tabaci. By contrast, Javed *et al*. [6] expressed a plant defence protein (a lectin) and a toxin (Hvt) from the venom of a spider to provide broad-spectrum resistance against multiple sap-sucking pests, including *B. tabaci*, mealybug, and aphids. Raza *et al*. [7] and Thakur *et al*. [9] used the RNAi strategy to kill insects by expressing dsRNAs in plants to silence vital *B. tabaci* osmotic regulators (aquaporin and alpha glucosidase) and an actin ortholog (*v-ATPase*). To maximize the protection, Raza *et al.* [7] simultaneously targeted two genes through RNAi by fusing RNA sequences derived from two *B. tabaci* genes. Collectively, these studies show a novel trend: targeting *B. tabaci* to obtain dual begomovirus and *B. tabaci* resistance (Figure 1).

Engineering Durable *B. tabaci* Resistance

Genetically modified (GM) crops expressing *Bacillus thuringiensis* (Bt) toxins, which are insecticidal endotoxins of bacterial origin, is the most widely applied technology in the history of GM crops; several Bt transgenic crop species are grown worldwide over a vast area. This indicates that the use of an effective toxin is by far the most practical approach in the development of insect-resistant plants. Lessons learned from the commercialization of Bt crops in the past must be considered before taking any other GM crops expressing a toxin to the field. Pyramiding multiple toxins and/or dsRNAs to interfere with different pathways of the target insect has been the most rational approach for developing long-term resistance in the past, and should be considered to engineer durable *B. tabaci* resistance. Moreover, the combined effect of Bt toxins (against chewing pests) and the recently investigated toxins (against sucking pests) should ideally provide broad-spectrum resistance.

Bemisia tabaci is a phloem feeder (Figure 1) and most begomoviruses are phloem restricted. For this reason, expressing toxins against *B. tabaci* under the control of a phloem-specific promoter should be more effective [6] and be more acceptable from a biosafety standpoint. At least one toxin, Tma12, has provided dual begomovirus-*B. tabaci* resistance; other toxins and RNAi plants remain to be tested for effectiveness against begomoviruses. Shukla *et al*. [8] tested resistance to the important *B. tabaci*-transmitted viruses causing cotton leaf curl virus disease [8]. The possible mechanism of action of Tma12 is binding to the chitin polymers that are a major component of the exoskeleton of insects [8]. However, the precise mechanism of action of Tma12 needs to be determined. Other economically important begomovirus diseases, such as tomato yellow leaf curl disease and cassava mosaic disease, remain to be evaluated.

Taken together, these findings not only provide exciting ideas for controlling *B. tabaci* and begomoviruses, but also raise interesting research questions (Figure 1). If followed through, this trend of engineering dual *B. tabaci*–begomovirus resistance using novel toxins and dsRNA could result in a breakthrough as important as once provided by crops expressing Bt toxins.

Resources

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References

1. Gilbertson, R.L. *et al.* (2015) Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. *Annu. Rev. Virol.* 2, 67–93
2. Mansoor, S. *et al.* (2006) Geminivirus disease complexes: the threat is spreading. *Trends Plant Sci.* 11, 209–212
3. Fondong, V.N. *et al.* (2016) Novel functional genomics approaches: a promising future in the combat against plant viruses. *Phytopathology* 106, 1231–1239
4. Zaidi, S.S. *et al.* (2016) Engineering plants for geminivirus resistance with CRISPR/Cas9 system. *Trends Plant Sci.* 21, 279–281
5. Hanley-Bowdoin, L. *et al.* (2013) Geminiviruses: masters at redirecting and reprogramming plant processes. *Nat. Rev. Microbiol.* 11, 777–788
6. Javed, S. *et al.* (2016) A transgenic approach to control hemipteran insects by expressing insecticidal genes under phloem-specific promoters. *Sci. Rep.* 6, 34706
7. Raza, A. *et al.* (2016) RNA interference based approach to down regulate osmoregulators of whitefly (*Bemisia tabaci*): potential technology for the control of whitefly. *PLoS ONE* 11, e0153883
8. Shukla, A.K. *et al.* (2016) Expression of an insecticidal fern protein in cotton protects against whitefly. *Nat. Biotechnol.* 34, 1046–1051
9. Thakur, N. *et al.* (2014) Enhanced whitefly resistance in transgenic tobacco plants expressing double stranded RNA of v-ATPase A gene. *PLoS ONE* 9, e87235
10. Whitfield, A.E. *et al.* (2015) Insect vector-mediated transmission of plant viruses. *Virology* 479-480, 278–289

Spotlight

Petal, Sepal, or Tepal? B-Genes and Monocot Flowers

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In petaloid monocots expansion of B-gene expression into whorl 1 of the flower results in two whorls of petaloid organs (tepals), as opposed to sepals in whorl 1 of typical eudicot flowers. Recently, new gene-silencing technologies have provided the first functional data to support this, in the genus *Tricyrtis* (Liliaceae).

The ABC Model of Flower Development

Since its inception in the 1990s, modifications to the iconic ABC model of floral organ development have been pouring in through studying species far removed from the original eudicot models thale cress (*Arabidopsis thaliana)* and garden snapdragon (*Antirrhinum majus)*. Expression studies across a diversity of angiosperm species have shown that minor alterations in patterns of MADS-box (ABC) gene expression result in substantial homeotic changes to flowers (organ transformation). These in turn can account for a great proportion of floral diversity seen in nature. In the typical ABC model,

A-function defines sepals – leaf-like floral organs of the perianth in whorl 1 of the flower. In whorl 2, the combined expression of A- and B-function genes defines petals; in whorl 3 both B- and C-genes define stamens (male organs), and in whorl 4 C-function alone dictates carpels (female organs) [1].

Two further classes of genes were since added to this simple scheme (classes D and E). D-class genes are involved in ovule development, and act in combination with C-class genes. Phylogenetically they are closely related, and D-genes may be best thought to represent the subfunctionalisation of C-function with a role in ovule development only. A similar situation occurs in B-genes, where *TM6* genes have a subfunctionalised B-function role in stamen development alone. *TM6* copies have been lost from both *Arabidopsis* and *Antirrhinum*, but they are found in many other eudicots including all Solanaceae (tomatoes and relatives).

A Revised ABC Model

E-class genes (*SEPALLATA*) are required for conferring floral identity, and expression of ABC genes in combination with E-genes is sufficient to produce ectopic flowers instead of vegetative organs [2]. Determination of the floral state, in other words floral meristem identity, is inextricably linked with the ground-state of floral organs, in other words sepals. A-function itself appears to be independently acquired in *Arabidopsis* and, in all other species tested, A-function mutations affect sepal identity and meristem identity together [3]. Further evidence for a revised A-function comes from the fact that the A-function genes are broadly expressed, and indeed this is also the case in monocots, where A-function genes are expressed in most whorls of the flower and other vegetative organs [4–6]. E-genes are also closely related to A-genes phylogenetically. Therefore, A and E-function can be categorised within a broad class of 'A'-function (floral meristem identity genes); C- and D-function can be categorised within a broad class of 'C'-function (female reproductive organ identity genes) (the revised ABC model has been recently reviewed [7]).

Petaloid Monocots: Expansion of B-Gene Expression

Several groups of monocots possess two whorls of petaloid organs (tepals) that vary from identical in tulips to highly differentiated in orchids. Expansion of B-gene expression correlates with petaloid tepals in the first whorl of these monocot flowers. There have been several studies of B-gene expression in other petaloid monocots from different orders including the Liliales, Asparagales, Alismatales, Commelinales, and Zingiberales [4,5,7,8]. Expansion of the B-gene expression domain is a common feature of floral development in monocots that have whorl 1 tepals as opposed to sepals (for example tulips, muscari, agapanthus, lilies, orchids). However, without functional data to back them up, these expression studies could be seen as merely correlative. Otani *et al*. [6] provide the first functional data for a modified ABC model in the genus *Tricyrtis* – toad lilies (Liliaceae; Figure 1A,B). Using chimeric repressor gene-silencing technology (CRES-T) and *Agrobacterium*-mediated genetic transformation, they developed several transgenic lines of *Tricyrtis* with partial to severe suppression of B-gene expression (reduced to 7–8% of the expression level in wild-type plants). This resulted in the conversion of whorl 1 and 2 floral organs from petaloid tepals to greenish sepal-like organs, and, in addition, the whorl 3 stamens were converted to carpeloid structures [6], as predicted by the modified ABC model.

More B-Genes, More Petaloid Possibilities?

In other monocots, paralogous (duplicate) B-genes often display unique expression

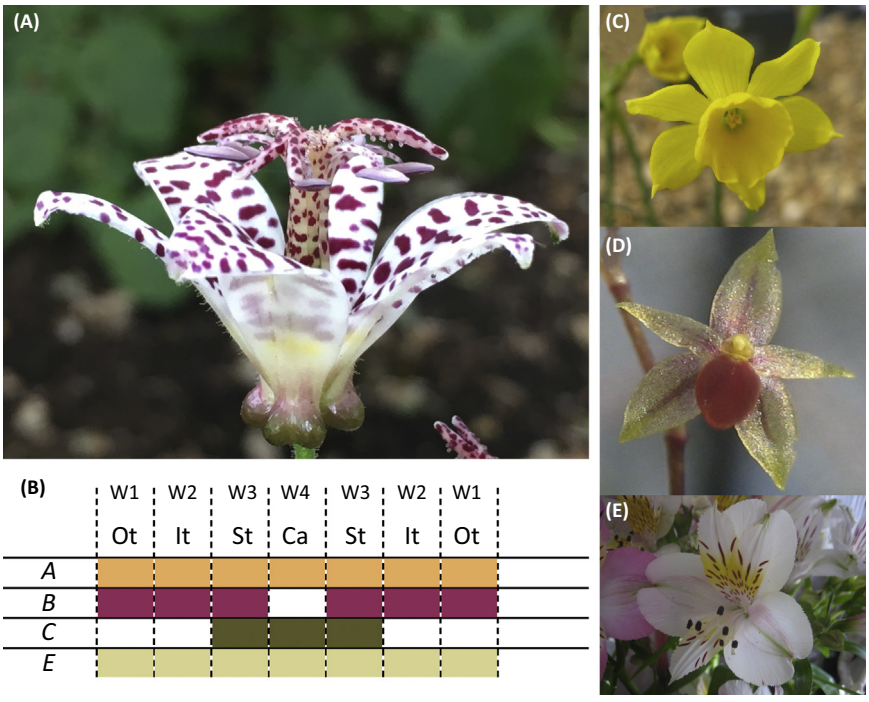

Figure 1. (A) Floral morphology of *Tricyrtis* sp. 'Jasmin', and (B) modified ABC model adapted from Otani *et al*. (2016) showing the different expression domains of ABC genes in outer tepals (Ot), inner tepals (It), stamens (St), and carpels (Ca), in whorls 1–4 of the flower. Further examples of petaloid monocot floral variation: (C) *Narcissus fernandesii* (Amaryllidaceae), (D) *Platystele misera* (Orchidaceae), (E) *Alstroemeria ligtu* cultivar (Alstroemeriaceae). Photography: S.D.

patterns between (and even within) whorls of the flower, permitting differentiated tepal morphologies and novel elaborations within an overall petaloid identity programme. In the Peruvian lily (*Alstroemeria ligtu*), for example, three B-function genes have been isolated with different expression patterns. *AlsDEFb* and *AlsGLO* were found to be expressed in whorls 1, 2, and 3, which correspond to outer tepals, inner tepals, and stamens, respectively. *AlsDEFa*, by contrast, was only expressed in whorls 2 and 3 [9]. The unique expression pattern of *AlsDEFa* may result in the unique morphology of the whorl 2 tepals, i.e. regulating the narrower and often highly ornamented inner tepals (Figure 1E). In orchids, different subsets of B-genes act to define multiple distinct morphologies of outer tepal versus inner tepal versus labellum [9,10] (Figure 1D). In several monocots this fine-tuning of B-gene expression may impact on other downstream morphologies, for example different pigmentation patterns [9] and even novel organs such as nectar spurs or lobed petals/tepals [10,11].

Some of these downstream morphologies may involve, for example, the differential expression of *KNOX* genes, which are known to have a role in forming dissected morphologies (such as compound leaves) and are thought to have a potential role in floral elaborations including lobed petals and spurs [12]. Developmental flexibility of B-genes has a crucial role in the evolution and morphological diversification of the petaloid monocot flower; B-genes also highlight the influential role of gene duplication in morphological evolution. Advances in gene-silencing technologies and transformation protocols for non-model plants now enable the full extent of MADS-box gene function to be tested instead of merely being speculated about. One particularly exciting advance will be to test how paralogous B-gene copies may be responsible for downstream morphological innovation, such as nectar spurs (Figure 1A), the corona of daffodils (Figure 1C), or the elaborations of the labellum (lip) in orchids (Figure 1D).

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References

1. Coen, E.S. and Meyerowitz, E.M. (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* 353, 31–37
2. Ditta, G. *et al.* (2004) The SEP4 gene of *Arabidopsis thaliana* functions in floral organ and meristem identity. *Curr. Biol.* 14, 1935–1940
3. Litt, A. (2007) An evaluation of A-function: evidence from the APETALA1 and APETALA2 gene lineages. *Int. J. Plant Sci.* 168, 73–91
4. Kanno, A. *et al.* (2007) Class B gene expression and the modified ABC model in nongrass monocots. *Scientific World Journal* 7, 268–279
5. Kanno, A. (2015) Molecular mechanism regulating floral architecture in monocotyledonous ornamental plants. *Hortic. J.* 85, 8–22
6. Otani, M. *et al.* (2016) Suppression of B function strongly supports the modified ABCE model in Tricyrtis sp. (Liliaceae). *Sci. Rep.* 6, 24549
7. Theissen, G. *et al.* (2016) MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. *Development* 143, 3259–3271
8. Hsu, H.-F. *et al.* (2015) Model for perianth formation in orchids. *Nat. Plants* 1, 15046
9. Hirai, M. *et al.* (2007) The expression patterns of three class B genes in two distinctive whorls of petaloid tepals in *Alstroemeria ligtu*. *Plant Cell Physiol.* 48, 310–321
10. Mondragón-palomino, M. *et al.* (2013) Perspectives on MADS-box expression during orchid flower evolution and development. *Front. Plant Sci.* 4, 1–9
11. Mondragón-Palomino, M. and Theißen, G. (2008) MADS about the evolution of orchid flowers. *Trends Plant Sci.* 13, 51–59
12. Box, M.S. *et al.* (2012) Flower-specific KNOX phenotype in the orchid *Dactylorhiza fuchsii*. *J. Exp. Bot.* 63, 4811–4819