, so that at maturity, epidermal and sub-epidermal cell types and structures such as columella are absent. Consequently, mucilage synthesis, storage and secretion is absent or very limited in the seed coat of *ap2* seeds [3,37,38].
In similar studies, about the same time, two groups reported that $AtAP2$ influenced seed shape, size, mass, content and yield in *Arabidopsis* [39,40]. Seeds of *ap2* mutant plants were larger in size and had more weight compared to wild type seeds. Increase in seed weight and size in *ap2* plants were also accompanied by increase in total seed protein and total seed oils content compared to wild type seeds [39].

Ohto *et al.*, (2005) [40], also reported that *ap2* mutant embryos were larger and irregularly shaped compared to wild type embryos. They concluded that *AP2* affects embryo cell number and size.

**Heteroblasty**

Heteroblasty can be defined as the occurrence of plant organs that bear anatomical, physiological and morphological differences compared to identical organs earlier formed on same plant [41,42]. Following germination, an *Arabidopsis* plant usually produces rosette leaves separated by short internodes. Then the internode elongates and producing cauline leaves along the way before terminating in inflorescence [43]. The differences between *Arabidopsis* rosette and cauline leaves demonstrates heteroblasty.

*EuAP2* genes have been associated with leaf heteroblasty in *Arabidopsis* and maize [16,17,41,44]. *Arabidopsis* null mutants for *euAP2* genes produce lesser number of rosette leaves compared to wild-type plants. This was observed in single and multiple null *ap2* mutants. However, the number of cauline leaves produced were identical between multiple null *ap2* mutants and wild-type plants. In addition, hexuple null *ap2* mutant plants showed early formation of trichomes on their lower leaf surface signifying precocious transition from vegetative to reproductive phase [17].

Heteroblastic effects of Glossy15 (*GL15*), an *euAP* gene, on maize leaves is well documented [41,44]. Post-germination, a maize plant will first produce 5-6 juvenile leaves. Subsequent leaves are called adult leaves. Maize juvenile and adult leaves are distinct in some features such as cell wall characteristics, epidermal cell morphology, fine structure and histochemistry of epicuticular waxes. Over-expression of *gl15* leads to increase in the number of juvenile leaves consequently delaying transition from vegetative to reproductive phase [41].

**Inflorescence Architecture and Meristem determinacy**

The activities of *euAP2* genes in determining inflorescence architecture in grass species have been reported in maize, rice, wheat and barley [20,24-27]. See Bommert and Whipple (2017) [45], for a discussion of the role of *euAP2* genes in grass inflorescence architecture and meristem determinacy.

**Plant Height**
Plant height has been reported to be affected by the $Q$ gene in wheat [31]. The $Q$ gene gives rise to shorter plants by producing shorter culm lengths [46,47].

**Cleistogamy**

Flowers that are self-pollinated because they remain closed at maturity are cleistogamous. In cereals like barley, wheat and rice a pair of lodicule lie below the carpel and swell at maturity, forcing the lemma and ovary apart, which results in open (chasmogamous) flowers at maturity. Over-expression of *Cleistogamy 1* (*Cly1*), an euAP2 gene in barley has been shown to lead to cleistogamy [48,49]. In *Cly1* mutants, the lodicule does not enlarge at maturity but remain small leaving the florets tightly closed.

**Conclusion**

*EuAP2* genes are broadly expressed in plants. They have been shown to play roles in the development of different plant parts. It is possible that many other developmental effects of *euAP2* are not yet reported because of researchers’ focus on specific tissues. We therefore encourage a more holistic approach in characterization of AP2 mutants. Such an approach will facilitate the understanding of their roles in plant development, and exploitation for domestication and biotechnological purposes.

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