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Review

Importance of genetic transformation in ornamental plant breeding

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Abstract Most of the economically important ornamental plants are cut flowers, which are produced by vegetative propagation. For many years, new varieties of ornamental plants have been produced by cross-hybridization and mutation breeding techniques, separately or in combination. Similar to mutation breeding, genetic transformation would also be a useful way of making a one-point improvement of a trait in original cultivars bred by cross-hybridization. Mutation breeding can change a dominant trait to a recessive one mostly. In other words, genetic transformation produces an "additive" one-point improvement, whereas mutation breeding produces a "subtractive" one-point improvement. Furthermore, genetic transformation can modify target traits by direct incorporation of related genes. Genetic transformation methods will be used in the near future as standard breeding tools in combination with traditional breeding methods.

Key words: Genetic transformation, ornamental plants, cross-hybridization, mutation breeding, chrysanthemum.

Characteristics of ornamental plants

The total area of cultivated ornamental plants in Japan was 21,889 ha in 2005. Japan is one of the leading floricultural countries in the world. The top 20 ornamental plants, based on wholesale price, are shown in Table 1 (Division of Statistics, MAFF, 2007). Chrysanthemum is the most important flower in Japan, followed by lily, rose, carnation, orchid (*Phalaenopsis*), and prairie gentian, in that order. Out of the top 20

Table 1. Top twenty ornamental plants evaluating their wholesale amount in Japan (2005)

No.	Ornamental plants	Gross (million)	Type	Propagation style
1	*Chrysanthemum*	100,940	cut flower	V
2	*Lilium*	30,012	cut flower	V
3	*Rosa*	28,999	cut flower	V
4	*Dianthus*	21,855	cut flower	V, S
5	*Phalaenopsis*	15,265	potted flower	V
6	*Eustoma*	12,834	cut flower	S
7	*Cyclamen*	6,996	potted flower	S, V
8	*Limonium*	6,942	cut flower	V, S
9	*Cymbidium*	6,053	potted flower	V
10	*Gerbera*	5,878	cut flower	V
11	*Gypsophila*	5,813	cut flower	V
12	*Alstroemeria*	5,518	cut flower	V
13	*Dendrobium*	4,730	cut flower	V
14	*Matthiola*	4,491	cut flower	S
15	*Gentiana*	4,403	cut flower	S
16	*Tulipa*	4,111	cut flower	V
17	*Lathyrus*	4,021	cut flower	S
18	*Viola*	3,979	bedding plants	S
19	*Delphinium*	3,899	cut flower	S, V
20	*Cymbidium*	3,868	cut flower	V

This table provides the data obtained by the Division of Statistics, MAFF (2007), along with propagation style, V: vegetatively propagated; S: seed propagated; V, S or S, V: though both, but primarily the first species, 16 are used as cut flowers, of which 11 are mainly produced by vegetative propagation. Thus, most of the economically important ornamental plants in Japan, and perhaps in the rest of the world, are asexually propagated cut flowers.

In a broad sense, ornamental plants include all plants used for ornamental purposes. Consumers demand new types of floricultural crops and are eager for new cultivars with ornamental value, such as flowers with new shapes and colors. Therefore, the lifespan of ornamental plant cultivars is much shorter than that of other crops. However excellent a cultivar is, it eventually loses market share. Because consumers lose interesting as familiarity wears its freshness off. This is the fate of most ornamental plants, and hence breeders or breeding companies have to continuously develop and release new cultivars.

Practical ornamental plant breeding

For many years, various types of ornamental plants have been produced by cross-hybridization and mutation breeding techniques, separately or in combination. A typical breeding method, as used for chrysanthemum, is outlined below.

Many ornamental plants originate from interspecific hybridization, which leads to a high degree of heterozygosity in the resulting hybrids. Often, polyploidy and aneuploidy also occur (Horn 2002). Chrysanthemum cultivars originated from an interspecific hybrid among wild species native to China (Machin and Scope 1978). The contemporary cultivars are hexaploids with loss or gain of several chromosomes. Their mode of inheritance is very complex and breeders sow a large number of

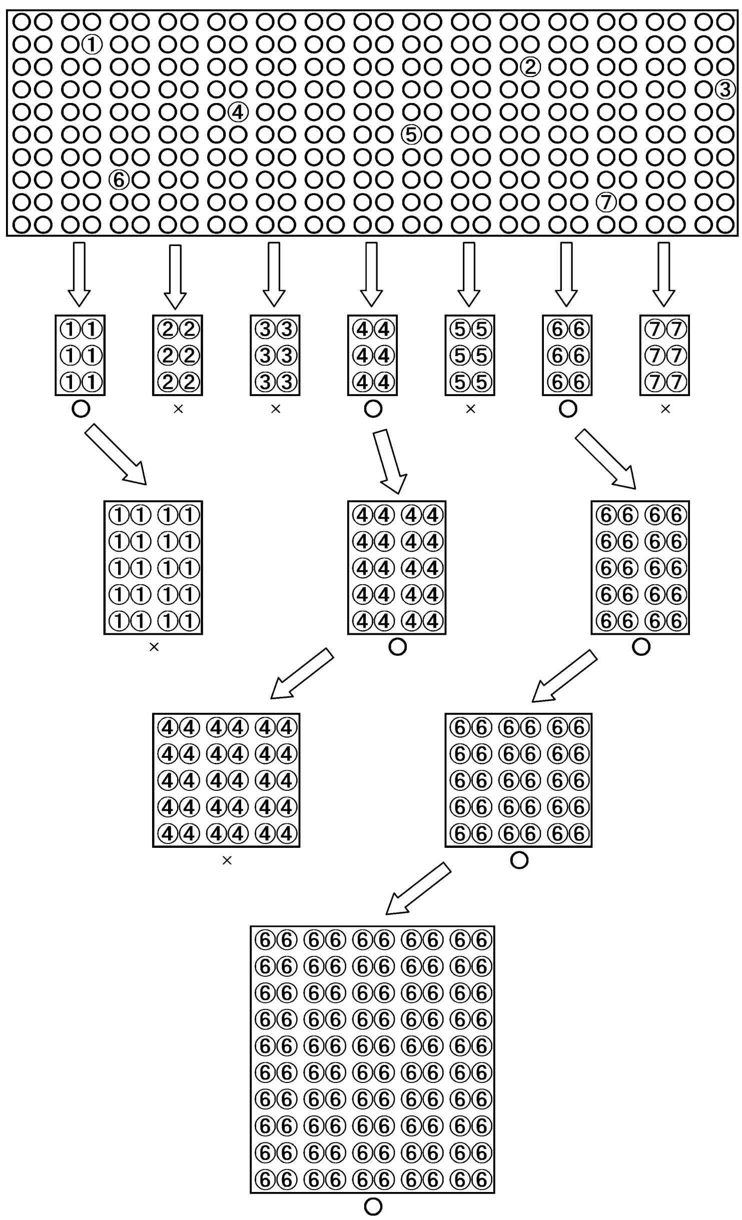

Figure 1. Basic scheme for breeding chrysanthemum cultivars.

Figure 2. Breeding process from initial cross to flowering of progeny in chrysanthemum. (A) Left, before pollination; right, shortened style 1 day after pollination. (B) Harvested fertile seeds. (C) Germinated seedlings. (D) Seedlings planted in the field. (E) Flowering progeny. The bars represent 10 mm (A, B, C).

seedlings in order to select the superior ones. The largest scale of chrysanthemum breeding is nearly one million seedlings per year in Japan.

Chrysanthemums are propagated asexually by cutting for commercial production; however, sexual reproduction by cross-hybridization is essential for breeding. The basic scheme for the breeding of chrysanthemum cultivars is shown in Figure 1. The first breeding process from the initial cross to the flowering of progeny is shown in Figure 2.

Cross-pollination by suitable parents is usually conducted in autumn. When fertilization is successful, the style of the pistil is shortened the day after pollination (Figure 2A). Fertile seeds ripen on the capitulum after 2 months (Figure 2B). The seeds are harvested and stored to be sown in early summer of the following year. Seedlings germinate within a week (Figure 2C); they are planted in the field in summer, a month after sowing (Figure 2D), and the plants flower in autumn (Figure 2E).

The second process involves the cycled selection of seedlings by cutting (Figure 1). Superior individuals are selected from the seedling population and screened in several cycles. Selected plants are asexually propagated and tested in replicated trials, and the finally selected clone is vegetatively propagated for marketing as a new

Figure 3. Cultivar “Reagan Elite White.”

cultivar clone (Horn 2002).

The ideal model for year-round production of chrysanthemum, as proposed by Langton and Cockshull (1976), is as follows: (1) Strong apical dominance during vegetative growth; (2) Immediate and rapid flower bud initiation under short days in both apical and axillary meristems; (3) A very high maximum leaf number, giving marked delay of bud initiation under long days (this helps prevent budding on stock plants); (4) A very high leaf initiation rate under long days; (5) Long internodes and rapid internode extension under short days; (6) Extremely rapid flower development under short days; (7) Moderate peduncle extension under short days; (8) A "thermozero" temperature response showing little or no delay in flowering at temperatures above and below 15.6°C; (9) Easily rooted cuttings that can withstand cold storage for at least 10 days; (10) Strong peduncles and stems that take up water adequately; (11) Large horizontally displayed leaves; (12) Pink flowers that can give rise to all other colors by mutation; (13) Low competitive ability in the flowering area; (14) Uniform and rapid flower bud formation at night temperature 10–13°C in the winter season; (15) Small to medium-sized leaves, resulting in a low competitive ability in the winter season; and (16) Flower characteristics, such as color, form, and size, as required by the consumer. It is difficult to produce the perfect cultivar with ideal characteristics by the process of selection.

In nature, spontaneous mutations for all kinds of traits occur at a low frequency. Such spontaneous mutants, often called "sports" or "bud sports," have long been of interest to ornamental plant breeders. The contribution of sports to the market for a number of economically important ornamental plants was noted by Wasscher (1956). The percentage growth for chrysanthemum, carnation, rose, and winter-flowering begonia was 30%, 25%, 40%, and 70%, respectively, during this period. Recently, artificial mutation breeding methods have been applied by irradiation using X-ray or gamma rays. Both spontaneous sports and artificially induced mutants have contributed to the flower industry in the Netherlands. The most successful cultivar and "mutant family" in chrysanthemum was the cultivar "Reagan" ("Sei-Rosa" in Japan, Figure 3) and its family, with more than 20 mutant cultivars and 400 million stems (representing 35–40% of the total Dutch market) being sold annually from 1992 to 1993 (Van Harten 2002).

Most customers like to choose from a wide variety of flower types and colors. However, breeders dislike growing a large number of cultivars because different growing regimes are required for different cultivars. By applying mutation breeding methods, new mutant cultivars with genetic variation for traits of interest to the customer may be obtained in a relatively easy way, while maintaining the required growing condition of the original cultivar (Van Harten 2002).

As mentioned above, the combination of cross-hybridization and mutation breeding has worked well in chrysanthemum breeding. Cross-hybridization is important in developing original cultivars that are close to the ideal model to the maximum extent. Mutation breeding can then be used for a one-point improvement of traits, such as flower color, morphology of flowers and inflorescence, leaf characteristics (form and size), growth habit characteristics (compact and branching types), and physiological traits (including photoperiodic response, early flowering, maintenance of flower quality, and tolerance to biotic and abiotic stresses). Improvement of flower color and size is comparatively easy to accomplish. Ornamentals are ideally suited for mutation breeding, because many economically important traits, such as flower characteristics, can be easily monitored after a mutagenic treatment.

Advantage of genetic transformation

Genetic transformation in higher plants was first reported by Zambryski et al. (1983). Meyer et al. (1987) reported that the introduced dihydroflavonol 4-reductase gene derived from *Zea mays* changed the color of petunia flowers to red, which was the first reported instance of flower color alteration by genetic transformation. Flower color is one of the most successfully improved target traits using the gene transfer technique. In 1997, the first genetically modified blue carnation was introduced in the market (Tanaka et al. 2005). Genetic transformation can improve traits derived from one or several kinds of genes. Thus, the technique would be useful in producing a one-point improvement of traits in original cultivars bred by cross-hybridization in a manner similar to the mutation breeding method. The advantage of genetic transformation compared with the traditional breeding method is shown in Figure 4.

As most mutations involve a change from dominance to recessiveness, mutation breeding has to start from plant material in which the target genes are known to be present in a recessive condition. For example, the direction of flower color mutations in chrysanthemum is known (Machin and Scope 1978; Figure 5). Pink is dominant and yellow is recessive; therefore, pink-flowered cultivars are thought to be useful as mutation breeding materials. White-flowered cultivars frequently give rise to yellow-flowered mutants, but the reverse mutation has rarely been observed. Therefore, mutation breeding is "subtractive" one-point improvement. On the other hand, genetic transformation can produce changes from dominance to recessiveness and vice versa. Genetic transformation is thus "additive" one-point improvement, which is a great advantage.

Genetic transformation is superior to mutation breed-

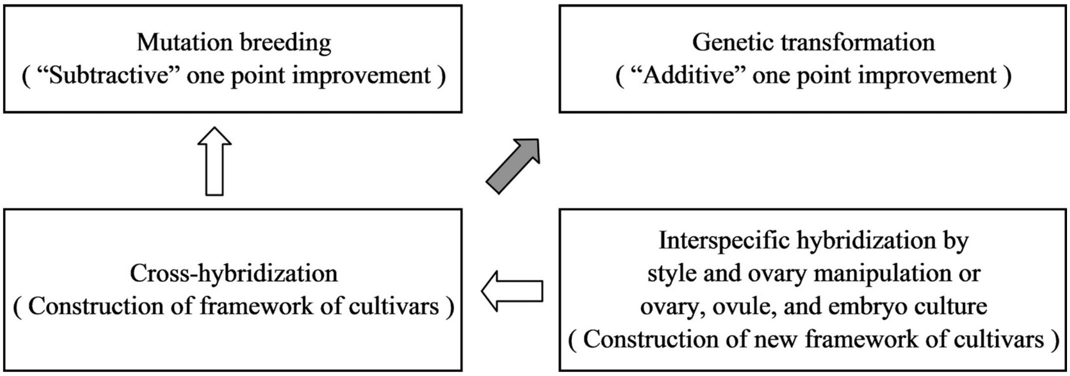

Figure 4. Combinations of breeding methods for vegetatively propagated ornamental plants.

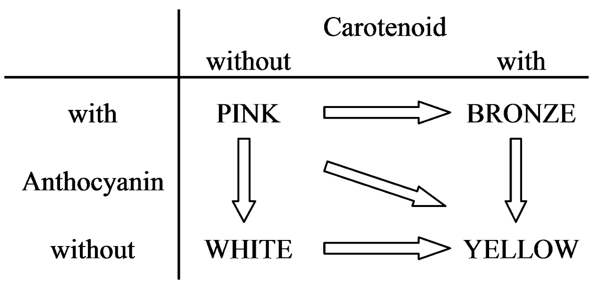

Figure 5. Directions and relative frequency of flower color mutations (Machin and Scope, 1978).

ing because the target trait can be modified directly by incorporating related genes. In mutation breeding, the desirable traits can be obtained only when the genes related to the targeted traits are altered accidentally.

Interspecific hybridization has also contributed much to the production of a broad range of genetic variation in cultivated plants. It has been successfully performed using style and ovary manipulation, or ovary, ovule, and embryo culture. However, interspecific hybridization introduces unexpected traits along with desirable ones. Because only one gene or several genes are introduced directly in genetic transformation, the possibility of incorporating unexpected traits is much lower than in interspecific hybridization. Furthermore, genetic transformation is a method with essentially infinite possibilities because the necessary resources are available in every living being on earth.

Future Prospects for Genetic Transformation

The species in which transgenic plants have been reported are shown in Table 2 (Bajaj 2001; Deroles et al. 2002; Chandler and Tanaka 2007). Since the transformation system is highly cultivar-dependent, which is a drawback, it is necessary to develop transformation systems in more species and cultivars. Moreover, it is important to develop more efficient

Table 2. Ornamental species for which trangenic plants have been generated (revised from Bajaj 2001; Deroles et al. 2002; and Chandler and Tanaka 2007)

Species	Species
Agapanthus sp.	*Gladiolus grandiflorus*
Alstroemeria spp.	*Hemerocallis* sp.
Antirrhinum majus	*Ipomea nil*
Anthurium andraeanum	*Iris germanica*
Begonia spp.	*Kalanchoe* spp.
Calanthe sp.	*Lavatera* sp.
Campanula spp.	*Lentopodium alpinum*
Cattleya spp.	*Linum* spp.
Chrysanthemum morifolium	*Lilium* spp.
Cyclamen persicum	*Lobelia erinus*
Cymbidium spp.	*Nierembergia scoparia*
Datura spp.	*Ornithogalum* spp.
Delphinium spp.	*Osteospermum* spp.
Dendrobium spp.	*Pelargonium* spp.
Dianthus caryophyllus	*Petunia hybrida*
Doritaenopsis hybrids	*Phalaenopsis* spp.
Eschscholzia californica	*Rhododendron* spp.
Euphorbia pulcherrima	*Rosa* spp.
Eustoma grandiflorum	*Rudbeckia hirta*
Forsythia×intermedia	*Saintpaulia ionantha*
Gentiana spp.	*Torenia fournieri*
Geranium spp.	*Verbena hybrida*
Gerbera hybrida	*Verticordia grandis*

transformation systems.

Another problem is the potential for gene flow from transgenic plants to native wild species. It is known that most spontaneous and artificial mutants are periclinal chimeras in which the mutant genes exist only in the epidermal layer (L-1). In several kinds of ornamental plants, periclinal chimeras generally represent a stable situation, and when cuttings are made from side shoots, the resulting plants will all be identical and similar to the original plants (Van Harten 2002). If transgenic periclinal chimeras could be formed, this might be useful in preventing gene flow to native wild species. Research on periclinal chimerism is an important task for the future.

Several kinds of useful genes have been isolated and analyzed in ornamental crops, e.g., floral pigmentation by anthocyanins and carotenoids (Ben-Meir et al. 2002; Cunningham and Gantt 2002), flower development

(Vishnevetsky and Meyerowitz 2002), floral fragrance (Dudareva 2002), floral senescence (Thompson and Wang 2002), light sensing in plant development (Samach and Pineiro 2002), and plant resistance to biotic and abiotic stresses (Lorito et al. 2002). Genetic transformation is certain to join traditional breeding methods as a standard tool for ornamental plant breeding in the near future.

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