Numerous studies have suggested various genetic models for flower color in alfalfa. Tetraploidy and inadequate color classification have resulted in paradoxical explanations for flower color inheritance. Inheritance studies and fertility relationships in crosses between Medicago sativa L. and M. falcata and in elucidating the type of ploidy in alfalfa. It is the purpose of this paper to report the results of a study on the inheritance of flower color in a diploid M. falcata x M. sativa cross.

LITERATURE REVIEW

Westgate (13) noted that in 1783 Thomas LeBlanc transplanted to his garden a single alfalfa plant with varied flower color which he found while gathering seed of yellow medic. Seedlings from this plant included individuals with flowers which were almost yellow, almost white, and various shades of purple. Atwood and Grun (1) reported that alfalfa flower color had been studied more than any other character. In the F1 of a cross between M. media and M. falcata, Moe8 recovered pure yellows, blues, and creamy whites. Burton (3) in summarizing his results of M. falcata x M. sativa crosses suggested that the number of yellow-flowered individuals indicated the character was controlled by three factors. Lepper and Odland (6) reported the hybrid from a purple x yellow cross gave light purple flowers. Purple-, yellow-, and white-flowered individuals were recorded in the F2 however, 20% of the yellow F1's x white M. sativa gave between purple and yellow plants (also referred to as variegated) and a white flowered plant gave a purple F3, changing with age to dark green. In 273 F2 plants the color varied from purple through green and yellow to white.

Weising (12) found both purple and yellow dominant to white. He did not study the cross between purple- and yellow-flowered plants. Using diploids, Twamley (10) studied the F2 segregation of crosses between purple and yellow plants. He classified his plants as having either purple or nonpurple (yellow) flowers. Twelve families fit a 3:1 genetic ratio and 3 families fit a 9:7 ratio. He interpreted these data to indicate that the anthocyanin pigmentation was controlled by 1 dominant factor in some plants and 2 dominant factors in others. In similar crosses, Lesins (8) expanded the two class segregation used by Twamley (10) and classified some F2 plants as near white. Lesins (7) concluded that a number of anthocyanins in alfalfa flowers behave genetically as a single block. All 150 flowers examined chromatographically contained the same 4 or 5 anthocyanin pigments. Hydrolysis of these pigments revealed that the aglycone portion was delphinidin, malvidin, and petunidin.

Peach (9) reported both carotenoids and flavonoids occurred in most yellow-flowered species. He also reported that genetic factors had been detected which modified the pigment, produced co-pigments, or changed the cell conditions in such a way as to produce a wide range of tones from one type of pigment. Genes have been found which alter the acidity of cell sap, thereby modifying flavonoid colors. Generally, plastid pigment, yellow flavonoid, and anthocyanin production were dominant over their absence. According to Geissman (5), flavones and flavonols were readily detected in white or pale yellow flowers by the ammonia test which turned white flowers yellow and intensified yellow flowers.

Bate-Smith (2) suggested that, even without exact identification of all the substances responsible, the spots seen on the chromatogram provided a means of rapidly characterizing genetic material and of tracing relationships among genotypes.

MATERIALS AND METHODS

A yellow-flowered plant, 27, was obtained from crossing 2 diploid M. falcata L. plants from seed received from J. L. Bolton, Canada. Plant 27 was crossed reciprocally to a M. sativa L. identified as 4463. The diploidy was confirmed cytologically. 27 x 4463 was identified as cross 9. The reciprocal was cross 10.

Seven F1 plants with good self-fertility, 4 from cross 9 and 3 from 10, were self pollinated. Three hundred twenty-eight F2 plants were classified. Those flowers which appeared green at some stage and also contained anthocyanin pigmentation were classified as green. Any open flower which contained anthocyanin pigmentation but which did not appear green at any stage of development was classified as purple. Blush purple, maroon red, light purple, and dark purple were included in this class. Any open flower without anthocyanin pigmentation that was similar in pigmentation to the yellow parent was classified as yellow. Those decidedly less intensely pigmented than the yellow parents were classified as cream. Forty of these F2 plants, approximately equal numbers for each of the major color classes, green, purple, yellow, and cream, were selected to represent the variation existing within each of the major color classes. The F3 population was produced by selling these 40 selected plants. Only 27 of these 40 plants produced progenies because of self-incompatibility in 13.

The flowers were sealed by rolling the racemes between the fingers. Crosses were made after emasculating with 57% alcohol as described by Tysdal and Garl (11). The study was conducted in a screened greenhouse.

Paper chromatograms were used to trace the relationship between genotypes without exact identification of the substances responsible. The standards of single flowers were smashed directly onto Whatman No. 1 filter paper. This method, which was used by Buzzati-Traverso (4) on other types of biological material, provided the least possible opportunity for chemical changes to occur during the "extraction" procedure. Irrigating solvents included one or more of the following: distilled water, normal butyl alcohol, glacial acetic acid, ammonium hydroxide, and hexane.

Spray reagents of 1% HCl and vapors of concentrated NH4OH were used to locate pigmented spots on the paper chromatograms. An ultraviolet lamp was used to locate fluorescent spots.

RESULTS

S1, F1, F2, and F3 Data

When selfed, 4463 bred true for purple flowers and 27 bred true for yellow flowers. The cross 4463 x 27 and reciprocal produced all green flowers. Table 1 shows the F2 segregation. The total progenies did not deviate significantly from a 9 green, 3 purple, 3 yellow, and 1 cream expected from 2 independent genes (P = .06). One F1 family, 9-7 deviated from the expected. These F1, F2, and F3 data suggested that a dominant gene (P) conditioned purple pigments and a dominant gene (Y) conditioned yellow pigments. The presence of both P and Y gave green flowers and the absence of P and Y gave cream flowers.

The chi-square test for heterogeneity indicated that the data from each of the seven F1 families were homogeneous.

1. Contribution from Department of Agronomy, Purdue University, Agricultural Experiment Station, Journal Paper No. 1740. Part of a thesis submitted by senior author in partial fulfillment of the requirements of the M.S. degree. Received April 14, 1961.
2. Instructor and Professor of Agronomy.

Published November, 1961