Inheritance of Carotenoid Pigmentation in Flower Color of Chrysanthemum

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Experiments were carried out to analyse the inheritance of the flower color, especially carotenoid pigmentation, in chrysanthemum. Such as determination can be performed easily by measuring the amount of light transmitted in fresh, intact petals, using spectrophotometry regardless of the presence of carotenoid pigments in the petals. Based on the results of the genetic background analysis of the inheritance of carotenoid pigmentation, chrysanthemum cultivars used in this experiment were classified into three groups. In the first group a heterozygous dominant inhibitor genes for carotenoid pigment biosynthesis was identified while in the second group no such dominant inhibitor gene was present and in the third group the gene constitution was complex and characterized by the presence of a heterozygous dominant inhibitor gene and heterozygous dominant gene for carotenoid pigmentation. The wild species, *Chrysanthemum japonense*, was different from the chrysanthemum cultivars used in this experiment in that species harboured a homozygous dominant inhibitor gene at least a single locus. Therefore, in all the F₁ progenies derived from the crossing of this species with several varieties no plants with yellow flowers were observed. Moreover, it is suggested that a chimerical structure should be considered in analyses of the flower color inheritance.

KEY WORDS: *Chrysanthemum morifolium*, carotenoid pigmentation, spectrophotometry, periclinal chimera, flower color, inheritance.

Introduction

Cultivated chrysanthemum, *Chrysanthemum morifolium* Ramat., belong to the family *Compositae* and have a $2n=6x=54$ chromosome number (DOWRICK, 1953). They display a genetically controlled sporophytic self-incompatibility like many other plants of the family *Compositae* (DRELOW *et al.*, 1973, ZAGORSKI *et al.*, 1983, STEPHENS *et al.*, 1984). Moreover, though it appears easy to obtain seeds through selfing or crossing in the first generation, it is difficult to obtain seeds from the selfed S₁ or F₁ progenies (KAWASE and TSUKAMOTO, 1977). STEWARD and DERMEN (1970) reported that the 'Indianapolis' family of chrysanthemum sports displayed periclinal chimeras associated with the three layers in the apical meristem. LANGTON (1980) who reported that in floriests' chrysanthemums, the segregation of carotenoid pigmentation depended on the action of a single dominant gene I (inhibitor) suggested that a chimerical structure should be considered in the analysis of flower color inheritance data. Therefore, little progress has been made in the genetical studies on chrysanthemum. WATANABE (1977) observed that in the polyploid genus *Chrysanthemum*, bivalent formation was normal and multivalents seldom appeared at the meiosis, especially in the plants with even ploidy. He therefore concluded that these multivalents may be autopolyploids and that a genic mechanism for the stabilization of pairing was involved because they behave like diploids at the meiosis and it appeared that genetic segregation was not complex.

In chrysanthemum, the flower color mainly depends on the presence of anthocyanin pig-

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ments in the vacuoles of the epidermis of ray florets and carotenoid pigments in the chromoplastid of whole ray floret tissues. The flower color is controlled by the equilibrium in the amount of these two kinds of pigments (KAWASE and TSUKAMOTO, 1974 and 1976). Thus, visual evaluation of the flower color based on these two pigments is very difficult. SHIBATA (1985) developed that opal glass transmission method for spectrophotometric studies of fresh petals. This method is very convenient since the main pigments related to flower color can be identified.

In this paper, the author describes the experiments carried out to analyse the flower color inheritance, especially in the case of the carotenoid pigment of hexaploid chrysanthemums by spectrophotometric analysis of fresh and intact petals. The presence of chimerical structures in chrysanthemum is also discussed.

Materials and Methods

Plant materials used in this experiment are shown in Table 1. Bonnie Jean (BJ) and Yellow Bonnie Jean (YBJ) belong to the same sport family. Also, Pink Marble (PM), White Marble (WM) and Blue Marble (BM) belong to another identical sport family. Yellow Delaware (YD) has been considered to be a spontaneous mutant of Delaware (D). *Chrysanthemum japonense* (Ch. j.) is one of the wild chrysanthemum occurring in Japan with white flowers and a chromosome number of $2n=6x=54$.

The S_1 and F_1 seeds were obtained by selfing and crossing of non-emasculated or emasculated disc florets, but not of ray florets of the flower head of chrysanthemums grown in greenhouses. These seeds were sown and cultivated in pots. At flowering time, all the ray florets were removed from the inflorescence of each plant.

To determine whether these fresh petals contained carotenoid pigments or not, absorption curves of the petals were analysed with a Hitach two wavelength double beam spectrophotometer (model:356) or UV-visible recording spectrophotometer (Shimadzu:UV-160) using the opal glass transmission method (SHIBATA, 1958). According to KAWASE and TSUKAMOTO (1974), these absorption curves showed *in vivo* maxima of anthocyanin absorption in the range of 530~560nm, carotenoid pigments in the range of 420~490nm and flavonol in the range of 350~370nm. Therefore, in this paper, the amount of carotenoid pigments

in each petal was determined by measuring the absorption peak in the range of 420~490nm.

Table 1. Materials used in this experiment

Strain	Abbreviation
Pink Daisy	PD
Bonnie Jean	BJ
Yellow Bonnie Jean	YBJ
Pink Marble	PM
White Marble	WM
Blue Marble	BM
Delaware	D
Yellow Delaware	YD
<i>Chrysanthemum japonense</i>	Ch.j.

Results

For the analysis of the flower color, examples of absorption curves are shown in Fig. 1 (a-d). The white flower that contains neither carotenoid nor anthocyanin pigments is shown in Fig. 1 (a). Fig. 1 (b), Fig. 1 (c), and Fig. 1 (d) show the petals that contained anthocyanin pigments, carote-

noid, and both anthocyanin and carotenoid pigments, respectively. These results clearly indicate that it is easy to determine whether the petals contained carotenoid and/or, anthocyanin pigments. Thus, the determination of the presence of carotenoid pigments was carried by measuring the absorption peak at about 450nm.

The results obtained in the S_1 progenies are showing Table 2. PD S_1 and BJ S_1 showed a ratio of Carotenoid +(existence): -(none) = 1:3 with a high probability in the chi-square

Fig. 1. Absorption spectra of intact petals of flowers with various colors in chrysanthemum
 (a): White flower lacking both anthocyanin and carotenoid pigments
 (b): Pink flower with only anthocyanin pigments
 (c): Yellow flower with only carotenoid pigments
 (d): Red flower with both anthocyanin and carotenoid pigments

Table 2. Carotenoid segregation in S_1 progenies derived from selfing of various varieties

Line	Progenies Carotenoid		Tested ratio	χ^2 -value	Probability
	+ ¹⁾	-			
PD S_1	22	60	1: 3	0.1463	0.7021
BJ S_1	57	185	1: 3	0.2700	0.6034
YBJ S_1	61	260	1: 3	6.1568	0.0131
			3: 13	0.0135	0.9075
PM S_1	14	58	1: 3	1.1852	0.2763
			3: 13	0.0228	0.8800
Ch.j. S_1	0	230			

¹⁾ + : contained carotenoid pigments

- : no

carotenoid

pigments

test. These findings suggested that PD and BJ harboured a heterozygous dominant inhibitor gene with one allele for carotenoid biosynthesis because in the progenies carotenoid pigments were detected only in the recessive homozygote. YBJ S_1 showed a $+: - = 3:13$ ratio but not $1:3$. PM S_1 showed a higher probability of a $3:13$ ratio than $1:3$. It was considered that in both varieties carotenoid pigmentation is controlled by two independent alleles, namely, YBJ and PM harboured a heterozygous dominant inhibitor gene with a single allele, and also heterozygous dominant gene for carotenoid pigmentation with another allele. Flowers in the S_1 progenies of Ch. j. were all white. It was demonstrated that Ch. j. carried at least one pair of carotenoid inhibitor genes with a homozygous dominant gene, or did not have genes related to carotenoid biosynthesis.

Table 3 shows the results obtained after the crossing of F_1 progenies. Crossing of all the F_1 progenies except for the D X BJ F_1 and YD X BJ F_1 progenies showed that carotenoid segregation agreed with the $+: - = 1:3$ ratio with a high probability in the chi-square test. For the YBJ X BJ F_1 and BJ X YBJ F_1 progenies, there was no difference in the carotenoid pigmentation segregation between reciprocal crosses. Based on the ratio of the carotenoid segregation from the S_1 progenies, it was suggested that BJ and YBJ harboured a heterozygous dominant inhibitor gene of the same locus. Furthermore, it is considered that BJ harboured a homozygous dominant gene for carotenoid biosynthesis, because YBJ harboured the heterozygous dominant gene for carotenoid pigmentation.

Similarly, based on the segregation ratio obtained after the crossing of WM and BM with BJ or YBJ, it was found that WM and BM harboured the heterozygous dominant inhibitor gene.

Based on the segregation ratio of the D X BJ F_1 and YD X BJ F_1 progenies, it is considered that D and YD did not harbour the dominant inhibitory gene for carotenoid pigmentation. However these varieties may harbour the gene for carotenoid biosynthesis because

Table 3. Carotenoid segregation in F_1 progenies derived from crossing of various varieties

Line	Progenies Carotenoid		Tested ratio	χ^2 -value	Probability
	¹⁾ +	-			
YBJ X BJ F_1	9	24	1:3	0.0909	0.7630
BJ X YBJ F_1	9	18	1:3	1.000	0.3173
PD X BJ F_1	47	127	1:3	0.3755	0.5400
PM X BJ F_1	30	103	1:3	0.4236	0.5152
WM X BJ F_1	71	204	1:3	0.0982	0.7540
D X BJ F_1	17	19	1:1	0.1111	0.7389
YD X BJ F_1	54	44	1:1	1.0204	0.3124
BM X BJ F_1	28	80	1:3	0.0494	0.8241
WM X YBJ F_1	75	247	1:3	0.5010	0.4790
BM X YBJ F_1	57	157	1:3	0.3053	0.5806
PM X PD F_1	9	27	1:3	0.000	1.0000

¹⁾ + : contained carotenoid pigments

- : no carotenoid pigments

carotenoid pigment(s) were detected in the ray floret. Seed could not be obtained through selfing in these varieties due to the high self-incompatibility and therefore the exact genetical background could not be clarified.

Data in the F_1 progenies derived from the crossing of Ch. j. with several varieties are shown in Table 4. None of the F_1 progenies, showed yellow flowers, suggesting that Ch. J. harboured the homozygous dominant inhibitor gene at least at a single locus.

Results of the genetic background analysis on the inheritance of carotenoid pigmentation are summarized in Table 5. Based on this table, the chrysanthemum cultivars used in this experiment, except WM and BM, were classified into three groups. PD and BJ which belonged to the first group harboured the heterozygous dominant inhibitor gene for carotenoid pigment biosynthesis. The second group which included D and YD, had no dominant inhibitor. YBJ and PM which belonged to the third group harboured the heterozygous dominant inhibitor gene and the heterozygous dominant gene for carotenoid pigmentation. This group was characterized by a complex gene constitution. Moreover, it remained to be determined

Table 4. Carotenoid segregation in progenies derived from crossing of Ch.j. and several varieties

Line	Progenies Carotenoid	
	+ ¹⁾	—
Ch.j. X YD F_1	0	48
YD X Ch.j. F_1	0	91
Ch.j. X D F_1	0	68
D X Ch.j. F_1	0	52
WM X Ch.j. F_1	0	120
PM X Ch.j. F_1	0	223
BM X Ch.j. F_1	0	149
BJ X Ch.j. F_1	0	249
YBJ X Ch.j. F_1	0	153

¹⁾ + : contained carotenoid pigments

— : no carotenoid pigments

Table 5. Genetic background of the inheritance of carotenoid pigmentation in chrysanthemums

Variety	State of dominant inhibitor for carotenoid biosynthesis	State of dominant gene for carotenoid pigmentation
Group I		
PD, BJ,	Heterozygous	Homozygous for dominant gene
Group II		
D, YD	Homozygous for recessive gene	—
Group III		
YBJ, PM	Heterozygous	Heterozygous

whether the dominant gene for carotenoid pigmentation harboured by each variety was located at the same locus.

The genetic background of the inheritance of carotenoid pigmentation of WM and BM is not clear because these two varieties were obtained by sport mutation from PM which had two different alleles for carotenoid pigmentation. WM and BM may carry the heterozygous dominant inhibitor gene for carotenoid pigmentation as well as the heterozygous dominant gene for carotenoid pigmentation at a different locus from that of YBJ, since the F_1 progenies derived from the crossing of these two varieties and YBJ showed a $+$: $-$ = 1 : 3 ratio for carotenoid segregation. When WM and BM harboured the homozygous dominant gene for carotenoid biosynthesis, in the variety PM, the mutation from homozygous to heterozygous dominant genes for carotenoid biosynthesis was induced after sport mutation occurred. Since the sport family, to which these three varieties belong usually exhibited male sterility, further genetic analysis could not be pursued.

Discussion

To identify the pigment related to flower color in chrysanthemum the opal glass transmission method developed by SHIBATA (1958) was used in the experiments. This method is very simple and it took only a short time to analyse each sample. Moreover, this method is very convenient for the analysis of the flower color even for a large number of samples. Using this method, SAITO (1967) reported that the absorption spectra of fresh flowers of many species of chrysanthemum could be classified into four groups according to the number and position of maxima in the visible region. Such an analysis may provide information on the chemical basis of color variation in flowers. KAWASE and TSUKAMOTO (1974) analysed the absorption curves of fresh and intact petals of *Chrysanthemum morifolium* (68 cultivars) using this method. Based on their results, four patterns of absorption spectra in the visible range were observed. These patterns were classified according to the presence or absence of flavonols, anthocyanin pigments and carotenoid pigments. Group (1) contained only flavonols and the flower color of this group was white. Group (2) contained flavonols, anthocyanin pigments and the color of the flowers was pink or red-purple. Group (3) showed the three characteristic absorption peaks of carotenoid pigments of yellow flowers. Group (4) displayed combined absorption of flavonols, anthocyanin and carotenoid pigments, and the flower color varied from orange to deep red.

The absorption curves of ray florets of many progenies derived from the selfing and crossing of chrysanthemum cultivars and one wild species were analysed. The flower color of the progenies derived from selfing and crossing varied widely and based on the absorption curves, the presence of carotenoid pigments was determined. Further studies are needed to identify the gene controlling anthocyanin pigmentation.

It was demonstrated that the segregation for carotenoid pigmentation in the progeny derived from the selfing and crossing of several chrysanthemum cultivars and one wild chrysanthemum species (*Chrysanthemum japonense*) fitted to a simple ratio. The cultivated chrysanthemum is usually considered to be a hexaploid, $2n=6x=54$ (DOWRICK, 1953). WATANABE (1977) reported that in all the polyploid genera of *Chrysanthemum* bivalent forma-

tion was usual, and multivalents were rare. It was considered that a simple segregation ratio was correct although the cultivated chrysanthemum was a hexaploid.

Except for YBJ and PM, it was also confirmed that a single dominant inhibitor gene mainly controlled the carotenoid pigmentation of ray florets as reported by LANGTON (1980). However an other single dominant gene was present in YBJ and PM. It is considered that the dominant inhibitor gene blocked the carotenoid biosynthesis while the dominant gene in YBJ and PM may act directly on carotenoid biosynthesis since carotenoid pigmentation was observed in ray florets in the absence of the dominant inhibitor gene but in the presence of the dominant gene for carotenoid biosynthesis.

It is considered that this dominant gene for carotenoid biosynthesis is very important for chrysanthemum crossing breeding programs. Many studies have been carried out on the gene controlling carotenoid biosynthesis. For example, BUISHAND and GABELMAN (1979 and 1980) reported that the inheritance of the carrot root color was controlled by three dominant inhibitor genes (Y , Y_1 , Y_2) and that these genes existed at independent loci. Furthermore, these dominant inhibitor genes did not completely inhibit carotenoid biosynthesis. In this paper, we could not determine whether the dominant inhibitor gene (I) inhibited any steps of biosynthesis, that is, any kind of carotenoid pigments synthesized so that exact pigment analysis could not be performed. Indeed it is very difficult to analyze many samples within a short period of time to determine the segregation ratio.

DREWLOW *et al.* (1973), KAWASE and TSUKAMOTO (1977), ZAGORSKI *et al.* (1983) and STEPHENS *et al.* (1984) reported that cultivated chrysanthemums showed a sporophytic self-incompatibility controlled by S genes at more than one locus.

In this experiment, the S_1 and F_1 progenies were used to observe the flower color segregation. Progenies could not be derived from S_1 and F_1 plants. Although the S_1 progenies derived from YBJ indicated that YBJ harboured two independent genes for carotenoid pigmentation, since it was very difficult to obtain the S_2 progenies from this varieties, further progeny tests were not carried out. From preliminary observations only a small amount of seeds derived from yellow flowered S_1 plants harboured the dominant gene for carotenoid pigmentation but no dominant inhibitor and a few white-flowered plants were obtained although the segregation ratio could no be determined. It is considered that YBJ harboured the heterozygous dominant gene for carotenoid biosynthesis. Further studies should be carried out identify the gene controlling carotenoid pigmentation (biosynthesis).

LANGTON (1980) also reported that yellow-flowered chrysanthemum sports with carotenoid pigment only in L1 are indistinguishable from their white progenitors in breeding behavior, which may account for the periclinal chimera structure of the corolla. Chimerical structure has, therefore, to be considered in the analysis of flower color inheritance data. Since L1 forms the epidermis and reproductive cells originate from inner cells, the breeding behavior deviates from the phenotypic expectation. Yellow-flowered YBJ carotenoid pigment only in the epidermis of ray florets segregates white- and yellow-flowered progenies derived from selfing. YBJ is a sport mutant derived from white-flowered BJ which harboured the heterozygous dominant inhibitory gene for carotenoid biosynthesis. In the case of YBJ, it is considered that sport mutation and gene mutation were induced in BJ.

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キクのカロチノイドによる花色の遺伝

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栽培ギク (*Chrysanthemum morifolium*) は $2n=6\times=54$ の染色体数を示す高次倍数体であり, 自家不和合性や雄性不稔性の発現などにより明確な遺伝子分析は困難であるとされてきた。また, キクの花色の発現に関与する主な色素は花卉の構成細胞中の色素体に存在するカロチノイド色素および向軸・背軸側の表皮細胞のみの液胞中に存在するアントシアニン色素の2種類であり, これらの色素の存否および量的な組合せにより様々な花色を発現しているものと考えられる。

本研究ではキクの花色に関する遺伝的な背景を明らかにするために自殖および交雑後代の色素, 特にカロチノイド類色素の存否について分離比を調査した。調査にあたっては, 多数の個体を扱う必要があるために SHIBATA (1958) により開発された Opal glass transmission method により生花卉の吸収スペクトルを計測し, そのパターンによりカロチノイドの存否を検定した。供試した材料は名古屋大学農学部で保存中の, 自殖や交雑により比較的種子の得やすい, 野生のノジギク (*Chrysanthemum japonense*) を含む8品種・系統である。これらの品種・系統の開花時に, 頭状花序から舌状花を切除し, 交雑を行う場合には, 自家不和合性や雄性不稔性を示す品種においても除雄を行い交雑種子のみを得るために万全を期し, 自殖の場合には除雄することなく同一花序内で人工受粉を行った。このようにして得られた種子を常法にしたがって播種し, 植木鉢に移植し栽培した。これらの各個体の開花時に1個体当たり数枚の花弁(舌状花)を採取し分光光度計により吸収スペクトルを測定した。吸収スペクトルのうち450 nm 付近の特徴的な3つのピークの有無を調査することによりカロチノイドの存否を判定した。

その結果, キクにはカロチノイド生合成に関与する2つの遺伝子, すなわち, 生合成を抑制する優性の抑制遺伝子と生合成そのものに関与する優性で正常に生合成を進行させる遺伝子の存在することが確認された。これらの遺伝子の存在状態により, 本研究に用いた栽培ギクのうち WM と BM を除く6品種は3つのグループに類別された。第1グループには PD・BJ の2品種が含まれ, これらの品種には優性の抑制遺伝子はヘテロで, 生合成そのものに関与する遺伝子については優性ホモで存在していた。D・YD が第2グループを形成し, これらの品種には優性抑制遺伝子については劣性ホモであることが確認されたが, 生合成に関与する遺伝子については明らかにできなかった。最後のグループには YBJ と PM が含まれ, このグループは抑制遺伝子・生合成に関与する遺伝子ともにヘテロで持ち, そのため自殖後代での分離比が他の品種に比べいくらか複雑であった。またこのような分離比の確認のためには自殖種子を得ることが必要となり, 自家不和合性を有する品種での確認はできなかった。さらに, 野生のノジギクは少なくとも1遺伝子座の優性抑制遺伝子をホモで持ち, 他の品種とは異なっていた。そのためにこの種との交雑後代にはカロチノイドによる着色は全く認められなかった。

以上のようなカロチノイドの発現に関する遺伝的な背景についての結果と親として用いた品種の表現型との間に矛盾するような場合が観察された。すなわち, YBJ は遺伝的には優性抑制遺伝子をヘテロで持つために白花色を発現するはずであるが, 実際には黄花色を示し矛盾するように思われる。しかしながら, この品種では花卉の表皮細胞のみにカロチノイドを含み内側の柔細胞にはカロチノイドを持っていない周縁キメラ性を示していた。このようなことから, キクの花色を考察する場合には周縁キメラ性について十分考慮する必要があることが示唆された。