Intervarietal Chimera Formation in Cabbage (*Brassica oleracea* L.)

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A histological and genetical study was done on intervarietal graft chimeras between "Yoshin kanran" (green colored) and "Murasaki kanran" (purple colored) in cabbage (*Brassica oleracea*). When the anthocyanin pigmentation pattern of the chimeric tissues was observed, epidermal and subepidermal cells were mosaic and recognized to be more complicated than the external appearance. The genotypic constitution of germ cells in chimera plants (V_0 , V_1) was studied through the segregation pattern of anthocyanin pigmentation in progeny plants derived from each capsule obtained by geitonogamy of chimeras and back crosses with the green parent "Yoshin kanran" and purple parent "Murasaki kanran". No segregation of plant color was observed in most of the progenies of two V_0 chimera plants as well as their vegetatively propagated V_1 chimera plants. However, segregations were observed in the progeny plants from two capsules of one V_0 chimera plant crossed with "Murasaki kanran" and from two capsules of one V_1 chimera plant by geitonogamy.

These segregation phenomena in the progeny plants suggest two possibilities. The first possibility is the chimericity of the germ cell layer in a single flower of the parental chimera plant, the second is gene transfer between different tissues or cells.

KEY WORDS: *Brassica oleracea*, graft, chimera, mosaic, anthocyanin.

Introduction

Graft chimeras in plants have not been much studied after the classical works of DARWIN (1868), WINKLER (1907) and JØRGENSEN and CRANE (1927) except for improvement of fruit and ornamental plants. An important question remaining unresolved is whether or not genetic changes exist in chimeras (NEILSON, 1963).

We have studied graft-induced genetic changes in pepper (YAGISHITA, 1961a, 1961b; YAGISHITA *et al.* 1985; YAGISHITA and HIRATA, 1986, 1987), eggplant (HIRATA, 1979), tomato (HIRATA, 1980) and soybean (HIRATA, 1986). These results suggest that the mechanism of such changes is the transmission of DNA of degraded cells in the stock plant to scion germ cells via its vascular system and the integration of the DNA into chromosome (s) (HIRATA and YAGISHITA, 1989) in accordance with the characteristics of genetic changes induced by grafting in red pepper (OHTA and CHUONG, 1975a,b), and confirming the mechanisms of "graft-transformation" (PANDEY, 1976; OHTA, 1977). Chimera plants are usually produced from grafting. Therefore, the occurrence of graft-induced genetic change is naturally expected. However, there have been only few reports on this topic (GLUSCHCHENKO, 1974, 1975; KUMAGAI and USAMI, 1964, 1966; MASUBUCHI, 1971, 1977) and no resolution has been made.

The present study was begun to establish a method of chimera formation as a new means of obtaining such genetic changes as graft-induced change and transformation.

Here, the method of chimera formation and some histological and genetical characteristics

Table 1. Segregation pattern of plant color in the progenies from geitonogamy of chimera plants (V_0) and from their crosses with "Yoshin kanran" and "Murasaki kanran"

Cross combination	(No. of crosses)	Segregation in progenies		
		Green	Light purple	Purple
Chimera-green (geitonogamy)	(6)	43	0	0
Chimera-purple (geitonogamy)	(14)	0	0	70
Chimera-green $\times$ Y	(15)	65	0	0
Y $\times$ Chimera-green	(3)	17	0	0
Chimera-green $\times$ M	(1)	0	20	10
Chimera-green $\times$ M	(1)	0	18	11
Chimera* { green capsule part $\times$ Y	(1)	0	1	0
purple capsule part $\times$ Y		0	10	0

Asterisk indicates an externally chimeric capsule of which one half was purple and the other half green. Abbreviations Y and M show "Yoshin kanran" and "Murasaki kanran", respectively. Light purple color plant is F_1 type between "Yoshin kanran" and "Murasaki kanran".

Table 2. Plant color in the progenies from geitonogamy of V_1 chimeras and crosses between V_1 chimeras and "Yoshin kanran"

Cross combination	Segregation pattern of plant color					
	G+LP	P+LP	G	P	LP	Total
Gei. (V_1)	6G+16LP (1, 1) 4G+15LP (1, 1)	—	429 (107, 20)	25 (4, 1)	7 (2, 2)	502 (115, 21)
(V_1) $\times$ Y	—	—	366 (71, 13)	—	11 (1, 1)	377 (72, 14)
Y $\times$ (V_1)	—	—	302 (53, 9)	—	162 (7, 1)	464 (60, 10)

Each datum is shown as no. of progeny plants (no. of capsules, no. of plants) G: green, P: purple, LP: light purple (hybrid type), G+LP and P+LP show segregation into green and light purple plants, and into purple and light purple plants, respectively. Gei.: geitonogamy, Y: "Yoshin kanran". 21 V_1 chimera plants used for this cross experiment were vegetatively propagated from 2 V_0 chimera plants.

of graft chimeras as well as their progenies are described.

Materials and Methods

The purple cultivar "Murasaki kanran" whose anthocyanin pigmentation is controlled by single incompletely dominant gene and the recessive non-purple, green one "Yoshin kanran" of *Brassica oleracea* were used for grafting. WINKLER's method (1907) was applied for obtaining graft chimera. Materials in the present study were chimeric plants (V_0) obtained by the methods, as well as vegetatively propagated plants (V_1) from the V_0 plants. Histological observation was done mainly on V_0 chimera plants.

Graft chimera plants, V_0 and V_1 and their progenies by geitonogamy of V_0 and V_1 plants and by back crosses with "Yoshin kanran" and "Murasaki kanran" were genetically studied.

Fig. 1. Grafting method to obtain chimeric plant. A three-month old "Murasaki kanran" plant with ten leaves was cleft-grafted on the same age plant of "Yoshin kanran". The grafted portion was tightly bound with woolen yarn.

Fig. 2. Two weeks after grafting, union was tightly formed and the graft portion was then transversely cut.

Fig. 3. Adventitious shoots regenerated from the cut part.

Fig. 4. Chimeric plant with green and purple stripes or sectors was selected from the many regenerants.

Fig. 5. A typical chimera was selected and used for this experiment.

- Fig. 6. Histological observation of leaves in chimera plants. A: A chimeric leaf whose vein of both sides were purple-striped was served for microscopic observation. In striped tissue, pigmented and non-pigmented cells were more or less mosaic. B: A chimeric leaf whose vein of one side was purple-striped was served for microscopic observation. In this case, the mosaic pattern was also observed in the anthocyanin pigmentation layers.
- Fig. 7. One chimeric capsule of which the half was green, "Yoshin kanran" origin and the other half purple, "Murasaki kanran" origin. However, the progeny from this capsule was all light purple, showing no chimericity of germ cell layer.

Results

Three-month old plants of "Murasaki kanran" which had ten leaves were cleft-grafted onto the same age plants of "Yoshin kanran" (Fig. 1). Two weeks after grafting the union was tightly formed and the grafted portion was transversely cut. Adventitious shoots were regenerated from the cut part (Fig. 2) and chimeric regenerants showing green and purple stripes or sectors were selected from the shoots and grown (Figs. 3 and 4).

Eleven chimeric plants were obtained from 212 grafted plants (5.2%). Three chimeric plants were grown in all (Fig. 5). Two of these (V_0) were histologically observed. Anthocyanin pigmentation patterns of the epidermis and subepidermis of leaves showed mosaicism and the pattern of histological observation was more complicated than that of the external appearance of plants (Figs. 6 and 7).

To clarify the genotypic constitution of the germ cell layer, the plant coloration pattern of the progenies from geitonogamy of V_0 chimeras and from back crosses with "Yoshin kanran" and "Murasaki kanran" was pursued (Table 1). All of the progeny plants from geitonogamy of green parts (V_0) were green, while the those from purple parts (V_0) were all purple. These results show no segregation of plant color and no chimericity in V_0 germ cell layers. The same tendency was found also in the back crosses with "Yoshin kanran". However, two crosses of chimera-green $\times$ "Murasaki", produced light purple and purple plants, showing chimericity of the V_0 germ cell layers (Table 1).

Another interesting phenomenon was the occurrence of one chimeric capsule whose half part was green and the other half purple (Fig. 7). This capsule had been pollinated with "Yoshin kanran" in its flowering stage. The progeny plants were all light purple like the F_1 type between "Yoshin kanran" and "Murasaki kanran" (Table 1) and no segregation.

V_1 chimeras with green and purple stripes on stems and leaves flowered later than green-dominating chimera plants and "Yoshin kanran". Purple-dominating chimeras had also intermediate external characteristics between two cultivars in general.

To further clarify the genotypic constitution of germ cell layers in V_1 chimeras, segregation pattern for anthocyanin coloration was studied in the progenies from each capsule of 21 V_1 plants by geitonogamy and back cross with "Yoshin kanran" (Table 2). Three types of chimeras, sectorial, "Yoshin kanran" and "Murasaki kanran" types, were served for this experiment. When green V_1 plants or green parts of V_1 chimeras were used, almost all the progenies by geitonogamy and test cross were green plants. When purple V_1 plants or purple parts of V_1 chimeras were used, most of the progenies were also purple or light purple. Twenty out of 21 plants showed no segregation in the progenies from each capsule, showing no chimericity of their germ cell layers. However, segregation was observed in the progenies from two capsules (2 of 247 capsules) by geitonogamy of one V_1 plant (Table 2).

Discussion

Histological observation of graft chimera plants showed that anthocyanin pigmented and non-pigmented cells were mixed in chimeric tissues. Externally purple-dominating chimeras in V_1 plants had intermediate characteristics such as intermediate flowering date and flow-

er size between "Yoshin kanran" and "Murasaki kanran". Such phenomena and physiological interactions between chimeric tissues are already known in other plants (KUMAGAI and USAMI, 1964; MASUBUCHI, 1961, 1977). The chimeric or mosaic nature of the inner tissues was more complicated than that of observation from the external appearance.

Almost no progenies from geitonogamy of V_0 and V_1 chimera plants and back cross showed segregation of anthocyanin pigmentation pattern. However, the progenies from two capsules of one V_0 chimera showed segregations (Table 1), suggesting that the germ cell layers of the externally chimera-green parts had dominant and recessive genes in egg cells. In the case of the two capsules in one V_1 plant, it is also suggested that male or female germ cells had both dominant and recessive genes (Table 2). Therefore, in most flowers the genotype of germ cells in a single flower was genetically uniform. In four capsules of the V_0 and V_1 chimeras, however, there existed two germ cell genotypes in a single flower ($4/288=1.4\%$). This shows the same case of chimericity of germ cell layer in a single flower.

Thus, the chimera plants in this experiment were principally the periclinal-sectorial type and two genetically different tissues were only connected independently with each other in a single plant, although the pigmented and non-pigmented cells were mixed in their border tissues. Hence, it can be concluded that the general uniformity of genotypic constitution exists in the germ cell layer of each flower of chimera plant. Chimericity of germ cells within one flower is considered as an exceptional case.

There might be three possible explanations for segregation phenomena of progeny plants from one capsule. The first possibility is the *in situ* occurrence of two types of germ cells in a single flower, the second one is gene transfer between two different tissues or cells of a chimera plant, and the third one is mutation. To elucidate the first possibility eggs (embryosacs) or pollen grains (anthers) from a chimera plant flower must be cultured and their genotypic constitution should be directly observed. The second possibility could be clarified by examining the transfer of DNA between two different tissues or cells using a gene such as GUS gene (JEFFERSON *et al.* 1987). The third possibility cannot be excluded completely, but it is difficult to think because gene mutation from recessiveness to dominance must be supposed.

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キャベツにおける品種間接木キメラ形成

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接木キメラ組織間の物質的相互作用を明らかにするとともに, その育種的利用の可能性を追究する目的で, 緑色の「葉深カンラン」と紫色の「紫カンラン」のキャベツ二品種を用いて作出した接木キメラに関して組織学的, 遺伝学的解析を行った。

外部形態的に紫色の卓越するキメラ植物は, 緑色卓越型キメラや「葉深カンラン」に比べて開花期が遅くて「紫カンラン」の開花期に近く, 花の形も, 両品種の中間的性質を持っていた。また, キメラ植物組織では, アントシアニン色素を発現している細胞と発現していない細胞が複雑なモザイク状態を呈していた。

これと平行して, キメラ植物の生殖細胞層の遺伝子型の存在状態を調べるために, キメラ植物の諸部位に着く花の柱頭に, アントシアニンを発現しない劣性ホモ型の「葉深カンラン」および優性親の「紫カンラン」とを交配して, 各莢から得られた F_1 植物のアントシアニン色素の発現状態より, キメラ植物における生殖細胞層の遺伝子型を推定した。その結果, ほとんどの場合に生殖細胞は, 一つの花の中では, 「葉深カンラン」型もしくは「紫カンラン」型のみで安定しており, 生殖細胞層のキメラ性は認められなかった。しかし, キメラ当代植物 V_0 の緑色部位に「紫カンラン」を交配した場合に, その同一莢に由来する種子世代 B_1 で, 薄紫色と紫色植物への分離が2例認められた。また, V_1 植物の隣花受粉の場合にも2莢に由来する種子世代において薄紫色と緑色植物への分離が認められた。これらのことは, きわめて低い頻度 ($1.4\% = 4/288$) ではあるが, 一つの花の中の生殖細胞層の遺伝子型が, キメラ性を持つことを示している。

この原因には, キメラ植物の生殖細胞層そのものが形成過程でキメラとなっているか, キメラ組織間の遺伝子の移動による形質転換による可能性が考えられる。