A System for Visually Classifying Alfalfa Flower Color

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A System for Visually Classifying Alfalfa Flower Color

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INTRODUCTION

Flower color inheritance in alfalfa has been studied more extensively than any of its other qualitative characters. Alfalfa flower color, because of its extensive variation, has been used to identify variety, plant breeding and genetic materials, and to distinguish between self-pollinated plants and those resulting from cross-pollination (*6*, *8*).[1] According to Clement (*3*) and Pedersen (*11*), flower color is often an important factor in the attractiveness of alfalfa clones to insect pollinators. The usefulness and importance of alfalfa flower color has been established; but the many hues, patterns, and intensities of purple, yellow, and combinations of purple and yellow pigments have caused difficulty in phenotypic classification.

This handbook briefly describes the genetics of alflafa flower color, discusses factors that influence alfalfa flower color classification, presents a system for visually classifying flower color, and presents color prints to illustrate the various flower color classes.

FLOWER COLOR INHERITANCE

Cultivated alfalfa is an autotetraploid. Difficulties in working with tetraploid genetics have limited the number of inheritance studies in alfalfa, especially on traits controlled by two or more genes, such as flower color. Results from genetic studies have demonstrated a close relationship between the inheritance patterns for purple and yellow flower color in diploid and tetraploid alfalfa. Therefore, in many instances, diploid species of *Medicago falcata* L. and *M. sativa* L. have been utilized to help interpret the more complicated inheritance patterns. All available information on the inheritance of alfalfa flower color was summarized and reevaluated by Barnes (*1*). The data indicated that flower color in alfalfa was conditioned by three types of major gene effects and several types of modifying genes.

The major genes include one recessive gene (*c*) with tetrasomic inheritance. The *c* gene is a basic color factor that, in the homozygous recessive condition, produces white-flowered plants that

[1] Italic numbers in parentheses refer to Literature Cited, p. 11.

are devoid of all anthocyanin pigmentation in flowers, seeds, stems, leaves, and roots. Purple flower color pigments are expressed in the presence of one or more dominant *C* alleles. Chemical analyses of diploid alfalfa indicated that purple flower color is due to a group of three anthocyanin pigments identified as malvidin, petunidin, and delphinidin diglucosides. These three pigments are inherited as a unit and are expressed when one or more dominant alleles of *P* gene are present. The tetrasomically inherited *P* gene is present in purple-flowered *M. sativa* plants. The homozygous recessive *p* genotype in the presence of dominant *C* alleles produces a cream flower color that does not interfere with anthocyanin pigmentation in floral veins, seeds, leaves, stems, and roots.

Genes responsible for producing yellow pigments can be classified as the third major type of alfalfa flower color pigmentation. The origin of yellow flower color in alfalfa can be traced to *M. falcata*. In diploid alfalfa the yellow pigments have been identified as being primarily xanthophyll with a small amount of *B* carotene present. Diploid genetic data suggest that yellow flower color is controlled by at least three and probably four genes with accumulative effects. Only limited data are available concerning the inheritance of yellow flower color pigments in tetraploid alfalfa. However, a pattern of tetrasomic inheritance for at least two genes (Y_1 and Y_2) with accumulative effects is hypothesized.[2] The homozygous recessive condition of both the *P* and *Y* genes produces a cream-flowered plant. Joint pigmentation of the *P* and *Y* genes produces phenotypes that have been commonly referred to as variegated flowers. Variegated flower colors may range from a very dark blue color to a green or yellow green.

Essentially no information is available regarding the inheritance of color intensity. Data presented by Soudah[3] suggested that the effects of the *P* gene were accumulative and that the various intensities of purple could be due to specific genotypes; that is, *pppp* = cream, *Pppp* = light purple, *PPpp* = purple, *PPPp* = deep purple, and *PPPP* = very deep purple. However, progeny tests needed to verify or disprove the theory have not been conducted. Other evidence is available that suggests accumu-

[2] Several loci are responsible for the expression of yellow flower color pigments, but for brevity in the following text, only one representative gene will be used.

[3] Soudah, R. E. TETRASOMIC INHERITANCE OF FLOWER COLOR IN ALFALFA, *Medicago sativa* L. (Unpublished Ph.D. thesis. Copy on file Dept. of Soils and Crops, Rutgers Univ., New Brunswick, N. J.). 1962.

lative effects of dominant alleles do exist in alfalfa flower color; that is, crosses between cream-flowered plants and dark-orange-yellow plants produce progenies with intermediate yellow flower color, and subsequent F_2 segregations produce various intensities of yellow flower color that can best be explained by assuming accumulative effects of the *Y* genes. Modifying genes must be considered as another source of factors that can affect flower color intensity.

Several types of modifying factors are known to exist in alfalfa. Nine anthoxanthin pigments have been chemically identified as flower color modifying pigments in diploid alfalfa (*4*). Six of the anthoxanthins were quercetin glycosides, and three were kaempferol glycosides. None of these pigments appeared to impart a phenotypically significant color of its own, but they tended to act as modifying genes when copigmented with the *P* or *Y* genes. No association or linkage has been shown between any of the modifying genes (*5*).

Two additional types of modifying flower color characteristics are purple bud color and floral vein color. Barnes and Cleveland (*2*) reported that in diploids they had not observed the purple bud color trait in unadulterated *M. falcata*, but it was often observed in yellow- and cream-flowered progeny from advanced generations of hybrids between *M. falcata* and *M. sativa*. The trait was shown to be conditioned by two complementary genes. The assumed genotype of pure *M. falcata* was *BfBf bsbs* and the assumed genotype of *M. sativa* was *bfbf BsBs*. Purple bud color is produced by *Bf*__*Bs*__genotypes. Barnes and Cleveland also established that the presence of pigmented veins in the standard petals of yellow-flowered diploid alfalfa was controlled by the presence of one or more dominant alleles of two duplicate genes (Vs_1 and Vs_2). When both genes were homozygous recessive, no floral vein pigmentation was observed. Barnes and Cleveland suggested that vein color in the wing petals was conditioned by a separate gene.

FACTORS INFLUENCING FLOWER COLOR CLASSIFICATION

Joint Pigmentation

Except for the pure purple, yellow, cream and white flower colors, most of the flower color phenotypes are caused by varying concentrations of two or more pigments being present in the same flower. The most distinctive differences in alfalfa are due to the joint pigmentation of purple and yellow pigments. Lesins (*9*) suggested that in copigmentation the purple pigments were in the epidermal layer of the flower, which in turn was over a background of yellow. However, Buker[4] suggested that the yellow and purple pigments were both present in the same cells and that the mosaic effect observed by Lesins was caused by cell injury that occurred when the epidermal cells were removed.

Extremely large numbers of copigmented genotypes are possible when it is considered that as many as five genes with major effects and numerous genes with modifying effects, all with tetrasomic inheritance, exist. However, many of the differences between phenotypes are very subtle and not obvious to the naked eye. Therefore, when differences in joint pigmentation are considered without the benefit of chemical analyses, similar phenotypes can be placed into several relatively distinct groups. These groups, generally, can be described as blue, blue green, green, and yellow green.

Color Intensity

Intensity of flower color varies considerably, regardless of the type of pigments present in the flower. Purple pigmentation varies among plants—from a very deep purple to a very light purple. Similarly, yellow pigmentation can vary from a dark orange yellow (typical of *M. falcata)* to a very light yellow. The inheritance of flower color intensity is not fully understood, but it has been suggested that flower color intensity is influenced by the accumulative effects of the dominant alleles of the *P* and *Y* genes. Sheridan and McKee (*13*) used colorimetric measurements to determine the effect of soil fertility, soil pH, and light on the intensity of purple flower color. Only light appeared to be a significant factor. Purple flowers from field-grown plants were generally lighter than flowers grown in the greenhouse. Even though environment can influence them, flower color intensities are stable enough to be used in establishing subclasses within the primary classes of purple, yellow, and variegated. However, color intensity could be responsible for misclassification of flower color if a plant has a very intense purple color that masks a very low level of yellow pigmentation. Such a plant would be classified as purple instead of variegated.

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[4] Buker, R. J. FLOWER COLOR INHERITANCE IN DIPLOID ALFALFA. (Unpublished M.S. thesis. Copy on file Dept. of Agronomy, Purdue Univ., Lafayette, Ind.) 1960.

Bud Color

Purple bud color is not conditioned by the major purple flower color gene (*P*); but to avoid misclassification, bud color should be considered when flower colors are observed. Purple bud color is characterized as having visible anthocyanin pigments at the tip of the flower bud (pl. 1, *A*). This pigmentation usually disappears as the flower opens. The purple bud trait is masked in purple-flowered plants but is very obvious on plants that have yellow or cream flower colors. Because the pigments are faint and disappear rapidly, they do not usually interfere with flower color classification. However, cream- and yellow-flowered plants have been observed in which the purple bud color is very intense and traces of purple pigments are visible several days after the flowers have opened. If this occurs, a decision must be made to determine whether the purple pigment is caused by the bud color or by the basic flower color gene. The best method for determining the cause is to observe the distribution of the pigment in the flower. If the purple pigment is uniformly distributed throughout the opened flower, it can be assumed that the color is attributed to the *P* gene. However, if the color is concentrated only at the tip and edges of the standard petal and wings, the color can be attributed to the bud color genes.

Aging

The life of an untripped alfalfa flower ranges from 8 to 15 days, according to Hanson (*7*). During this period, the flower color of each floret is continually changing. Many, if not all, of the purple pigments are very sensitive to sunlight and will begin to fade soon after the flower opens. The yellow pigments appear to be more stable than the purple pigments. Because of the aging process in alfalfa flowers, the same age flowers must be used for all flower color classification. Flower age can be readily established by the position of a floret on a raceme (pl. 1, C). One-day-old flowers are the best age for flower color classification in the field. In the greenhouse, flower colors may remain stable for 2 or 3 days, depending on the conditions. Even though 1- or 2-day-old flowers are used for classification, it is a good practice to observe several faded flowers on the same plant for traces of yellow pigments. This essentially eliminates the misclassification of plants that may appear to be purple at first glance, but are actually variegated (joint pigmentation of purple and yellow pigments).

Irregular Petal Color Patterns

Alfalfa flowers are formed by five petals: two wing petals, two keel petals, and a standard petal. All of the petals are uniformly colored in most alfalfa flowers. However, irregular color patterns can be observed in some purple pigmented flowers. The most frequently observed irregularity is in keel petals whose tops are more or less intensely pigmented than the rest of the flower (pl. 1, B). An unusual mutant, characterized by darkly pigmented wing petals, cream-colored keel, and standard petals, is shown in plate 2, A. Partial pigmentation of the standard petal (pl. 2, B) is another unusual color pattern. None of these traits can be related to known flower color patterns, because their inheritance has not been studied. Practical judgment must be used when classifying unusual flower colors. The flowers in plates 2, A and B were classified as having purple flower color, because they lacked all traces of yellow pigments and could not be classified as either cream or white.

Vein Color

Most alfalfa flowers have pigmented veins in the standard petals, and a few plants have pigmented veins in the wing petals. In yellow and cream-colored flowers the veins are usually brown (pl. 2, C), while in purple pigmented flowers the veins are purple (pl. 2, D). Vein color usually does not contribute a great deal to the total flower color, but the presence or absence of pigmented veins or degree of vein pigmentation can be very useful identification characteristics. The presence of brown pigmented floral veins in an otherwise 'white' flower denotes cream flower color. The presence of purple pigmented floral veins in an otherwise 'white' or cream flower generally denotes that the flower color should be classified as a very light purple (pl. 2, D). However, it should be pointed out that even though purple vein color and purple flower color are closely associated, no studies have yet been conducted to demonstrate pleiotropy.

SYSTEMS FOR FLOWER COLOR CLASSIFICATION

Previous Classification Systems

A number of methods have been used for scoring alfalfa flower color, such as the British Colour Council Horticultural Colour Chart (*5*), Nickerson Color Fan (*10*), and the Munsel Color Key

(*3*). However, such systems based on solid colors have usually proved difficult to use, because age of flowers, variable color patterns, and vein pigmentation tend to influence the results and make classification very confusing.

Arbitrary systems of visual classification have often been originated by researchers for special studies. Three of these systems are presented in table 1. Several additional schemes no doubt can be found in the literature. In all of the classification systems described, flower colors have been arranged according to shade and intensity of purple, yellow, and variegation (joint pigmentation of yellow and purple pigments). Each scheme phenotypically classified plants, but the numerical scores usually did not reflect any genotypic relationship among the various colors. Another difficulty with the arbitrary classification systems is the discrepancy in descriptions and interpretations of colors among workers. What is called blue by one worker may be considered as purple by another.

New Classification System

Before attempting to design a new system for classifying alfalfa flower color, I asked alfalfa scientists what type systems would be most useful to them. The consensus of opinion was that we needed a system of only a few phenotypic classes, each represent-

TABLE 1.—*Three previously proposed systems of flower color rating for visually classifying alfalfa flower color*[1]

Numerical rating	System 1	System 2	System 3
1	Deep reddish purple	Purple	—
2	Deep purple	Lilac	Yellow
3	Strong purple	Cream	—
4	Moderate purple	Light variegated	Green-blue green
5	Light purple	—	—
6	Light violet	Much variegated	White or cream
7	Strong yellow green	—	—
8	Strong greenish yellow	Light yellow	Light purple
9	Brilliant yellow	—	—
10	White	Dark yellow	Dark purple

[1] 1, System proposed by Nittler, McKee, and Newcomer (*10*); 2, system used by North Central Regional Plant Introduction Station, Ames, Iowa; 3, unpublished system used by D. K. Barnes, L. J. Elling, and A. G. Peterson in studies on the attractiveness of alfalfa clones to pollinating insects, Univ. of Minnesota.

ing specific genotypes arranged in order of their genetic relationship. The system should easily and accurately classify the flower color of individual alfalfa plants or plant populations by a numerical rating so that data could be arrayed according to percent of plants in each flower color class; population means could be obtained and analyzed statistically; or flower color data could be stored in a data retrieval system for future reference.

After considering the proposed requirements for a flower color classification system and the problems involved with phenotypic classification of alfalfa flower color, the writer designed the visual classification system in table 2. The rating scale is similar in some respects to those of previous systems; however, primary emphasis is given to the effects of major genes and less emphasis to effects of modifying genes. The proposed system is intended to be more meaningful than previous systems for comparing or averaging flower color scores of individual plants of a variety or plant introduction. The proposed system deals more with gene frequency of several major loci than with various allelic interactions at any one locus. Also, the system is flexible because it can be used either as a five-class system for general studies; or, in cases of clonal identification and critical genetic studies, the secondary classes can be used.

As mentioned earlier, word descriptions of color can sometimes be misleading because all people do not associate colors in the same manner. In this handbook, photographs of each flower color subclass serve as color guides. Variations for color hue and color patterns occur within nearly all subclasses; therefore, it was postulated that groups of racemes illustrated the normal variability within each subclass better than could be done by using only one typical raceme.

The theory for arranging the order of the primary flower color groups as presented in table 2 was based on the fact that pure *M. sativa* and pure *M. falcata* have dark purple and dark yellow flowers, respectively. Variegated colors result from intercrosses of purple and yellow flower colors. Therefore, purple and yellow flower color classes should be located on opposite ends of any color scale with the variegated types in the center. The next consideration was where to place white- and cream-flowered plants. Usually these plants make up less than 1 percent of a population, but they each represent distinct genotypes and must be given a place in any flower color classification.

White flower color is produced by the homozygous recessive genotype of the basic color factor (*c*) which blocks the expression

TABLE 2.—*Proposed scale for visually scoring alfalfa flower color. Scale is based on genotypic as well as phenotypic characteristics of the alfalfa plant*

Numerical rating	Flower color class and subclass	Probable genotype
1	Purple or violet	C___ P___ yyyy
1.1	Dark	
1.2	Moderately dark	
1.3	Light	
1.4	Very light	
2	Variegated	C___ P___ Y___
2.1	Dark purple variegated	
2.2	Maroon	
2.3	Dark blue	
2.4	Light blue	
2.5	Dark blue-green	
2.6	Light blue-green	
2.7	Green	
2.8	Dark yellow-green	
2.9	Light yellow-green	
3	Cream	C___ pppp yyyy
4	Yellow	C___ pppp Y___
4.1	Very light	
4.2	Light	
4.3	Moderately dark	
4.4	Orange	
5	White	cccc P___ yyyy

of all purple and yellow flower color pigments. Since white flower color does not give any identity of the genotypes for either the *P* or *Y* genes, it should be placed at one end of the scale to denote a special genotype. All reported cases of naturally occurring white flower color have been found in *M. sativa.* For this reason, the white-flowered class could be placed at the purple end of the color scale. However, it has been the consensus of a number of plant breeders that they would prefer purple to be the number 1 class, because of prior usage. Therefore, white flower color was placed at the other end of the scale in class 5. The cream-flowered class was placed between the variegated and yellow-flowered groups because of its assumed hybrid origin and similarity to the very light yellow flower color class.

The only primary flower color classes used in table 2 that may be troublesome to correctly identify are the creams (pl. 3, A) and the whites (pl. 3, B). White-flowered plants lack the basic color factor and so are devoid of anthocyanin pigmentation in all tissues.

The genotype of the cream-flowered plants does not affect color production of any organs of the plant except the flower. In direct comparison of white and cream flowers, the cream flowers usually will be more ivory colored than true white. However, in cases when white flowers are not available for comparisons, the presence of pigmented floral veins or purple bud color can be used as evidence of cream flower color. White-flowered plants occur very seldom in natural populations, so as a general rule, plants lacking purple and yellow pigments are usually cream rather than white. If white-flowered types are found that lack pigmented floral veins, positive identification can be made by producing a few seed to check for white seed color or by testing stem tissue for the presence of anthocyanin.

Most of the flower color subclasses used in table 2 are relatively easy to identify. The four purple subclasses (pl. 3, C, D, E, and F) are differentiated according to color intensity variations; differences in hues of purple or violet are not considered. The variegated class consists of nine heterogeneous subclasses, all of which are characterized by varying degrees of purple and yellow copigmentation. Flowers in the dark purple variegated subclass (pl. 4, A) are usually dark purple when they first open, but the purple pigments fade with age and the flowers become a smudgy purple with faint traces of yellow pigmentation. The maroon class (pl. 4, B) occurs rather infrequently and is characterized by obvious joint purple and yellow pigmentation that lacks any trace of blue or green color. The two blue subclasses (pl. 4, C and D) are differentiated from each other on the basis of color intensity. The blue-green subclasses (pl. 4, E and F) are quite variable in color patterns, but they have a decided green color over a blue background.

The blue-green flower colors are also separated by color intensity into dark and light subclasses. Plate 5, A, illustrates the green subclass. The green color is typical of F_1 hybrids between *M. sativa* and *M. falcata*. The yellow-green subclasses (pl. 5, B and C) are predominantly yellow pigmented flowers with varying degrees of purple and green pigmentation. Intensity of yellow color separates the dark and light yellow-green subclasses. The four yellow subclasses (pl. 6, A, B, C, and D), like the purple subclasses, are differentiated only according to color intensity. The orange-yellow subclass is characteristic of unadulterated *M. falcata* flowers.

Differences in scores among observers occurred infrequently during trial runs with the new classification system. No differences appeared when the five-class primary scale was used, and usually only differences of 0.1 were observed when the secondary scale was used.

The system was adopted by the Twenty-first Alfalfa Improvement Conference.[5] It is presently being used by the North Central Regional Plant Introduction Station of the U. S. Department of Agriculture in their uniform recording and retrieval system for use in evaluating alfalfa. The system was successfully used by the NC–83 technical committee in a study to determine whether associations exist between seed yield in the North Central States, California, and Idaho.

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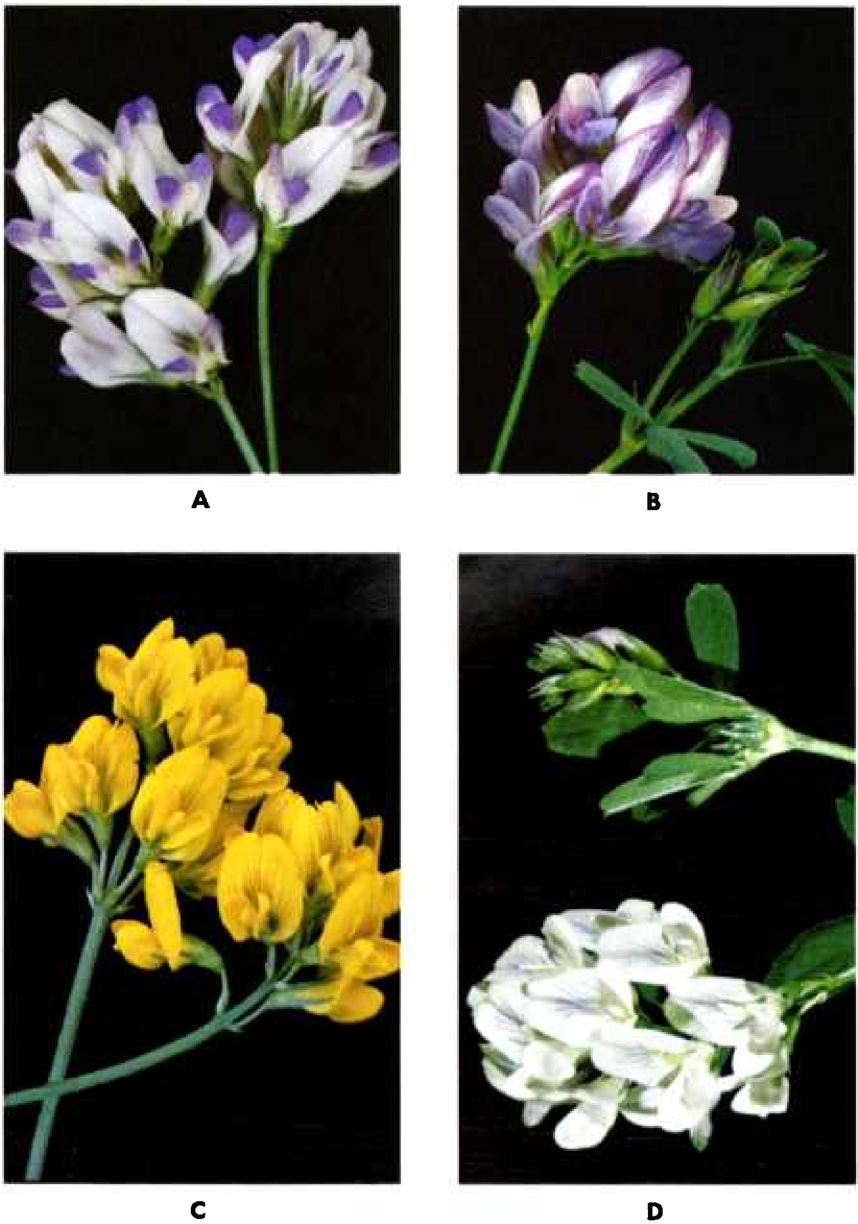
A

B

C

A, Purple bud color (right); open flowers from same plant with no obvious purple pigmentation resulting from bud color (left); *B*, three racemes demonstrating that color of keel petals can vary from color of wing and standard petal; *C*, three racemes from same plant demonstrating effect of age on color changes. Raceme on right just opening, raceme on left 5-6 days old.



A, Unusual type of irregular flower color pigmentation in which wing petals are purple while keel and standard petals are cream; *B*, unusual type of irregular flower color pigmentation in which pigmentation pattern on standard petal is uneven; *C*, brown vein color usually associated with yellow and cream-colored flowers; *D*, purple vein color usually associated with purple pigmented flowers.

A

B

C

D

E

F

A, Cream- or ivory-colored flowers—*class 3*; *B*, white flowers lacking basic color factor for all types of floral pigmentation—*class 5*; *C*, dark purple flowers—*subclass 1.1*; *D*, moderately dark purple flowers—*subclass 1.2*; *E*, light purple flowers—*subclass 1.3*; *F*, very light purple flowers—*subclass 1.4*.

A

B

C

D

E

F

A, Dark purple variegated flowers: newly opened flowers are dark purple color, older flowers smudgy purple showing traces of yellow pigments—*subclass 2.1; B*, maroon flowers—*subclass 2.2; C*, dark blue flowers: newly opened flowers show purple color, older flowers are dark blue—*subclass 2.3; D*, light blue flowers: newly opened flowers tend to show purple color, older flowers are light blue—*subclass 2.4*; *E*, dark blue-green flowers: various dark shades of green with blue background colors—*subclass 2.5; F*, light blue-green flowers: various light shades of green with blue background colors—*subclass 2.6*.

A

B

C

A, Green flowers: some purple evident in newly opened flowers, blue colors are not apparent—*subclass 2.7*; *B*, dark yellow-green flowers: darkly pigmented yellow flowers with varying degrees of light purple pigmentation—*subclass 2.8*; *C*, light yellow-green flowers: lightly pigmented yellow flowers with varying degrees of light pigmentation—*subclass 2.9*.

A

B

C

D

A, Very light yellow flowers—*subclass 4.1*; *B*, light yellow flowers—*subclass 4.2*; *C*, moderately dark yellow flowers—*subclass 4.3*; *D*, orange-yellow flowers—*subclass 4.4*.

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