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Floral Genetics of African Nightshade (*Solanum* section *Solanum*)

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ABSTRACT

Solanum section *Solanum*, centering on the species commonly known as African nightshade, and botanically known as the "*Solanum nigrum* complex" is composed of a large number of morphogenetically distinct taxa, with certain common features. Their wide tolerance of habitat types, early flowering and prolific fruit production are adaptive features for success in the wild, semi-wild or weedy forms. In most parts of Africa and south-east Asia, their consumption, demand and market value as leafy vegetables have been on the rapid and steady rise in recent years due to their high nutritional and health benefits. However, production of these vegetables has traditionally remained on kitchen-garden scales with very low leaf yields. Notably, competition between vegetative and reproductive functions accounts for this low yield. Accurate manipulation of the switch from vegetative to reproductive development or elimination of the latter would potentially delay, reduce or eliminate competition from excess fruit load. A thermosensitive abnormal floral organ mutant (T-5) with sepaloid, stamenless and indeterminate phases has been induced in the sub-taxon *S. villosum*. The mutant will form an important basis for understanding reproductive developmental steps, such as floral induction, meristem formation, and organ development in African nightshade. This review explores the established floral genetic models as a basis to elucidate the aspects of floral genetics of African nightshade, with special reference to the T-5 mutant.

Keywords: abnormal floral organ, floral architecture, restoration, *Solanum nigrum*, temperature-dependent

CONTENTS

INTRODUCTION

Solanum L., to which African nightshade (*Solanum nigrum*-related species) belongs, is the largest and most diverse genus in the *Solanaceae* family, containing many essential food plants such as potato (*S. tuberosum* L.), egg plant (*S. melongena* L.) and naranjilla (*S. quitoense* Lam.); horticulturally useful plants such as winter cherry (*S. pseudocapsicum* L.) and jasmine nightshade (*S. jasminoides* Paxt.); medicinal plants such as bittersweet (*S. dulcamara* L.) and *S. viarum* Dun., both used as sources of corticosteroids. The section *Solanum*, centering on the species commonly known as the black, garden or common nightshade, *Solanum nigrum* L., is one of the most ubiquitous, largest and most variable species groups of the genus. Although the *S. nigrum*-related species are distributed throughout the world, they occur in their greatest concentrations in tropical and warm temperate regions with centres of diversity occurring in South America, Australia and Africa, with relatively few and less diverse species being found in Europe and Asia (Symon 1981; D'Arcy 1991).

The genus *Solanum* is distinguished from most of the other genera in the tribe *Solaneae* by its poricidal anther dehiscence. Although the species belonging to the section *Solanum* have been subjected to extensive taxonomic studies and many morphogenetically distinct taxa identified, this species group is still often referred to as the *Solanum nigrum*-complex by many scientists. Edmonds and Chweya (1997) proposed that the taxa of African origin, referred to as African nightshade, require urgent taxonomic revision. While members of this section play an important role in agricultural and nutritional systems of developing countries in Africa, South East Asia, Central and Southern America as indigenous/traditional leafy vegetables and medicinal herbs, they are treated as weeds in most developed countries. Unfortunately, there is also widespread confusion over the precise taxonomic identification, especially in those areas in which the species are most commonly used as food sources. In some developed countries, they are generally considered poisonous. Coupled with the lack of documentation of their total yields and sales in the past, these traditional leafy vegetables were given low priority in most agronomic, not to mention genetic, research and development programmes (Brown 1983; Prescott-Allen and Prescott-Allen 1990).

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Hypothesis Paper

Consumption, demand and economic value of these traditional leafy vegetables have been on a rapid and steady rise, especially in the urban centres such as Nairobi, Kenya where the leaves are sold in supermarkets as found in recent surveys (Masinde and Agong, unpublished). However, the leaf yields have been relatively low due to prolific early flowering and excessive fruit-set, which competes with leaf productivity. There are many reports indicating that male-sterile (non-fruiting) plants have more vigorous vegetative growth than those with excessive fruit-load (Hurd *et al*. 1979; Eckhart 1992; Heuvelink and Buiskool 1995; Poot 1997). A proper understanding of the flowering habit, the flower morphology, and pollination characteristics is indispensable for manipulation of the switch from vegetative to reproductive development or elimination of the latter. This would in effect help to delay the onset of reproductive growth, reduce or eliminate competition from excess fruit load and adjust the distribution of biomass to a favourable harvest index.

An abnormal floral organ mutant, T-5, with temperature-dependent structural phases was developed in *S. villosum* (the most common and popular taxon in Kenya) through $^{12}C^{5+}$ ion-beam irradiation (Ojiewo *et al*. 2006a). The T-5 mutant flowers are sepaloid from winter to mid-spring, stamenless in late-spring, indeterminate in summer and partially restored in autumn. These characteristics are replicable through all the mutant generations so far (M_5), making the mutant unique, with stable flowering pattern, potential for seed propagation and superior vegetative growth characteristics (Ojiewo *et al*. 2007). Besides being an important step towards circumventing the vegetative-reproductive imbalances that occur after anthesis, the T-5 mutant is a potentially versatile material expected to contribute significantly in the understanding of evolutionary features of flower development in the section *Solanum*. This could be a good starting point for taxonomists in delineating the distinguishing features of various *Solanum* taxa of African origin. This review uses the gene-based ABCDE and protein-based quartet models as the framework for elucidating the putative mutations responsible for the T-5 features. The underlying assumption is that there is considerable conservation in the genetics of floral initiation and development between floral model plants (*Arabidopsis thaliana*, *Antirrhinum majus* and *Petunia hybrida*) and the section *Solanum*.

FLORAL ARCHITECTURE OF *SOLANUM* SECTION *SOLANUM* (*SOLANUM NIGRUM*-RELATED SPECIES)

The gene-based ABC(D)E model

There is a wide variety in the number and position of organs among various plant species: some plants lack particular organ types, some show multiple whorls of certain organs, and some plants harbor organs of mixed characteristics such as petaloid sepals (Cronquist 1981). The basic architectural floral pattern is, however, remarkably invariant across all species (Lohman and Weigel 2002). In dicots, the flower is organized into four concentric rings of organs, termed whorls. The bauplan of a typical African nightshade flower consists of campanulate-stellate calyx, with 5 broadly triangular to ovate-lanceolate green leaf-like sepal lobes in the outermost whorl; white to purple corolla, a conspicuous basal star with 5 showy petals in the second whorl; 5 stamens with fused filaments and semi-fused oblong yellow anthers in the third whorl; and single carpel with straight exserted styles and capitate stigmas in the fourth, innermost whorl (**Fig. 1**). How do the floral meristems establish the precise flower pattern characteristics with proper positioning of the floral organs and specification of their identity in a position-dependent manner in this species group?

Genetic and molecular analyses of genes that control floral development in two model plants, *Arabidopsis thaliana* and *Antirrhinum majus* led to the proposal of a genetic model (Bowman and Meyerowitz 1991; Coen and Meyerowitz 1991; Ma 1994; Weigel and Meyerowitz 1994) that could account for the identity of the four whorls in typical eudicot, including *S. nigrum* flowers. Termed the ABC model, it states that the development of the four types of floral organs is governed by overlapping activities of three classes of regulatory genes A, B, and C, hereafter referred to as ABC functions. Expression of A-function genes in any one of the four flower whorls specifies the development of sepals, the combined expression of A-function and B-function genes specifies the development of petals, the combined expression of B-function and C-function genes specifies the development of stamens while the expression of C-function genes alone specifies the development of carpels. Further, the A-function and C-function genes are mutually exclusive and negatively regulate each other and the B function is restricted to the second and third whorls independent of A-function and C-function genes (Bowman *et al*. 1991; Coen and Meyerowitz 1991).

In *A. thaliana* the A-function is performed by APETALA1 (AP1) and APETALA2 (AP2); the B-function by APETALA3 (AP3) and PISTILLATA (PI); and the only C-function gene is AGAMOUS (AG) (Riechmann and Meyerowitz 1997). For simplicity, we would name the putative orthologs in *S. nigrum*-related species as SnAP1, SnAP2, SnAP3, SnPI and SnAG. However, acknowledging that there may be some variation in the homology of the genes controlling the floral architecture among the *S. nigrum*-related species, we will limit ourselves to the case study of *S. villosum* and name the corresponding genes as SvAP1, SvAP2, SvAP3, SvPI and SvAG.

Further analysis of two very closely related genes of *Petunia hybrida*, FLORAL BINDING PROTEIN7 (FBP7) and FBP11, resulted in the addition of D-class genes which specify ovule development (Colombo *et al*. 1995). Here, we will consider ovules as extra-floral organs; hence the D-function is not discussed further. Pelaz *et al*. (2000) observed that the expression of the ABC genes alone was not sufficient to superimpose floral organ identity in *A. thaliana*.

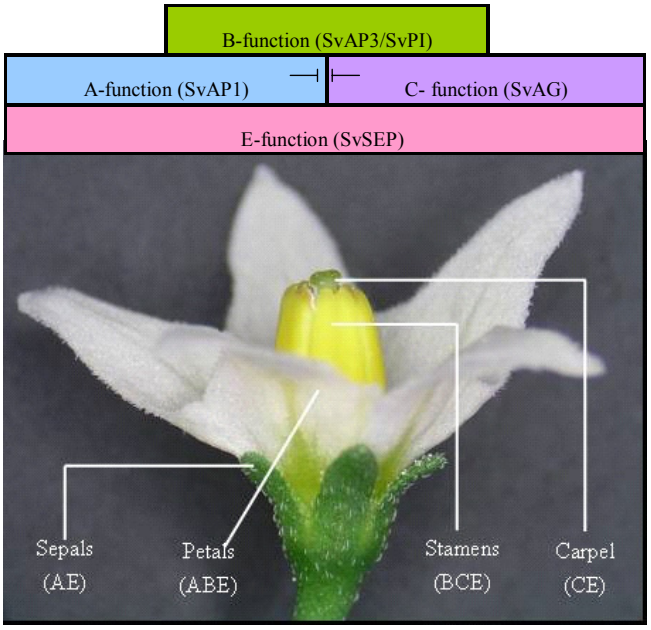

Fig. 1 The gene-based ABC(D)E model of floral organ identity showing the putative activity of 'floral organ identity genes' within adjacent whorls. A- and E-function genes specify the 5 sepals in whorl 1; A-, B- and E- function genes specify the 5 petals in whorl 2; B-, C- and E-function genes specify the 5 stamens in whorl 3; and C- and E- function genes specify the single carpel in whorl 4. Barred lines indicate the mutually antagonistic activities of A and C: A prevents the activity of C in whorls 1 and 2, and C prevents the activity of A in whorls 3 and 4. In *S. villosum*, the A-function is conferred by the putative gene SvAP1, B-function by SvAP3 and SvPI, C-function by SvAG and E function by SvAG.

In addition, AGAMOUSLIKE genes (AGL2, AGL4 and AGL9) which were first expressed in whorl 2, whorl 3 and whorl 4 before the onset of B and C gene expression were necessary. AGL2, AGL4 and AGL9 genes have since been renamed SEPALLATA (SEP1, SEP2 and SEP3), respectively, because the triple mutant flower consists of indeterminate flowers exclusively made of sepals. Considering the SEP genes as providing yet another floral homeotic function, Theißen (2001) suggested the term E-function, thus yielding an 'ABC(D)E model'. Additionally, Ditta *et al*. (2004) reported that AGL3 (SEP4) contributes to the development of sepals in addition to petals, stamens, and carpels. In *S. villosum*, the E-function would putatively be conferred by SvSEP genes. Thus, SvAP1 and SvSEP (A- and E-function genes) are needed for the 5 sepals; SvAP1, SvAP3/SvPI and SvSEP (A-, B- and E- function genes) for the 5 petals; SvAP3/SvPI, SvAG and SvSEP (B-, C- and E-function genes) for the 5 stamens; SvAG and SvSEP (C- and E-function genes) for the 1 carpel (**Fig. 1**).

The protein-based quartet model

Apart from AP2 in *A. thaliana* the rest of the ABCE genes regulating the floral organ development encode putative transcription factors that share a highly conserved domain called the MADS-box domain (Jofuku *et al*. 1994). MADS is an acronym for the first four members of the gene family: MiniChromosome Maintenance gene, **M**CM1 from *Saccharomyces cerevisiae* (Ammererer 1990; Passmore *et al*. 1989), **A**GAMOUS from *Arabidopsis thaliana* (Yanofsky *et al*. 1990), **D**EFICIENS from *Antirrhinum majus* (Sommer *et al*. 1990), and Serum Response Factor, **S**RF from *Homo sapiens* (Norman *et al*. 1988). The MADS-box genes encode transcription factors which play fundamental roles in developmental control of signal transduction processes in almost all eukaryotes (Messenguy and Dubois 2003).

All MADS-box genes have in common a highly conserved DNA sequence of about 180 base pairs length, which encodes the DNA-binding domain of MADS-domain proteins. In addition to the MADS-domain and unique to angiosperms is a moderately conserved extra domain called the K-box, after its homology to the coiled-coil structure of keratin (Ma *et al*. 1991). It mediates interactions between MADS-box proteins in conjunction with an intervening region (I-region), which separates it from the MADS-box (Davies *et al*. 1996; Fan *et al*. 1997). A C-terminal enhances/stabilizes interactions that are mediated by the K-domain (Fan *et al*. 1997; Pelaz *et al*. 2001). Genes encoding this type of protein have been termed MIKC-type MADS-box genes (Münster *et al*. 1997).

The K domain, which apparently is absent in animal and fungal MADS-domain proteins (Theißen and Saedler 1995; Theißen *et al*. 1996), has a conserved, regular spacing of hydrophobic residues that allows for the formation of an amphipathic helix. Such an amphipathic helix interacts with that of another K domain-containing protein to promote dimerization (Shore and Sharocks 1995; Riechmann and Meyerowitz 1997). Aided by the presence of the K-domain, the MIKC-type proteins, therefore, have a special potential to form complexes involving more than two homologous proteins constituting transcriptional regulators (Kaufmann *et al*. 2005).

Protein-protein interaction studies have revealed that the MADS-box transcription factors are active at the molecular level in a combinatorial manner. The protein–protein interactions between MADS dimers, mediated by the C-terminal domains result in a tetramer of MADS proteins that is simultaneously bound to a pair of diverse DNA-sequence elements, the CArG-boxes (CC-A rich-GG, e.g., $CC(A/T)_6GG$) (Riechmann and Meyerowitz 1997; Jack 2001). Each tetramer consists of two MADS dimers, each of which binds to a single CArG box. For example, the *Arabidopsis* PI and AP3 form a heterodimer to bind the CArG box *in vitro* (Goto and Meyerowitz 1994; Riechmann *et al*. 1996). The AP3 promoter has three CArG boxes, which are bound by the PI-AP3 complex, and AP3 is autoregulated by PI-AP3 (Krizek and Meyerowitz 1996; Tilly *et al*. 1998; Hill *et al* 1998; Honma and Goto 2000). It has been demonstrated that some homeotic MADS-box proteins from *Antirrhinum* form multimeric DNA-binding complexes (Egea-Cortines *et al*. 1999). Honma and Goto (2001) reported that *Arabidopsis* proteins also bind to DNA as multimeric complexes containing AP3 and PI (class B proteins), SEP3 (class E protein), and either AP1 (a class A protein) or AG (a class C protein). Higher-order complex formation results in enhanced stability in the binding to CArG-boxes, due to cooperative binding of two MADS dimers (Egea-Cortines *et al*. 1999). The protein-based floral 'quartet model' was introduced on the basis of these findings (Theißen and Saedler 2001). The model suggests that four different combinations of four different floral homeotic proteins (tetramer) determine the identity of the four different floral organs. In *S. villosum*, these combinations may be based on the putative formation of 4 different protein complexes. For the 5 sepals: SvAP1-SvAP1-SvSEP-SvSEP; for the 5 petals: SvAP1-SvAP3-SvPI-SvSEP; for the 5 stamens: SvAP3-SvPI-SvAG-SvSEP; and for the 1 carpel: SvAG-SvAG-SvSEP-SvSEP (**Fig. 2**).

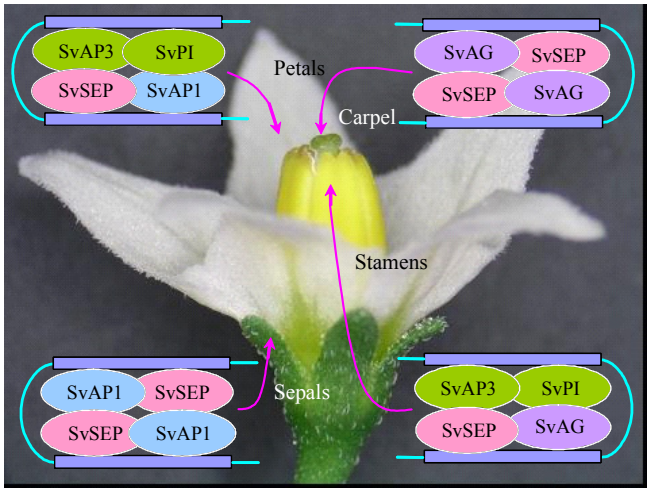

Fig. 2 *S. villosum* flower structure as related to the protein-based quartet model of floral organ specification. The protein complex transcription factors may operate by binding to two CArG-box sequences of a target promoter, either activating or repressing expression of the targeted gene. For example, in petals, the postulated heterodimer SvAP3-SvPI may bind to one CArG box as SvAP1-SvSEP heterodimer binds to a second CArG box.

Homo- or heterodimers of MADS protein transcription factors recognize and bind specific sequence of CArG boxes *in vitro* (Riechmann *et al* 1996). Interactions with either an unrelated protein or another MADS protein to form a tetramer of transcription factor, may be responsible for the modulation of DNA-binding specificity and transcriptional activity *in vivo* (Herskowitz 1989). The transcription factors operate by binding to two CArG-box sequences of a target promoter, either activating or repressing expression of the targeted gene for the development of the different floral organ identities (Riechmann *et al*. 1996; Jack 2001; Theißen 2001). For example, in petals, the SvAP3–SvPI heterodimer is postulated to bind one CArG box and an SvAP1–SvSEP3 heterodimer to a second CArG box. Likewise, in the stamens, the SvAP3–SvPI–SvSEP3–SvAG tetrameric complex may exert its function by specifically binding to the promoters of targeted genes (**Fig. 3**). Specificity of target gene selection may be achieved by the four complexes having different binding affinities for pairs of diverse DNA-sequences of the CArG-boxes and by the characteristic distribution of these pairs of CArG-boxes in the promoter regions of the different target genes. I-, K- and C-domains have been shown to mainly contribute tar-

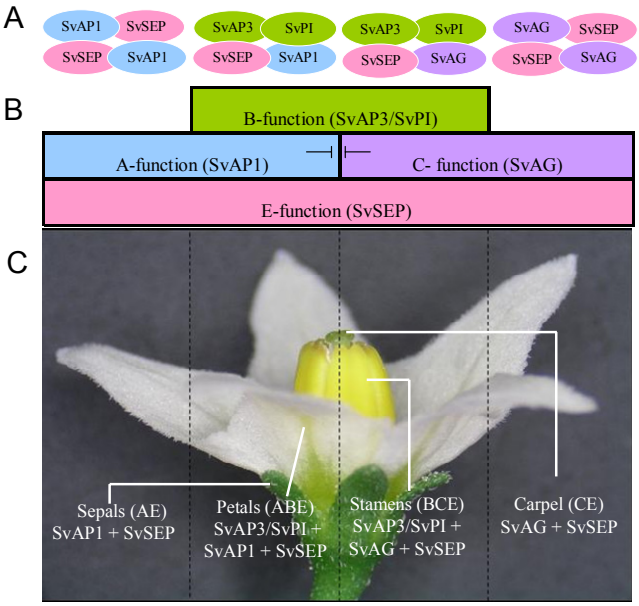

Fig. 3 The hypothetical gene-based ABC(D)E model and the protein-based quartet model. (**A**) The hypothesized transcription factor complexes, based on putative dimeric or multimeric protein interactions, necessary for each floral organ formation in *S. villosum*. (**B**) The hypothesized target genes activated or inhibited by the transcription factor complexes for the formation of sepals (A- and E-function genes), petals (A-, B- and E-function genes), stamens (B-, C- and E-function genes) and carpel (C- and E-function genes). (**C**) Determination of the four floral whorls as related to the ABC(D)E and the quartet models. The hypothesized transcription factor complexes and the target gene functions are shown.

get-gene specificity, implying an important role of protein interactions in the modification and specification of the binding to target-gene promoters. Since MADS-boxes encode DNA-binding domains which bind with a certain sequence specificity to CArG-boxes (Davies and Schwarz-Sommer 1994), it is conceivable, for example, that the SvAP1 encoded MADS-domain provides DNA-binding specificity to a SvAP1 gene product. The antagonism between the A and C function in the 'classical ABC model' could be due to the fact that protein complexes that contain AP1 repress the expression of the *AG* gene, and complexes that contain AG repress the *AP1* gene.

Temperature-sensitive floral mutant of *S. villosum*

In wild-type flowers, the A-function genes are expressed in the first and second floral whorls, the B-function genes in the second and third whorl, and the C-function genes in the third and fourth whorl, resulting in sepals, petals, stamens and carpels in whorls one, two, three and four, respectively (**Fig. 1**). The ABCE model was based on the phenotypic observations in a series of homeotic mutants in which floral organs developed in inappropriate whorls (Schwarz-Sommer *et al*. 1990). In *Arabidopsis* A-function mutants, (*ap1* and *ap2*) the first whorl organs develop as carpels instead of sepals and the second whorl organs develop as stamens in place of petals (Bowman *et al*. 1989, 1991, 1993). In B-function mutants (*pi* and *ap3*) sepals develop instead of petals in the second whorl and carpels instead of stamens in the third whorl (Hill and Lord 1989; Jack *et al*. 1992; Goto and Meyerowitz 1994). In C-function mutant (*ag*) petals develop in place of stamens in the third whorl and another flower replaces the carpels in the fourth whorl (Bowman *et al*. 1989; Yanofsky *et al*. 1990; Bowman *et al*. 1991). In E-function (*sep1 sep2 sep3*) triple mutants all whorls of the flower are converted into sepals (Pelaz *et al*. 2000) and the flower becomes indeterminate, a phenotype similar to B-function and C-function (*ap3 ag*, *pi ag*) double mutants. The *sep1 sep2 sep3 sep4* quadruple mutants develop vegetative leaves rather than sepals, petals, stamens, or carpels (Ditta *et al*. 2004).

The *S. villosum* T-5 mutant does not perfectly resemble any of the ABCE gene mutants because of structural dynamics which have been shown to be due to temperature sensitivity (Ojiewo *et al*. 2006b). Low growth chamber (10°C) and greenhouse (<15°C) night temperature favoured the formation of flowers with leaf-like organs only. High growth chamber (30°C) and greenhouse (>25°C) night temperatures favoured the formation of indeterminate flowers. Day/night temperatures of 30/20°C were found to favour formation of stamenless flowers in the growth chamber. The optimum temperatures for floral structure and fertility restoration were between 20-25°C (day) and 15-20°C (night). Temperature-sensitive floral organ mutant phenotypes have been reported in *Antirrhinum* (Schwarz-Sommer *et al*. 1992) and *Arabidopsis* (Bowman *et al*. 1989). The *Antirrhinum def-101* and *Arabidopsis ap3-1* mutant plants grown at low temperature (16°C and 15°C, respectively) produce nearly wild-type flowers, but growth at the non-permissive temperature (28 and 26°C, respectively) causes the second-whorl organs to develop as sepals (only A function present) and stamens to be replaced by carpelloid organs (only C function left in the third whorl).

The proposal that the K-box participates in protein–protein interactions was based on the temperature-sensitive defects of *def-101* (Davies and Schwarz-Sommer 1994). The mutant *def-101* has a lysine residue deleted at position 93 within the K domain of plant DEFICIENS (DEF) MIKC protein (Ma *et al*. 1991). Dimerization of DEF-101 with GLOBOSA (GLO) was shown to be temperature-sensitive *in vitro*, suggesting that unstable interaction with GLO was the basis for the temperature-dependent defect of DEF-101 *in vivo* (Zachgo *et al*. 1995). Arabidopsis *ap3-1* carries a missense mutation, resulting in a change from lysine to methionine at position 153 within the K-box of AP3 MIKC protein (Jack *et al*. 1992). At 25°C, exon 5 (which contains the mutation) is skipped by the splicing machinery, resulting in *AP3* cDNAs that fuse exon 4 directly to exon 6 (Jack *et al*. 1994). The fusion of exon 4 to exon 6 results in an in-frame 14–amino acid deletion in the AP3 protein, which results in a non-functional AP3 protein. This splicing defect is responsible for *ap3-1* phenotype (Sablowski and Meyerowitz 1998). Sensitivity to temperature may therefore be a property of a mutant protein which either loses its active conformation or the ability to interact functionally with other proteins under non-permissive conditions. Given the consistency of the function of the K box in protein–protein interactions, and the resulting sensitivity to temperature in mutations at the K-domain (Davies and Schwarz-Sommer 1994; Zachgo *et al*. 1995), it seems plausible to suggest that a MIKC protein mutant (probably at the K-box) could be responsible for the temperature-sensitive phenotypes of *S. villosum* T-5 mutant. The nature of the mutation and its molecular consequences are uncertain. Since the K domain also mediates either AP3/PI heterodimer formation or interaction with accessory factors (Ma *et al*. 1991; Yang *et al*. 2003; Kaufmann *et al*. 2005), we predict that a MIKC protein mutant that is non-functional at restrictive temperatures could result in inhibition of protein–protein interaction.

The T-5 mutant flowers remain 'vegetative' with large leaf-like structures in all the four floral whorls from winter to early-spring (**Fig. 4**), phenotype similar to the ABC triple loss of function mutants (Bowman *et al*. 1991) or *sep1 sep2 sep3 sep4* quadruple mutants (Ditta *et al*. 2004) in *Arabidopsis*. In A-function single mutants, the C function takes over the A function in whorls 1 and 2 resulting in a flower with carpels in whorl 1 and stamens in whorl 2. In the AB double mutant, only the C function is present, so the flowers are composed only of carpels. Mutations in all three functions lead to the transformation of all floral organs into leaf-like organs. The floral homeotic proteins require at least one of the four SEP proteins (E-function) to superimpose proper floral organ identity on vegetative leaf

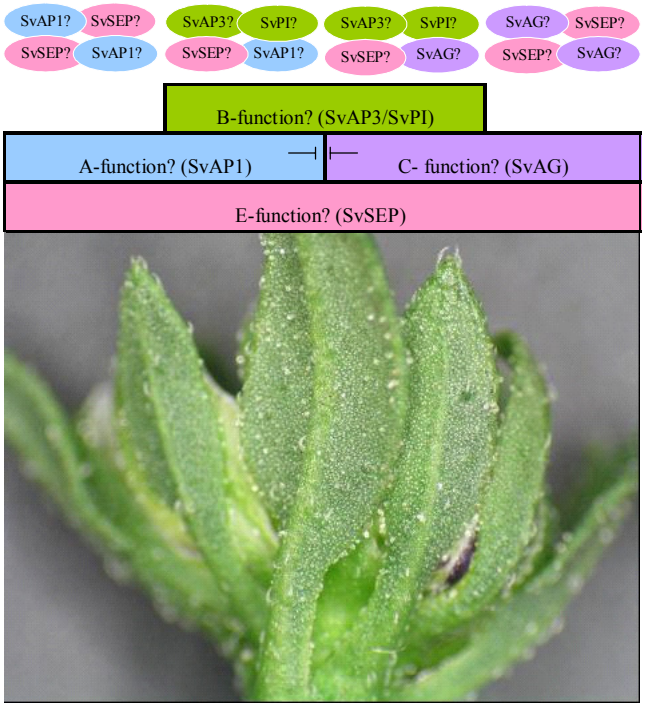

Fig. 4 Leaf-like organs of *S. villosum* T-5 mutant observed in all floral whorls under low temperatures (<15°C) in winter and early spring. This trait is similar to the phenotype displayed by *Arabidopsis* mutant flowers missing all the three ABC gene activities (Bowman *et al*. 1991). The quadruple *sep1/2/3/4 Arabidopsis* mutant displays indeterminate flowers composed only of leaf-like organs resembling those seen in ABC mutants (Ditta *et al*. 2004). The phenotype suggests that the putative A (SvAP1), B (SvAP3/SvPI) and C (SvAG) genes are not activated under low temperatures. The putative *Sv*SEP proteins are required as part of all four different protein complexes that would activate the putative ABC genes in the four whorls for the development of proper floral organs: sepals, petals, stamens and carpels.

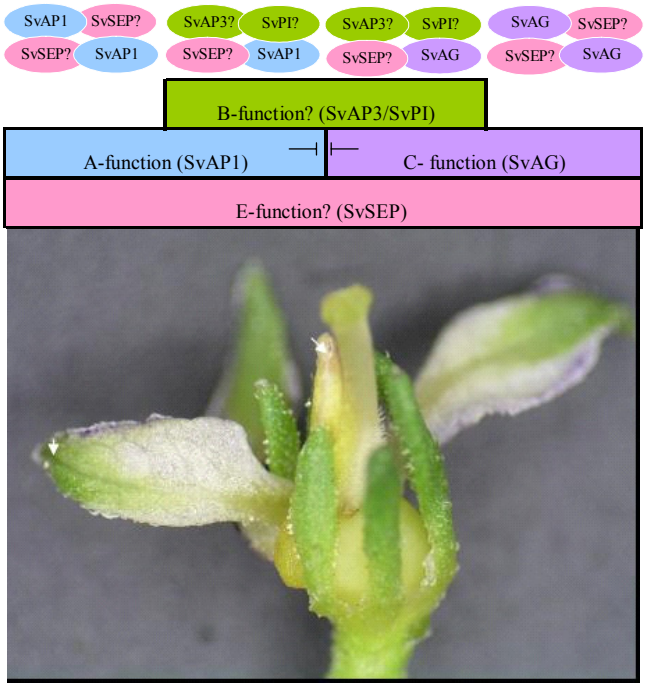

Fig. 5 Sepaloid petals and largely stamen-less *S. villosum* T-5 mutant flower observed under alternating high and low temperatures in the transition between late spring and summer. This phenotype resembles that of Arabidopsis B-function gene mutants where the flowers are composed of sepals-sepals-carpels-carpels in whorl1-whorl2-whorl3-whorl4, respectively. Arrows indicate petals transformed into sepaloid organs and stamens transformed into carpeloid organs, suggesting that the putative B (SvAP3/SvPI) gene is not activated under moderately high or alternating temperatures.

(Ditta *et al*. 2004). The phenotype displayed in T-5 mutant from winter to early spring, therefore, suggests a failure of activation of all A, B and C gene functions. This could imply a temperature-sensitive triple mutation of the putative homeotic proteins SvAP1 (A-function), SvAP3/SvPI (B-function) and SvAG (C-function) or a failure to interact functionally with the putative SvSEP proteins (E-function) under low temperature conditions.

From late-spring to early summer the whorl 2 organs maintain the general appearance of petals, but have sepaloid features (**Fig. 5**). Petals have greenish tips and margins, characteristic of sepals and leaves. The stamen-less trait seems to be a combined result of stamen abortion and homeotic transformation into petaloid and staminoid organs (Ojiewo *et al*. 2006b). The transformed stamens fuse with the true carpel in the fourth whorl, forming a unique gynoecium. In some flowers, semi-fused stamen tissue is observable in the central sector of each pistil; in others, the transformed carpels remain unfused and develop their own styles and stigmas, resulting in more than one carpel per flower (Ojiewo *et al*. 2006a). These homeotic transformations, mainly affecting the second and third whorls suggest a class-B gene mutation. In B mutants, the B-function is lacking in whorls 2 and 3 with the result that both whorls 1 and 2 express only the A- and E-functions, and whorls 3 and 4 express only the C- and E-functions (Pelaz *et al*. 2000). As a consequence, a flower is produced that consists of sepals, sepals, carpels and carpels, a phenotype that best describes T-5 in late spring. A change in the conformation of either SvAP3 or SvPI proteins (B-function) probably affecting their interaction (suppression) with each other or with SvSEP (E-function) could be responsible for sepaloid petals in whorl 2 and transformation of stamens into carpels in whorl 3. However, since the whorl 2 organs are not perfect sepals and some miniature stamens formed in whorl 3, we speculate that the B-function is not lost as such, rather the expression is inhibited.

The indeterminate phenotype of T-5 in summer (**Fig. 6**) is strikingly similar to *Arabidopsis* E-function (*sep1 sep2 sep3*) mutants or B- and C-function (*ap3 ag*, *pi ag*) double mutants (Pelaz *et al*. 2000). In *Arabidopsis BC* mutant, the A-function is active in all floral whorls, the B-function is absent, sepals are formed in the outer 3 whorls and the flower becomes indeterminate, resulting in an iteration of the floral programme and the production of a new floral bud from the centre of the flower. In the E triple mutant, only sepals are produced and, as in the BC double mutant, the flowers of E mutants become indeterminate and form a new floral bud from the central meristematic region (Ferrario *et al*. 2004). The T-5 phenotype in summer, therefore, suggests a change in the conformation of SvAG and SvAP3 /SvPI proteins (B- and C-functions) or SvSEP (E-function).

Partial restoration of the T-5 floral structure in autumn (**Fig. 7**) could be assumed to be due to the restoration in the function of the affected protein(s) and its interaction with other proteins. Most point mutations or short deletions of the K-domain lead to partial, but not complete, loss-of function. The resulting phenotypes may be due to temperature-dependent reduction/loss of specific protein interactions (Zachgo *et al*. 1995). At the restrictive temperature (26°C), the DEF-101 protein was unable to form DNA binding heterodimers with wild-type GLOBOSA (GLO; the *Antirrhinum* PI homolog) in a DNA binding assay. Zachgo *et al*. (1995) suggest that sensitivity to temperature is a property of a protein product which depending on temperature conditions, changes its active conformation and interacts (activation/inhibition) differently with other proteins. Yi and Jack (1998) isolated an intragenic suppressor, *ap3-11* (a weak *ap3* allele), that functions to suppress the splicing defects of *ap3-1*. In homozygous *ap3-11 ap3-1* flowers, the aberrant splicing patterns observed in *ap3-1* are observed much less frequently; the flowers exhibit very weak conversions of petals to sepals and stamens to carpels, and the flowers are self-fertile even at 29°C. In both *ap3-1* and *ap3-11 ap3-1* flowers, the percentage of correctly spliced

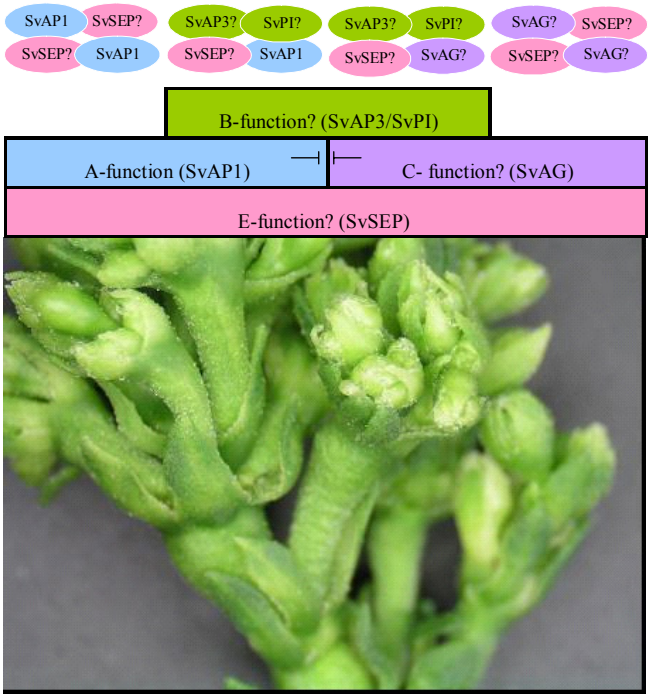

Fig. 6 Indeterminate *S. villosum* T-5 mutant flower with endless whorls of sepals as observed under high temperatures (>25°C) in summer. This trait is similar to that displayed by E-function (*sep1/2/3*) triple mutants (Pelaz *et al*. 2000), but also resembles the phenotype of the double *bc* (*ap3/pi ag*) mutants (Bowman *et al*. 1991) of *Arabidopsis*. The petals and the stamens are transformed into sepals and the carpels are replaced by another flower which repeats the same pattern, suggesting that the putative B (SvAP3/SvPI) and C (SvAG) genes are not activated under high temperatures.

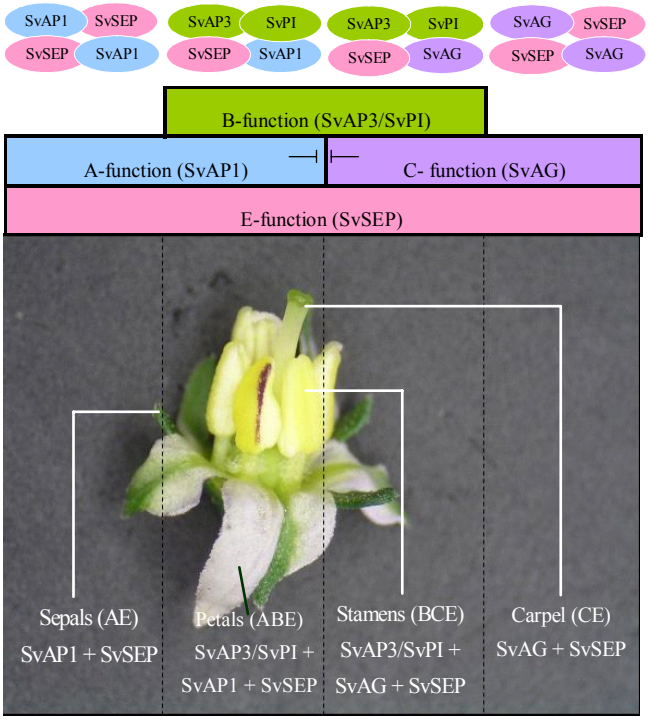

Fig. 7 Partially restored *S. villosum* T-5 mutant flower showing all the 4 floral whorls present in under optimum temperatures in autumn. The restoration of the normal pattern of floral organ initiation and differentiation to some degree suggests activation of all the homeotic genes under favorable temperatures probably due to improved functional interaction between the MADS-box protein complexes of transcription factors (Zachgo *et al*. 1995).

AP3 RNAs correlates with the phenotype of the flowers; *ap3-1* plants grown at 16°C had higher ratio of exon 5-containing to exon 5-deleted RNAs and exhibited a less severe *ap3* mutant phenotype than plants grown at 23°C or 29°C. Whether the partial restoration of the floral organ structure and fertility restoration in T-5 mutant under optimal temperature conditions is due to improved efficiency/accuracy of splicing or of protein-protein interactions is yet to be determined experimentally.

CONCLUSIONS AND PROSPECTS

To the best of our knowledge, no analysis of flowering characteristics of the section *Solanum* has been initiated at genetic or molecular level, so far. This may be due to lack of genetic resources such as a genetic map, developmental mutants or transposable elements that could be used as tools for generation of novel mutants (transposon mutagenesis) or isolation of genes (transposon tagging). Besides, the flower is very small, making it fairly difficult to collect particular tissue or organ for molecular analysis. With the isolation of the T-5 mutant, the major goals of future research will be to establish the exact structures of the putative transcription factor complexes and the target genes they control during the development of floral organ identity. Wild-type and T-5 mutant analysis involving gene cloning and sequencing, expression studies and phylogeny constructions, will make it possible to correlate putative MADS-box genes with flower morphology *in S. nigrum*-related species. Isolation of the MADS-box orthologs determining floral organ identity in *S. villosum* will enable the analysis of the effects of temperature on their expression or activity in the T-5 mutant. Through manipulation of the MADS-box transcription factors and the target genes, it is possible to turn floral organs into leaves and leaves into floral organs (Goto *et al*. 2001). The male function of a temperature-sensitive African nightshade mutant flower, such as T-5, can be eliminated when not needed by growing in restrictive temperatures and restored when needed by growing in permissive temperatures. Thus, production of fruit and seed (which are the major sink of the plant) can be excluded to improve leaf productivity and recovered for purposes of propagation.

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