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Project Review

Beyond the blue rose: Modification of floral architecture with plant-specific chimeric repressors

Norihiro Ohtsubo*

National Institute of Floricultural Science (NIFS), National Agriculture and Food Research Organization (NARO), Tsukuba, Ibaraki 305-8519, Japan
* E-mail: nohtsubo@affrc.go.jp Tel & Fax: +81-29-838-6822

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Abstract There is a significant difference between cereal crops and floricultural crops in the concept of genetic modification. This is due to the difference in requirements for these agricultural products; wide variation of flower color, shape and fragrance for floricultural plants in contrast to productivity improvements or fine-tuning of physiological traits for crop plants. Bringing GM flowers to the market against their short product life involves the following factors: (1) a high-efficiency production and screening system of elite lines, (2) reliable methods to minimize biodiversity impact and evaluation of their efficacy, and (3) social receptivity based on intensive education and information sharing. We have developed an efficient system by merging the genetic information resources of the *Arabidopsis* genome and a transcription factor-based gene silencing system called CRES-T through the Flower CRES-T Project. In this project, we have demonstrated the applicability and general versatility of CRES-T in various plant species through experiments on eight different flower species with over 100 transcription factors. In addition to providing phenotypic information via the original database, we are attempting to improve public knowledge, for example, through the production of resin-embedded specimens of GM-flowers and reviving lost garden varieties of morning glory and using them in educational programs, to gain public acceptance of GM plants. Multi-petal cyclamens with complete sterility, which have been produced by suppressing a pair of floral-organ identity genes, will be released in the near future as the third GM commodity following Suntory's blue carnations and rose. This cyclamen represents the best example of a GM product with a low diversity impact ever produced.

Key words: Cartagena Protocol, chimeric repressor, CRES-T, practical molecular breeding, transcription factor.

Crop improvement via genetic modification is now applied to several plant species mainly utilizing the *Agrobacterium*-mediated procedure. By conferring a new functionality that the host plants do not have, or by suppressing their negative traits, many GM crops have been commercialized to date. Flavr Savr^{TM} tomato (Kramer and Redenbaugh 1994) and the Roundup Ready^{TM} crops (Padgette et al. 1996) are representative examples of such crops, and now more than 100 GM crops are cultivated and distributed all over the world. Because commercialization of GM plants requires enormous investment in terms of cost, time, and effort, only those crops that have a huge market and long product life are targeted for the modification. Likewise, phenotypes that contribute to increased productivity such as resistance to herbicides or various diseases have been preferentially chosen as objectives, over those products that do not directly benefit producers. In contrast to these producer-oriented GM crops, a series of blue carnations named Moondust^{TM} (Tanaka et al. 2009) and a blue rose Applause^{TM} (Katsumoto et al. 2007) released in 1997 and 2009, respectively by Suntory, are the distinct examples in the point of consumer-oriented modification. However, the investment required for commercialization just the latter case is estimated to be more than 20 years and 30 million dollars. This review describes the concepts and research strategies for the Flower CRES-T Project, which has been launched in 2005 to overcome difficulties associated with the commercialization of GM flowers, through an overview of recent progress gathered in this special issue.

Particularity of floricultural plants as GM materials

A remarkable number of phenotypic changes concentrated in flowers, especially corollas, are observed in ion-beam irradiated torenia plants (Sasaki et al. 2008). This apparently paradoxical feature could be due to the particular and sensitive nature of our flower recognition,

Abbreviations: AG, AGAMOUS; AP1, APETALA1; DP, DUPLICATED; CaMV, cauliflower mosaic virus; CRES-T, chimeric repressor gene-silencing technology; EAR-motif, ETHYLENE RESPONSE FACTOR (ERF)-associated amphiphilic repression (EAR) motif; GMO, genetically-modified organism; SRDX, the optimized EAR-motif used for constructing the chimeric repressors; TCP, TEOSINTE BRANCHED1, CYCLOIDEA, and PCF
This article can be found at http://www.jspcmb.jp/

which enables us to detect slight changes in floral tissues but often leads us to overlook changes in other vegetative tissues, in addition to the complicated and unstable regulation of flower colors and shapes. This is similar to the case of personal identification by facial recognition, which depends on the fine distinction of the arrangement of facial parts, and how we draw a sharp contrast between one and the other (Blais et al. 2008). Several people unconsciously use corolla size and shape, petal color, and pattern preferentially to distinguish flowers. On the other hand, other nondescript phenotypes including leaf shape, stem thickness, internode length and overall shape might be used less frequently. The fact that social background affects facial recognition behavior (Matsuda et al. 2008) may also fit flower recognition. Changes in living environment may decrease our opportunities of seeing flowers in nature, but increases our chances of seeing them at florists or via TV screens, and this reinforces our tendency to focus on floral tissues. This characteristic of flower recognition provides us with important criteria to determine which phenotype we should target and how strongly to modify it, when we use floricultural crops as materials for genetic modification.

The most significant difference between cereal crops and floricultural crops is the difference in the concept of modification. The modification of floral traits includes multidirectional possibilities of commercial value, and crop breeding usually aims to produce new physiological characters to overcome decreasing yields, which might be caused by disease, environmental stress, or herbicides. At present, it is said that more than 3,000 new flower varieties are released every year but, of course, most have very short product lives. To commercialize GM flowers in such a situation, an efficient production/screening system that minimizes labor and the costs of product development is required. To this end, we are trying to develop systems to produce novel flowers by combining genetic modification and other recently developed techniques. One good example is heavy-ion beam irradiation, which is used to produce petal color-modified transgenic torenia plants (Sasaki et al. 2008). We have succeeded in producing over 200 novel torenia flowers in only 2 years using this system. Creating a simultaneous set of variations from an elite line will provide a significant benefit for producers because they can handle multiple items under the same cultivation conditions. However, in ion beam breeding, it is still difficult to determine the causative genes and replicate the phenotypes obtained without genomic information, especially in multiploid crops.

CRES-T breaks technical barriers in genetic modification

CRES-T is a unique gene-silencing method that turns transcriptional activators into transcriptional repressors by adding a plant-specific repression domain sequence called the EAR-motif (Hiratsu et al. 2003). The most significant advantage of this method is the dominant repressive activity, which overrides endogenous activity even when functionally redundant transcriptional activators are present. Therefore, chimeric repressors can preferentially suppress their target genes (for review Mitsuda and Ohme-Takagi 2009; Shikata and Ohme-Takagi 2008). In this, CRES-T has a great advantage over other sequence-dependent gene silencing procedures such as antisense RNA and RNAi. Many novel functions of *Arabidopsis* transcription factors were revealed by this method over the past years (Koyama et al. 2007, 2010; Matsui et al. 2005; Mitsuda et al. 2005, 2007; Shikata et al. 2009). Taking this approach, we started producing novel floral traits in various types of ornamental flowers in 2004. This is the Flower CRES-T Project.

CRES-T is basically only applicable for transcription factors. This target constraint is sometimes regarded as a shortcoming because the phenotypes obtained result only from the loss of gene function. On the other hand, signaling cascades regulating cellular events cross-talk in complicated ways, various types and kinds of unexpected phenotypes with new genetic features have been obtained as reported in this issue. The fact that most of the morphological mutants in *Arabidopsis* are caused by mutations in transcription factors supports their use as modification targets. In addition, *Arabidopsis* chimeric repressors are thought to be applicable to other plant species with little modification. Because we still do not have sequence information on the genomes of most of the agricultural plants that directly benefit us, isolation and functional confirmation of genes related to target phenotypes is a major problem. If we could control such genes by introducing *Arabidopsis* chimeric repressors, significant reductions in cost and labor could be achieved using resources that already exist. Takagi et al. in AIST, Japan began the functional analyses of whole transcription factor genes of *Arabidopsis* in 2004, which was followed by a launch of a project evaluating the applicability and versatility of CRES-T in 2005, utilizing the *Arabidopsis* resources. We applied this method to various genetically transformable floricultural plants including torenia (*Torenia fournieri*), chrysanthemum (*Chrysanthemum morifolium*), Japanese gentian (*Gentiana* spp.), cyclamen (*Cyclamen persicum*), Japanese morning glory (*Pharbitis nil*), lisianthus (*Eustoma grandiflorum*), tobacco (*Nicotiana tabacum*), petunia (*Petunia*×*hydrida*), carnation (*Dianthus caryophyllus*), and rose (*Rosa*× *hybrida*), through the support of the Grant-in-Aid "Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries" from the Research Council, Ministry of Agriculture, Forestry and Fisheries of Japan (grant no.1783) in 2005, and the Programme for

Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry in 2008.

TCP3 and AG

To facilitate the confirmation of the availability of CRES-T in every plant species, we carefully considered which gene should be used for the first experiment. The important requirements are as follows: (1) inducing visibly apparent phenotypes on juvenile vegetative tissues to enable early judgment even in Japanese gentian and cyclamen, which require a long time to flower, (2) targeting major phenotypes that are in great demand, and (3) providing benefits for the promotion of practical use of the resulting products. Based on these considerations, we chose *Arabidopsis TCP3* and *AG*. The former regulates cell proliferation in meristems, and repression of its function results in serration in the marginal region of the leaves and petals in addition to causing them to have wavy blades (Koyama et al. 2007, 2010). The strong phenotype of TCP3-SRDX *Arabidopsis* is represented by severely curled leaves in juvenile plants, therefore its effectiveness can be determined within a short period. *AGAMOUS* is a class C gene in the floral organ development ABC model, which contributes to the formation of stamens and carpels. Dysfunction of this gene in *Arabidopsis* results in the conversion of stamens into petals and carpels into sepals, and the flower continuously produces more flowers inside, thus producing a flower with numerous petals (Mitsuda et al. 2006; Yanofsky et al. 1990). Double flower is one of the most important phenotypes in floricultural crops, and the loss of fertility associated with it will reduce the biodiversity effects of GM flowers after commercialization. As a consequence, these two transcription genes were chosen to provide suggestive and contrasting results. All the flowers transformed with chimeric TCP-related repressors exhibited similar morphological changes, such as serrated margins of the vegetative tissues and unique petal color patterns (Gion et al. 2011; Narumi et al. 2011; Tanaka et al. 2011a, 2011b). In addition, we confirmed that repressive activity occurs only when the chimeric repressor has an intact repression domain, using diploid torenia and hexaploid chrysanthemum (Narumi et al. 2011). Thus, we succeeded in demonstrating the applicability and versatility of *Arabidopsis*-derived chimeric repressors in other plant species including polyploid species, during the early years of the project.

On the other hand, flowers of transgenic torenia expressing *Arabidopsis* AG-SRDX showed unexpected phenotypes such as serrated petals, unopened corollas and greenish petal edges without producing a multi-petal flower as in *Arabidopsis* (Narumi et al. 2008). Likewise, disruption of petal development and elongation of carpels are observed in AG-SRDX *Pharbitis* (Sage-Ono et al. 2011). At first, we thought these unexpected phenotypes were induced by aberrant function of the chimeric AG repressor suppressing some genes not under the control of endogenous AG homolog, or by ectopic expression of the chimeric repressor in tissues that usually express the AG-related activity of another function. However, intensive analysis in these plant species revealed that these unexpected phenotypes could be explained by their original class C gene function.

Technical improvement of CRES-T

Interestingly, the unexpected phenotype of AG-SRDX torenia was also observed in tobacco and lisianthus (M. Ikeda in AIST and K. Isuzugawa in Yamagata Integrated Agricultural Research Center, personal communication), as well as when the chimeric repressors of two class C genes, *TfPLE* and *TfFAR*, isolated from torenia, were independently expressed in torenia (Narumi et al. 2008). In addition, functional analyses of cyclamen class C genes, *CpAG1* and *CpAG2*, revealed that loss of CpAG1 function doubled the petal number to 10 by converting sepals to petals, and the additional loss of CpAG2 function increased the petal number to between 40 and 50, while the loss of CpAG2 did not affect the petal number (Sugiyama et al. in preparation). Functional divergence between the two class C genes might have occrred in these plant species, and their simultaneous repression must be required to obtain the multi-petal phenotype like the *Arabidopsis ag* mutant. Individual repression of these genes is required in such cases, even though the chimeric repressors have dominant-negative repression activity against multiple transcription factors in the same protein family. This kind of functional divergence was also observed in class B genes of torenia (Sasaki et al. 2010) in the development of petals and stamens. Expression of *Arabidopsis* AG-SRDX in *Pharbitis* also failed to induce the multi-petal phenotype, and plants exhibited short green edged petals and projecting carpels (Sage-Ono et al. 2011) like the *ap3* mutants in *Arabidopsis* (Bowman et al. 1989). On the other hand, a chimeric repressor of the *Pharbitis* double-flower gene *DP* (Nitasaka 2003) induced a multi-petal phenotype inside the flower buds, although the flowers never opened due to the growth inhibition effect of DP-SRDX. To try resolving this problem, combinations of the Gal4 system and two kinds of inducible promoters, heat-shock protein 18.2 (HSP18.2) and Alc promoter unit, were used. This enabled the flowers to bloom without growth inhibition. In addition, fertility, which had been disrupted by the conversion of stamens to petals by the transient suppression of DP-SRDX activity, was restored using the same system. This will facilitate the use of infertile phenotypes for breeding.

The usability of CRES-T is also improved by combining a floral organ-specific promoter with

development/differentiation-related transcription factors. Expression of *Arabidopsis* MYB24-SRDX, a chimeric repressor that result in curled leaves, unopened flowers, and modified petal color, using the *Arabidopsis* AP1 promoter allowed normal flowering with modified petal phenotypes without affecting the leaf morphology in torenia (Sasaki et al. 2011 in this issue). Expression of chimeric TCP3 repressor using various petal-specific promoters produced many kinds of novel petal phenotypes, which were not obtained with the CaMV35S promoter, without changing the phenotypes of other vegetative organs (Sasaki et al. in preparation). While we think that the distinct petal phenotype with a fringed margin and color pattern in 35Spro-TCP3-SRDX torenia (Narumi et al. 2011) is conferred by changes in cell identity, some transgenic plants expressing the same gene with a petal-specific promoter exhibited a change in petal color, suggesting the possibility of that transcription factor directly contributes to petal pigmentation biosynthesis. Plant-specific transgene inactivation by promoter methylation in the Japanese gentian is effectively avoided using *Arabidopsis* actin2 promoter, thus leading to the production of the first bi-color flower (Nakatsuka et al. 2011). Intensive analyses of this 35S promoter-specific methylation are also reported by Yamasaki et al. (2011) in this issue. Such in-depth examination of phenotypes and technical improvements focusing on flowers as commercial crops will accelerate the practical use of GM flowers.

We have developed the collective transformation system (CT system), which facilitates the screening of valuable traits of transgenic plants by examining up to 50 chimeric repressors at a time (Shikata et al. 2011). This is, therefore, expected to be a powerful tool in overcoming the difficulties in GMO commercialization mentioned above. The most interesting fact in the development/differentiation-related transcription factors that modify petal shape and flower color pattern was that transcription factors involved in the basal plant activities, which do not directly contribute to floral phenotypes, are also available for the modification of floral traits. We also identified genes that could be utilized for the pin-point modification of plant traits such as blotch size and adventitious shoot formation using this system. Transcription factor genes that make up the bulk sets were chosen on the basis of *Arabidopsis* microarray data and the previous results of transcription factor studies. This is an excellent example of practical development based on the basic research. FOX hunting, an overexpression-based bulk gene-screening system, which overexpresses a set of complete cDNAs and has already been used commercially (Ichikawa et al. 2006; Kondou et al 2009). This system may help to accelerate molecular breeding by screening biologically valuable genes beyond plant species, with the creative use with other procedures including CT and traditional breeding systems.

Improvement in basic techniques is also the great wealth of this project. Increase in translation efficiency by the modifications of the vector system (Sugio et al. 2008, 2010; Nagaya et al. 2010) greatly supported our project in terms of increasing the phenotype appearance ratio (Aida et al. 2008; Ohshima et al. 2011). Efficient translation without a corresponding increase in transcript amount has the advantage of resolving gene silencing, in addition to increasing the final product. Expression of the *FT* gene advanced flowering in Japanese gentian without affecting other morphological traits, unlike in other plant species. Its main merit is in confirming the gene effect within 12 months, instead of the 2 years that is usually required (Nakatsuka et al. 2009). Similarly, application of trehalose to the culture medium of torenia drastically elongated the culture period and reduced the costs and labor for the maintenance less than one third of those for the sucrose-based system (Yamaguchi et al. 2011).

A concept for addressing the biodiversity impact of GM flowers

To commercialize GM flowers, their evaluation as commercial products as well as their biodiversity impact according to the Cartagena Protocol and domestic law, are required (food and foodstuffs require additional procedures to assess their safety). As mentioned above, this process is responsible for a large part of the commercialization costs. There are two options to overcome this problem. One is to introduce male sterility to avoid the spread of pollen spreading (or completely abort sexual reproduction) and simplify the evaluation, and the other is to establish an evaluation procedure commonly applicable to all GM flowers. Although the latter is the final goal, we first have to commercialize a quantity of GM flowers without fertility and introduce them to the market to familiarize people with GM flowers and gain public acceptance. To produce GM flowers with minimal biodiversity impact, the starting plant material should be chosen on the basis of the following features: (1) should have no cross-fertilizable wild races in the field, (2) should produce pollen with low dispersal and sterility, and (3) should have reduced fertility caused by natural mutation or intensive domestication. This is the case with the blue carnations (Tanaka et al. 2009) and roses (Katsumoto et al. 2007) commercialized to date. The blue carnations are varieties of an incidental chimeric plant in which only the L1 layer has been transformed, therefore the transgenes are never introduced into germline cells. The transgenes never spread into the environment via their pollens and seeds in this case. On the other hand, all cultivated roses are tetraploid, while all wild races that can cross-fertilize with the transgenic rose are diploid. Therefore, the resulting triploid progeny are infertile (Nakamura et al.

2011a, 2011b). However, the evaluation of biodiversity impact in these plants consumed much time and incurred heavy costs. The biggest problems are the absence of evaluation criteria commonly applicable beyond plant species and the laborious event-based evaluation procedure defined by domestic law and the Cartagena Protocol on Biosafety. Of the flowers used in our projects, torenia, cyclamen and probably lisianthus have no effect on biodiversity because these plants have no cross-fertilizable wild races in Japan.

Besides the development of practical and versatile sterilization systems, the establishment of evaluation methods commonly applicable to various plant species might facilitate the practical use of GM flowers. The use of chrysanthemum and Japanese gentian, which have cross-fertilizable wild races, may be achieved by increasing empirical studies using torenia and cyclamen as models for evaluation and practical use, respectively. Sterilization should not be postulated as an indispensable prerequisite for commercialization for GM flowers, and we have to aim at establishing evaluation methods that appropriately estimates their biodiversity impact.

In 2010, the Gene Research Center of the University of Tsukuba in Japan established a nationwide research base for GM plants named the "PTraD initiative" (http://ptrad.gene.tsukuba.ac.jp/). This program might provide a good opportunity to discuss how we should define and evaluate biodiversity effects, through the production and evaluation of transgenic plants with practical applications. It is hoped that this will be an active consortium that can make appropriate proposals to the ministries concerned to improve the situation. In addition to these academic actions, there are two companies evaluating transgenic crops on a commercial basis in Japan, and one of them offers contract evaluation in foreign fields. While these companies may continue to provide effective data on field assessment, this should be regarded as a supplementary role to domestic assessment in the long run.

Publication and sharing of results and technical knowledge of previous assessments will help to improve the domestic rules for GMO evaluation. In this issue, Suntory provide their technical data for the biodiversity impact evaluation of the blue rose (Nakamura et al. 2011a, 2011b). Although this kind of work as well as the approaches to GMO production including isolation and characterization of applied genes as in this issue (Fukuchi-Mizutani et al. 2011; Togami et al. 2011) are sometimes difficult to publish as academic papers, we hope many scientific journals, including *Plant Biotechnology* will provide a place for the discussion of its importance as a part of academic research.

When introducing infertile phenotypes by genetic transformation, risk-adjusted approaches, such as providing extra safety on transgene effects, are preferred at present. Other than the conversion of stamens to petals by class C gene repression, we are now testing the prevention of pollen development by modifying the anther-specific flavonoid biosynthesis system, in addition to various combinations of stamen-specific promoters and cell death-inducing genes to prevent stamen development. In the former case, an anther-specific flavonoid biosynthesis gene *GtFLS* (Kimura et al. 2011) seems to be effective in establishing male sterility. On the other hand, we have not obtained plants with complete sterility from cell death gene-dependent inactivation of stamens. This might be due to the transgene expression being too short at the appropriate stage, tissue, and dose for prevention, due to differences in the expression profiles of the promoters. If we could obtain a stamen-specific promoter that did not have activity in other organs, it might be possible to produce infertile plants through the expression of strong cell death-inducing genes including barnase, as in the successful cases targeting tapetum and meiosis (Kobayashi et al. 2006; Mariani et al. 1990). However, as in our report on AP1 promoter (Sasaki et al. 2011), it is not easy to obtain promoters with complete specificity. Based on these results, we are now trying to develop a sterilization system using class C gene promoters, which expresses desirable properties targeting stamen development.

Educational approaches to establishing public acceptance

Educating the public and gaining their acceptance are important to accelerate commercial use of GM plants. In general, it is said that disclosing the behavior and associated risks of GM plants are the most important factors gaining public acceptance, in addition to help them to understand how these plants benefit consumers. We have found that the presentation of real transgenic plants in contained greenhouses has a profound impact on visitors, greater than any other presentation, including printed matter and slides. This shows that the level of impact is strongly affected by the amount of information, and there is a significant difference between two-dimensional presentation and the "real thing" in this case. Seeing the real thing face-to-face increases the time spent and depth of thinking, and therefore, strengthens the impression and understanding. From this standpoint, we are making two attempts to introduce GM flowers to the public. One is in the form of a resin-embedded specimen of transgenic torenia and the other is in developing common rules for displaying live GM plants in public (Figure 1).

The resin-embedded specimen (Figure 1A) fixes the natural floral morphology and petal colors by replacing the plant fluids with resins. This procedure is commonly used to produce specimens of animals and plants,

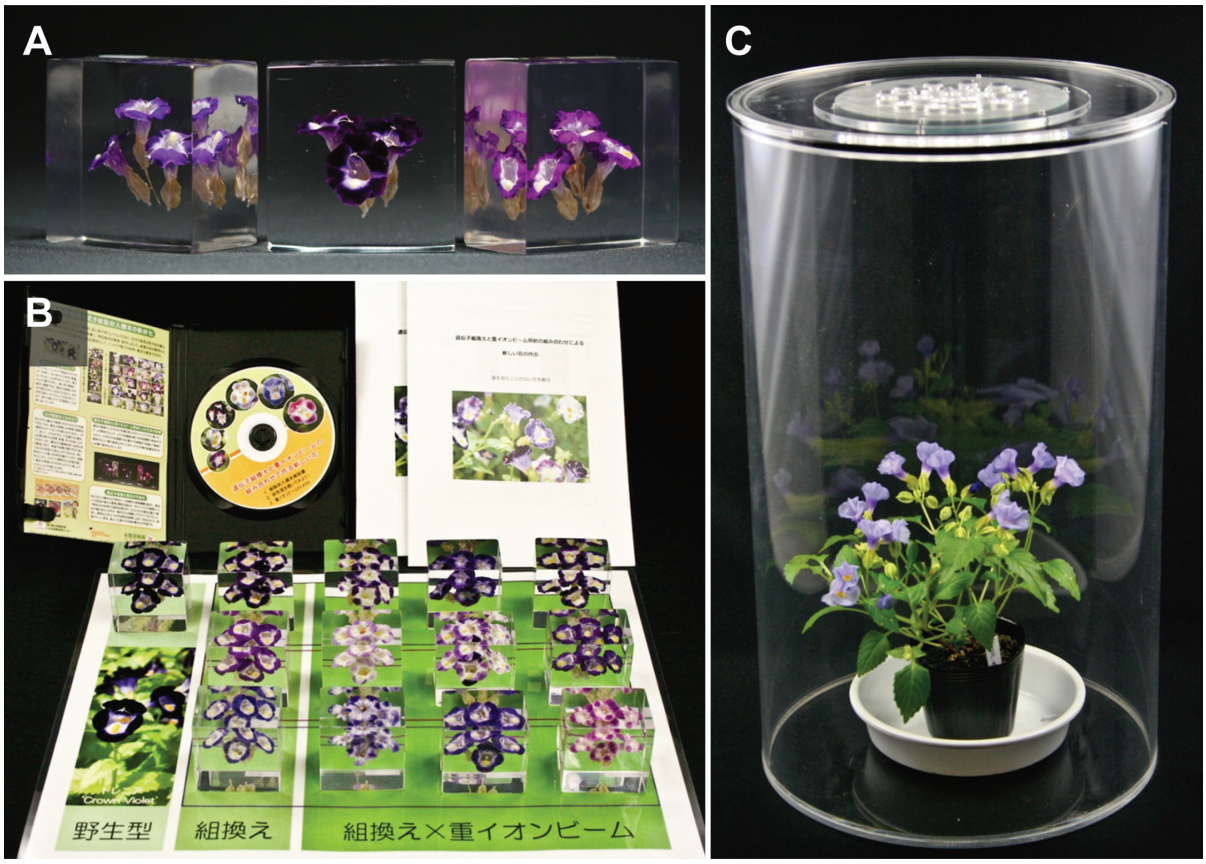

Figure 1. Educational tools for gaining public acceptance of GM flowers. (A) Examples of resin-embedded torenia specimens. Any plant materials, except for high water content tissues such as grape berries and tomato fruits, can be fixed without changing their natural shapes and colors. (B) An educational kit. This kit consisting of wild-type, GM, and ion-beam irradiated GM torenia specimens with a practical guide and DVD contents describing production of the transgenic plants. Twenty kits are used in many high schools and universities on a trial basis since 2009. (C) A prototype of the exhibition case. The top lid is equipped with a pollen filter that completely avoids pollen diffusion into the environment, without losing ventilation capacity. It is important to note that containment of GM plants using this case is not enough for public exhibitions under the regulation of the Cartagena Protocol and domestic law in Japan.

including insects and seaweeds, for display in museums. Transgenic plants are fixed during embedding, and therefore, are no longer classed as GMOs and can be distributed without following legal regulation. We have improved the facilities for making specimens of GM flowers with the help of a private company, which has the skills and technology required to fix the flowers without changing their morphology and colors. We have produced specimens of genetically modified torenia, chrysanthemum, Japanese gentian, and morning glory. These specimens are stable for over 10 years under fluorescent lamps, and are thus suitable as educational materials or interior accessories. These specimens and educational kits (Figure 1B) have been used in public exhibitions and classes at museums, universities, high schools, and symposia since 2007. In addition, we are organizing a contact manufacturing system for the commercial production of specimens that comply with the Japanese regulation of commercial GM handling under a containment system. This system will be in operation during 2011, and every researcher will have the opportunity to make GM specimens without complicated administrative procedures. Details of this educational trial will be reported elsewhere.

On the other hand, there is no clear guidance on the regulation of the public exhibition of GMOs in Japanese domestic law. Therefore, we have to install research grade contained facilities at exhibition sites according to the regulations for the research use of GMOs. To overcome this ridiculous situation, we are working to develop a contained exhibition case suitable for GM plants (Figure 1C) and to formulate a rule prescribing proper handling under current laws. As a first attempt, Ono et al. in University of Tsukuba started exhibiting transgenic *Pharbitis* flowers with lost garden variety phenotype, those that have been revived by the application of CRES-T, in their training program. Such educational approaches using attractive GM flowers will accelerate the understanding of and social receptivity to GM plants.

Understanding the nature of gene functions for crop design

Most of the working models of genetic control, frequently found in scientific papers, are still composed of two dimensional on/off signaling. Although this style is effective in providing a simplified explanation, it might not provide enough basis for the discussion of sensitive and sequential changes in agronomic traits directly reflected in commercial value. This is because many factors determine the optimum phenotype, and a slight change in the balance of these factors may result in a myriad of novel phenotypes. The production of various floral phenotypes through the expression of *TCP3-SRDX* using petal specific promoters is a good example of this concept (Sasaki et al. in preparation). Genetic regulation of a certain phenotype cannot be explained by a single-state working model, therefore we need to develop a strategy for crop design on the basis that the regulation depends on a number of factors, including developmental stages, tissue identities, physiological states and environmental conditions. Considering the fact that most morphological mutants in *Arabidopsis* arose from the functional disturbance of transcription factors, controlling the target phenotype via transcription factors seems to be the best option for effectively modifying floral phenotypes, rather than overexpressing or targeting their downstream structural genes.

In basic research, results from model organisms are postulated as standards, while phenotypes caused by ectopic gene expression in different organs and/or developmental stages are often regarded as aberrant and as having low academic value. However, such a perspective often limits the potential use of genes to create novel applications or phenotypes. Many of the variations in commercial flowers are composed of sequential slight variations, which have been created through repetitive crossing or natural mutations. Each of them reflects a balance selected from a myriad of possibility generated by a combination of timing and spacing in gene expression. Most of these slight phenotypic changes do not affect the survival potential of the plants and do not concentrate directionally. Therefore, if we try to add novel phenotypes to the floricultural crops, it is important to screen valuable phenotypes from many different combinations of gene functions irrespective of their 'standard' function. While molecular breeding is sometimes regarded as an extension of basic research, there is a significant difference in the concept of gene utilization between the two.

There are two different types of phenotypic change in CRES-T-applied plants. One is dose-independent and always exhibits the same phenotype. In torenia, chimeric repressors of *Arabidopsis* AtRL2, AP2, and AGL6 are categorized as this type. The other exhibits wide variations between the lines, probably by reflecting the strength of the transgene effect. TCP3 and AG belong to this category. While these two types seem to be explained by differences between qualitative and quantitative characters, and the latter seem more complicated in their response to dose, timing, and spacing, in addition to stability and threshold. The fact that phenotypic strength does not always reflects the mRNA level of transgenes (Narumi et al. 2008; 2011), supports this idea. Therefore, there must be a better way to screen valuable genes from a number of candidates by definitive phenotypes, even when the target phenotype is clearly defined. This approach may help to achieve the target phenotype, rather than giving up on the basis of the results of few genes driven by the 35S promoter. To our knowledge, activity of the CaMV35S promoter sometimes varies according to tissue or developmental stage, especially in floral organs (Sasaki et al. 2011). The combined use of various genes and promoters for screening valuable phenotypes might become common hereafter.

Future molecular breeding: Will there be no need for model plants or traditional breeding?

It is sometimes difficult to control plant phenotypes through the modification of target gene function, because of the unpredictability of the physiological response of plants to the modification as well as the difficulty in fine-tuning the transgenic effect. Most of the practical trials of genetic modification without long-term financial support therefore failed to reach their goal, thus leading many molecular breeding research projects to unsuccessful conclusions. Based on increasing genomic information on cultivated species, the mass production of transgenic plants followed by intensive screening of valuable traits will be standard procedures in the future, as was true for many transgenic crops commercialized to date. In addition to our flower project, a research project producing and distributing the FOX hunting lines and the CRES-T lines of rice has recently started in the National Institute of Agrobiological Sciences in Tsukuba (http://www.nias.affrc.go.jp/index_e.html). These research communities will become major information and resource providers.

Under these circumstances, is there any demand for progress in studies based on model plants or developments in traditional breeding? The answer is unequivocally yes because intensive studies on the model plants such as *Arabidopsis*, rice, and tomato, still form a powerful tool to providing basic information on gene functions applicable to other plant species. Utilization of such information in appropriate combination with existing resources will facilitate the production of useful novel plants, and the outcomes can be utilized in traditional systems. Skills in the effective use of CRES-T and FOX hunting, in combination with other gene-

screening systems and breeding methods should be required for molecular breeders. Of course, cooperation with the private sectors or administrative bodies is required for extensive application and practical use, in addition to making good business sense. We are confident that transgenic lines produced by these systems will produce many fruitful results, and will contribute to our lives in the near future.

The multi-flowered cyclamen, in which endogenous functions of class C genes, *CpAG1* and *CpAG2* have been suppressed by natural mutation and CRES-T, respectively, produced beautiful flowers with many petals not seen before (the cover photograph, press releases issued on Mar. 16, 2010 by Hokko Chem. and AIST). This transgenic cyclamen developed in our Flower CRES-T Project is an excellent example of a transgenic plant with a low impact on biodiversity. It will be ready for the market in the near future as the third GM flower to be produced. Based on the technical improvement in phenotypic modification and sterilization, several flowers with novel different traits will be released via our project in the near future.

Concurrent with the release of this issue in March 2011, we have released to the public domain the upgraded version of the flower CRES-T database FioreDB (http://www.cres-t.org/fiore/public_db/) in order to share the information on CRES-T-applied transgenic plant phenotypes and generate new users of CRES-T. Extensive modifications and upgrades of the contents have greatly improved usability and accessibility, especially for beginners. Please refer to the manuscript at the end of this issue (Mitsuda et al. 2011) for more information and visit our flower wonderland.

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