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Original Paper

FioreDB: a database of phenotypic information induced by the chimeric repressor silencing technology (CRES-T) in *Arabidopsis* and floricultural plants

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Abstract Manipulation of horticultural plants' traits using genetic engineering has been a challenge because of gene redundancy and limited information concerning genome or other factors necessary for successful engineering. Recently we have developed a powerful tool with potential to overcome these difficulties, a novel gene silencing technology targeting transcription factor, which is designated Chimeric REpressor gene-Silencing Technology (CRES-T). Using this system, we are now analyzing biological functions of transcription factors in *Arabidopsis* and trying to manipulate morphological traits of various floricultural plants. To provide these information for genetic engineering of horticultural plants, we have developed the 'FioreDB' database in a web-based interface (http://www.cres-t.org/fiore/public_db/), which stores phenotypic information induced by various chimeric repressors in *Arabidopsis* and six floricultural plants, namely torenia, chrysanthemum, gentian, cyclamen, eustoma, morning glory. Users can find gene constructs that induce their preferred phenotype in *Arabidopsis* using simple searches, and can browse induced phenotypes in floricultural plants. Most phenotypic information has photo data. FioreDB is continually updated by addition of new data derived from the CRES-T analyses. FioreDB will help to improve traits of horticultural plants using the CRES-T system.

Key words: *Arabidopsis*, chimeric repressor, CRES-T, database, floricultural plant.

Genetic engineering and functional analysis of genes in horticultural plants, including crops and floricultural plants have been a great challenge. Among strategies that aim to improve plant traits and analyze gene functions, there have been several successful applications to confer useful traits to plants by employing exogenous genes, such as genes encoding herbicide resistance (Comai and Shen 1983), pest resistance (Bates et al. 2005), and increased production of nutrients (Ye et al. 2000). Gene-knockout or knockdown technology is major strategies for functional analysis of genes, and can confer new traits on plants. However, these techniques have various difficulties mainly due to genetic redundancy, polyploidy, and limited information of genomic or transcriptomic information. Although RNA interference (RNAi) effectively knocks down the expression of the targeted genes in model plants such as *Arabidopsis thaliana* and rice, it is difficult to apply this technology to horticultural plants because sequence information of the target gene is required. Furthermore, even if the technique is successfully applied, genetic redundancy of genes often compensates for the specific gene that has been knocked-

Abbreviations: CRES-T, Chimeric REpressor gene-Silencing Technology; RNAi, RNA interference; CaMV, Cauliflower Mosaic Virus
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down.

Recently we developed a novel gene-silencing technology targeting transcription factor, designated Chimeric REpressor gene Silencing Technology (CRES-T), which has potential to overcome these problems (Hiratsu et al. 2003). In this system, a plant-specific transcriptional repression-domain named "SRDX", consisting of only 12 amino acids originally derived from SUPERMAN that acts as strong repressor (Hiratsu et al. 2002), is fused to the C-terminal of the preferred transcription factor. The resultant chimeric repressor is constitutively expressed in the plant. As a result, the chimeric repressor dominantly suppresses the target genes and the transgenic plant has a phenotype similar to loss-of-functional alleles of the transcription factors, even if there are endogenous and functionally redundant transcription factors (Hiratsu et al. 2003). We have shown that this CRES-T system functions in various transcription factors of *Arabidopsis* and rice, resulting in the induction of defective phenotypes that could not be revealed in knockout or knockdown lines (Fujita M et al. 2004; Fujita Y et al. 2005; Koyama et al. 2007; Kubo et al. 2005; Matsui et al. 2004, 2005; Mitsuda et al. 2005, 2006, 2007). There is high conservation of amino-acid sequences of each transcription factor and those of the 'repression domain' among plant species. We therefore expected that the chimeric repressors derived from *Arabidopsis* may function in other species, including horticultural plants. This may be one of the greatest advantages of this strategy. We expressed various chimeric repressors in floricultural plants in an attempt to improve flower traits and obtained many interesting results. Here we report on the database designated "FioreDB" that stores phenotypic information induced by the chimeric repressor in *Arabidopsis* and in six floricultural plants (http://www.cres-t.org/fiore/public_db/). FioreDB provides information concerning gene constructs of the chimeric repressors, and morphological phenotypes induced by these constructs in *Arabidopsis* and six different floricultural plants. Scientists who attempt to improve the traits of horticultural plants using CRES-T can search for particular gene constructs and phenotypes induced by them in *Arabidopsis* or in floricultural plants. This database will help scientists to improve traits of horticultural plants using the CRES-T system.

Materials and methods

Software used for the construction of the database

All CGI programs for dynamic contents were written in Perl. All web services are on the Apache server. The database is managed by MySQL.

Plant transformation

Arabidopsis plant was transformed by conventional floral dip method (Clough and Bent 1998). Transformation methods of *Torenia* (Aida and Shibata 2001), *Chrysanthemum* (Aida et al. 2004), *Pharbitis* (Kikuchi et al. 2005) and *Gentiana* (Mishiba et al. 2005) were described previously. Improved method of transformation of *Cyclamen* is reported in this issue (Terakawa et al. 2008). The transformation method of *Eustoma* is following. Leaf disc was excised from 60-day-old plant and pre-cultured on MS-based re-differentiating medium containing 0.5 mg/L benzylaminopurine (BA) for 8 days. Pre-cultured leaf disc was treated with *Agrobacterium* EHA101 and cultured together for 9 days to allow transformation. Leaf disc was washed to eliminate *Agrobacterium* and cultured on medium containing 50 mg L^{-1} hygromycin to select transformed shoot.

Results and discussion

Overview of data stored in the database

FioreDB stores more than 2,500 pieces of phenotypic information. Phenotypes have been induced by the expression of various chimeric repressors in *Arabidopsis* and other floricultural plants including *Torenia fournieri*, *Chrysanthemum morifolium*, *Gentiana triflora*×*G. scabra*, *Cyclamen persicum*, *Eustoma grandiflorum*, and *Pharbitis* (*Ipomea*) *nil* (Table 1). The chimeric repressors were driven by the CaMV 35S promoter. In *Arabidopsis*, more than 400 strains that express each individual chimeric repressor were grown and phenotypically examined. Visible phenotypes induced by the chimeric repressor in the transgenic *Arabidopsis* plants were classified into 79 terminologies as listed in Table 2. This terminology list is based on that of Kuromori et al. (2006) with slight modifications. This project is designated as the "Individual project" in contrast to the "Bulk project" described later. Remarkably, more than half the gene constructs encoding chimeric repressors induced visible phenotypes (Table 1). This indicates that CRES-T has potential to induce phenotypic change with high efficiency. We transformed approximately 40 gene constructs that induce visible phenotypes in *Arabidopsis* into various other floricultural plants. We found that the frequency of visible phenotypes induced by the chimeric repressor was highly variable among species (Table 1). For example, 9 of 15 gene constructs introduced into

Table 1. Summary of data stored in the database as of September 10, 2007.

Species	Number of transformed gene constructs	Number of genes showing phenotype
Chrysanthemum morifolium	36	11
Torenia fournieri	15	9
Gentiana triflora×*G. scabra*	11	2
Cyclamen persicum	13	0
Eustoma grandiflorum	8	2
Pharbitis nil	13	5
Arabidopsis thaliana	395	229

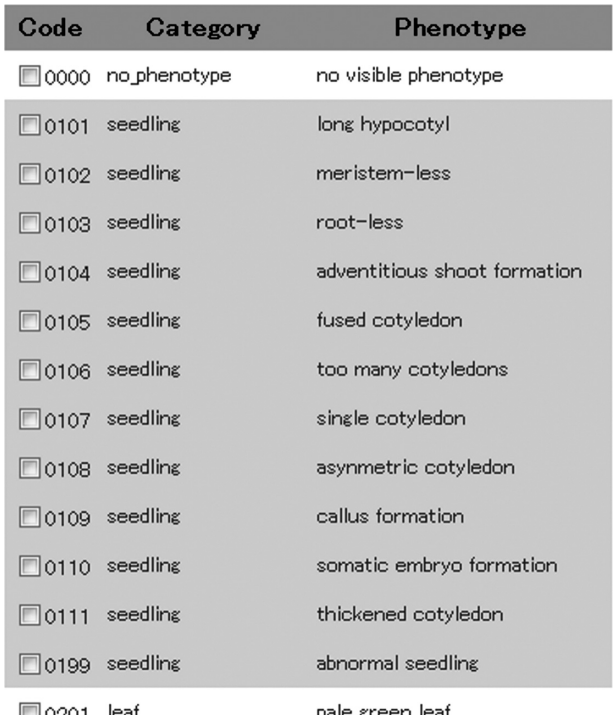
torenia induced morphological changes, indicating that CRES-T is effective in this species, whereas it was less effective in chrysanthemum and gentiana (Table 1). The small genome size of torenia (171 Mbp, which is slight bigger than that of *Arabidopsis*) may contribute to this high efficiency. The low efficiency of the chimeric repressor in chrysanthemum may be due to its polyploidy (hexaploid). Multi-copied endogenous genes may be a major obstacle in applying CRES-T, even though CRES-T has great potential to overcome gene redundancy. In gentiana, every gene construct was driven by the promoter of the *Arabidopsis ACTIN2* (*ACT2*) gene (An et al. 1996) instead of the CaMV 35S promoter, because the 35S promoter is known to be extensively methylated and inactivated in gentiana (Mishiba et al. 2005). The promoter activity of *ACT2* might be not strong enough for the chimeric repressor to induce morphological changes especially in flower. Among the chimeric repressors that induced visible phenotypes in *Arabidopsis*, not all induced similar phenotypic changes observed in *Arabidopsis* in other floricultural plants. This suggests that regulatory mechanisms, such as binding sequence of transcription factor, are not always conserved among different species. In some cases however, the gene construct used in *Arabidopsis* was similarly effective in other plants including chrysanthemum and gentiana without any changes, indicating the wide applicability of the CRES-T system.

To efficiently identify the chimeric repressors that may be useful for the improvement of floricultural plants, we performed large-scale screening of *Arabidopsis* using mixed gene constructs or T1 seeds in which around 40

Table 2. Classified terminology of phenotypes and the number assigned to each category.

Tissue	Phenotype	Individual project	Bulk project
Seedling	Long hypocotyl	6	8
	Meristem-less	19	12
	Root-less	3	8
	Adventitious shoot formation	16	4
	Fused cotyledon	15	0
	Too many cotyledons	3	0
	Single cotyledon	0	0
	Asymmetric cotyledon	7	0
	Callus formation	1	0
	Somatic embryo formation	2	0
	Thickened cotyledon	11	4
	Other	41	4
Leaf	Pale green leaf	12	14
	Dark green leaf	16	52
	Variegated leaf	0	2
	Hyponastic leaf	15	85
	Rough surface leaf	21	141
	Short petiole	0	22
	Long petiole	5	24
	Twisted petiole	0	8
	Abnormal phyllotaxis	22	3
	Too large leaf	11	18
	Glabra	6	36
	Narrow leaf	54	55
	Wide leaf	6	8
	Over-accumulated anthocyanin	2	14
	Serrated leaf	24	30
	Thickened leaf	0	14
	Too many rosettes	12	30
	Abnormal shaped leaf	51	90
	Other	19	45
Flowering and growth	Early flowering	12	29
	Late flowering	14	48
	Aerial rosette	8	11
	Dwarf	47	90
	Slow growth	21	27
	Loss of apical dominance	28	22
	Tall height	25	17
	Strong apical dominance	1	1
	Non-rosette growth	0	7
	Other	1	8
Stem	Waxy stem	0	2
	Thin stem	1	1
	Fasciated stem	2	0
	Fused stem	10	10
	Fragile stem	2	11
	Other	16	4
Branching	Bushy	47	16
	Short internode	2	5
	Too few branchings	0	3
	Too many branchings	1	0
	Abnormal branching	0	0
Flower	Short pedicel	24	32
	Abnormal sepal	21	12
	Abnormal petal	44	8
	Abnormal stamen	10	9
	Delayed growth of stamen	0	23
	Anther indehiscent	2	3
	Short stamen	6	24
	Pollen defective	4	13
	Abnormal carpel	7	11
	Other	87	49
Silique	Short silique	38	12
	Abnormal shaped silique	57	13
	Indehiscent silique	1	52
	Other	13	6
Seed	Sterile or low fertility	79	101
	Dark seed color	0	2
	Light seed color	8	15
	Abnormal shaped seed	3	2
	Embryonic lethal	4	1
	Viviparous	2	0
	Other	2	1
Root	Short root	16	0
	Root-hair-less	0	0
	Too many root hairs	6	0
	Few lateral roots	3	0
	Too many lateral roots	2	0
	Abnormal root	8	0
Total		1085	1432

Figure 1. Entry page of FioreDB. The database has two entry gates at the bottom of the page; "Search by phenotype" and "Browse constructs". The hyperlinks to other content are listed horizontally at the top of the page.



Code	Category	Phenotype
0000	no_phenotype	no visible phenotype
0101	seedling	long hypocotyl
0102	seedling	meristem-less
0103	seedling	root-less
0104	seedling	adventitious shoot formation
0105	seedling	fused cotyledon
0106	seedling	too many cotyledons
0107	seedling	single cotyledon
0108	seedling	asymmetric cotyledon
0109	seedling	callus formation
0110	seedling	somatic embryo formation
0111	seedling	thickened cotyledon
0199	seedling	abnormal seedling
0201	leaf	pale green leaf

Figure 2. Search by phenotype. The part of the page where users select their preferred phenotypes. Users access this page by clicking "Search by phenotype" in the entry page of FioreDB. "Category" indicates categorized organs, and "Phenotype" indicates phenotype observed in each organ.

kinds of them were mixed in *Arabidopsis*. From each batch, we grew approximately 200 independent transformants and examined phenotypes individually (Table 2). After harvesting T2 seeds, the genome of each plant showing a distinct phenotype was extracted from a stored leaf and the introduced gene construct was determined. This was designated as the "Bulk project". To date, more than 1,400 phenotypes from approximately 1,000 independent T1 plants are stored in the database. The frequency of each phenotype is shown in Table 2. We have determined the transgenes in more than half of the transgenic plants produced in the "Bulk project". The database contains all "Individual project" and "Bulk project" data from *Arabidopsis*, and the phenotypic data of other plants about maximum 40 genes.

The database has two entry gates for searching (Figure 1). One is "Search by phenotype", designed for finding gene constructs that induce preferred phenotypes in *Arabidopsis*. The other is "Browse constructs", designed for browsing a list of phenotypes and gene constructs introduced into *Arabidopsis* and various other plants. Most phenotype records also have photo data.

Searching for gene constructs that induce preferred phenotypes in Arabidopsis

As described above, we classified morphological phenotypes observed in *Arabidopsis* using terminology listed in Table 2. Database users can find the gene constructs that induce the preferred phenotype from these classifications using a simple interface with checkboxes (Figure 2). Searching can be carried out with "OR" searches but not "AND" searches. Users can simultaneously search both data of the "Individual project" and the "Bulk project". The search result is shown as two simple list-views representing the results from the "Individual project" and "Bulk project" (Figures 3A and 3B). The result from the "Individual project" shows all gene constructs inducing at least one of the phenotypes selected by the user in each row of the list-view (Figure 3A). This list-view contains data for the genes with hyperlinks to each gene's summary page. The summary includes data from other plants, if exist, and phenotypes associated with the gene with a hyperlink to a page showing a photograph of the phenotype, if photo data exists (Figure 3A). The search result shows not only the phenotype selected by the user, but also information of all the phenotypes associated with the gene construct. On the other hand, the search result from the "Bulk project" shows all individual T1 plants exhibiting phenotypes selected by the user in each row of the list-view (Figure 3B). This list-view contains an ID number unique to each set of transgenic plants growing on one tray (one set includes up to 51 T1 plants), the serial number within a set, and, if identified, the gene construct introduced into the plant. The gene construct is hyperlinked to a page showing a summary of the gene, and all phenotypes associated with the plant, with a hyperlink to a page showing photo data, if it exists. If

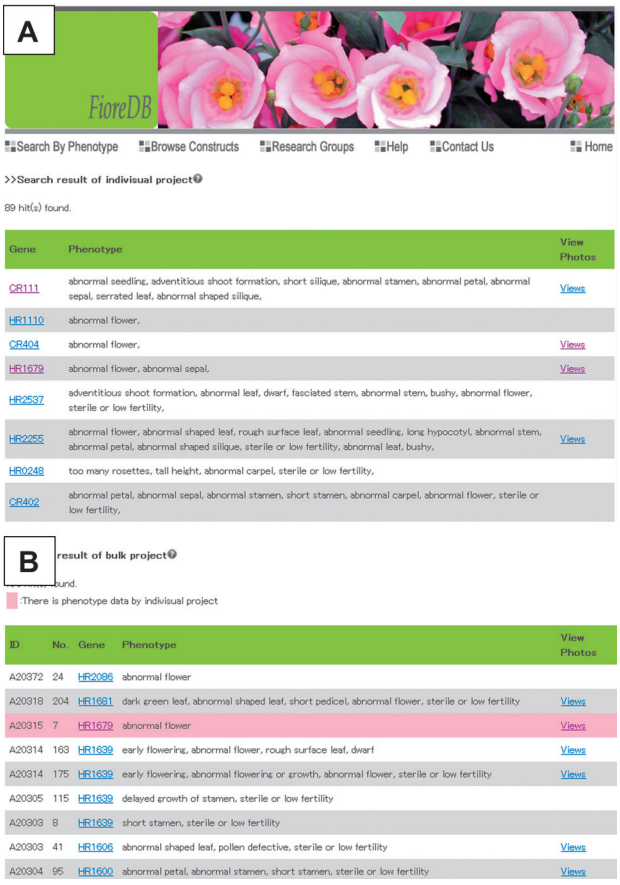


Figure 3. Search result of "Search by phenotype". (A) An example of search result of "Individual project". Gene constructs inducing the phenotype selected in the previous page (Figure 2) can be found. Each row shows the gene, phenotype, and the hyperlink to a page showing a photo, if it exists. (B) An example of a search result of "Bulk project". Each row shows the unique ID of the plants, transformed genes, phenotypes, and a hyperlink to a page showing photo data.

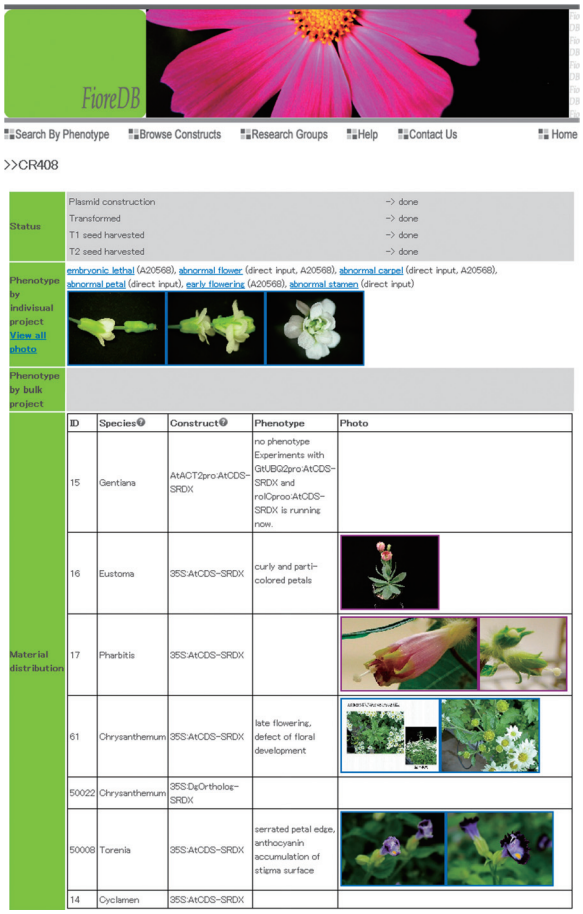


Figure 4. Page showing gene information. The page shows information regarding the status of the "Individual project", and the phenotype recorded by "Individual project" and "Bulk project" with photos, if they exist. It also shows information of floricultural plants, including species, gene constructs and phenotypes induced by them with photos, if they exist.

there is data from the "Individual project" concerning the identified gene, the row is colored pink, letting the user validate the data by checking the result from the "Individual project".

The user can browse a brief summary of each gene by clicking the hyperlink (Figure 4). This summary includes a report of the progress of the "Individual project", phenotypes recorded by the "Individual project" and the "Bulk project" with thumbnails of photo data, if they exist. There is also a list of phenotypes and gene constructs introduced into other species with thumbnails of photo data, if they exist.

Browsing phenotypes and gene constructs introduced into floricultural plants

Users can browse a list of gene constructs introduced into floricultural plants in addition to *Arabidopsis* (Figure 5A). We have already transformed approximately 40 gene constructs into floricultural plants (Table 1). This list-view shows the gene and the number of gene constructs transformed into each plant in each row. The list includes other gene constructs (e.g., over-expression) in addition to the chimeric repressors. In addition to this list-view, users can browse a more detailed list of phenotypes and gene constructs introduced into various plants in addition to *Arabidopsis* by clicking a hyperlink at the top of the first list-view (Figure 5B). In each row, this list-view shows a unique ID for all experiments, a short description of the introduced gene construct, species, phenotype with classified terminology (described in Table 2), and a hyperlink to a page showing photo data, if it exists. In most experiments, the gene construct used for *Arabidopsis* was employed for other plants directly without any modification. In some cases, the gene construct was optimized for a particular species. For example, the *AtACT2* promoter was used instead of the CaMV 35S promoter in gentiana, and in another case, an orthologous gene in the respective species was used instead of the *Arabidopsis* gene. From these list-views, users can browse a brief summary of

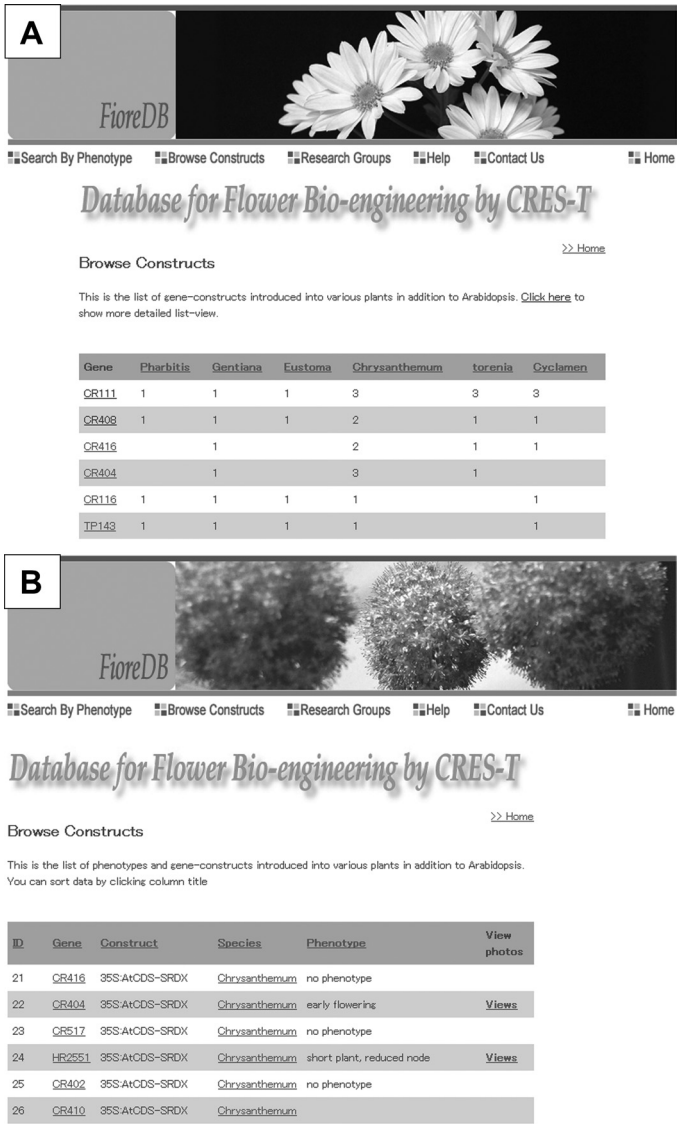


Figure 5. Browsing constructs. (A) The first page of "Browsing constructs". The list-view shows the gene constructs, which include not only constructs for chimeric repressors but also some other gene constructs such as over-expression, and number of gene constructs transformed into each of six floricultural plants in each row. (B) The second list-view, accessed by clicking "Click here" on the first page, displays gene constructs, their brief description, species they have been introduced into, phenotype, and a hyperlink to a page showing photo data in each row.

each gene as described in the previous section by clicking the hyperlink of the gene-code.

Conclusion

We have developed the FioreDB database, which stores phenotypic data mainly induced by CRES-T in six different floricultural plants and in *Arabidopsis*. At present, the database stores thousands of pieces of phenotypic data from *Arabidopsis* described by limited and classified terminology, and data from approximately 40 gene constructs in floricultural plants including torenia, pharbitis, gentiana, eustoma, cyclamen and chrysanthemum. The FioreDB database is continually updated by adding new data. We expect that scientists who want to improve morphological traits of horticultural plants will be users of the database. The user can find the gene constructs inducing preferred phenotypes in *Arabidopsis* and check the effect of the construct in other floricultural plants. The user can also request the gene construct from us. This database will facilitate genetic engineering and the study of gene function in horticultural plants using CRES-T.

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