GENETICS OF THE MONOECIOUS CHARACTER IN SPINACH[1]

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Received September 20, 1954

WHILE spinach (*Spinacia oleracea* L.) is usually considered to be dioecious, there is a continuous range of monoecious types as regards the proportion of pistillate to staminate flowers per plant. Sex determination in dioecious strains of spinach is controlled by a mechanism that acts as if it were a single factor pair; the pistillate plant is homozygous (XX), and the staminate plant is heterozygous (XY) (JANICK and STEVENSON 1954a). The inheritance of the monoecious character has been genetically interpreted by HIRATA and YAMAMOTO (1931), NEGODI (1934), MIRYUTA (1937), LORZ (unpub.), SUGUIMOTO (1948), and BEMIS and WILSON (1953). SUGIMOTO (1948) suggested that a single gene independent of the XY factors controls monoecism, while BEMIS and WILSON (1953) assume that there are two closely linked factors in addition to the XY factors. A study of progeny segregation from selected crosses involving pistillate, staminate, and monoecious types was attempted to clarify the genetic mechanisms that bring about the monoecious complex in spinach.

MATERIALS AND METHODS

The seed for the major portion of this study was harvested in 1947 and represented the third generation of selection within the variety Nobel. All plants were classified for femaleness[2] by estimating the percentage of pistillate flowers per plant, employing a system suggested by LORZ (unpub.). Seven classes were used, namely, 100, 95, 75, 50, 25, 5, and 0 percent female. The percentage of maleness was the difference between the percentage of femaleness and 100. A plant designated as 100 percent female was completely pistillate, and a plant classified as 0 percent female was completely staminate. Plants classified as 95, 75, 50, 25, or 5 percent female were monoecious, differing only in the ratio of pistillate to staminate flowers.

Selfing and crossing were facilitated by isolating selected plants in the greenhouse.

RESULTS

A number of lines grown in February 1951 were found to exhibit distinct patterns of inheritance, as can be seen in table 1. The variety Old Dominion segregated only staminate and pistillate plants; while the variety, New Giant Leaf, segregated all three types, pistillate, staminate, and monoecious. Two lines, 73A0,0,7 and 73A5,1,3, derived from monoecious selections, were particularly interesting. Line 73A5,1,3

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[1] Journal paper No. 816 of the Purdue University Agricultural Experiment Station, Lafayette, Indiana.

[2] The terms maleness and femaleness, while not botanically precise, were found to be convenient in referring to the proportions of pistillate to staminate flowers in spinach and consequently have been used in this study.

TABLE 1

Sex ratios of four lines of spinach showing distinct patterns of inheritance

Lines	Sex types		
	Staminate	Pistillate	Monoecious
Old Dominion	25	33	0
New Giant Leaf	5	3	1
73A5,1,3	0	11	9
73A0,0,7	0	0	24

TABLE 2

Distribution of progenies of monoecious plants derived from 73A0,0,7 when selfed and crossed with pistillate plants

	Monoecious plant selfed									× Pistillate (100 percent female)								
Parental phenotype	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean value in percent	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean value in percent
	100	95	75	50	25	5	0			100	95	75	50	25	5	0		
	percent									*percent*								
5				15.4	57.7	26.9		26	23.5		36.0	62.0	2.0				50	81.7
25			13.3	33.3	46.7	6.7		15	38.7		68.4	24.6	1.8	3.5	1.8		57	85.3
75			23.9	37.0	37.0	2.2		46	45.8		65.7	17.1	11.4	5.7			35	82.4

segregated into both monoecious and pistillate offspring, while 73A0,0,7 was found to be completely monoecious.

Some of the monoecious plants derived from 73A0,0,7 were subsequently selfed and in some cases used as the pollen parent in crosses with pistillate plants from the variety Long Standing Bloomsdale. The segregation of progenies from some of these selfed monoecious plants and crosses is presented in table 2. Selections derived from the line 73A0,0,7 were found to be true-breeding for the monoecious character. Significantly, the F_1 progenies from the pistillate × true-breeding monoecious crosses were also all monoecious. These progenies were, however, quite different from the monoecious ones obtained from selfing the monoecious pollen parents. They consisted of highly pistillate monoecious plants, the greater bulk of which were scored as 95 or 75 percent female. In contrast, the S_1 progenies of the selfed monoecious pollen parents consisted of highly staminate monoecious plants, the greater bulk of which were classified as 50, 25, or 5 percent female.

Some of the monoecious F_1 plants from one of the pistillate × true-breeding monoecious (5 percent female) crosses derived from 73A0,0,7, as well as some of the monoecious S_1 plants, were selfed. Seven selections from the subsequent F_2 populations were selfed to give F_3 lines. These results are presented in table 3.

The S_2 progenies derived from 73A0,0,7 consisted of highly staminate monoecious selections as did the S_1 progenies. The F_2 progenies segregated in a ratio of 3 monoecious to 1 pistillate. The monoecious segregates could be grouped into two classes. One class consisted of plants that were phenotypically indistinguishable from the

TABLE 3

Distribution of progenies from a monoecious selection derived from 73A0,0,7 when selfed and crossed with a pistillate plant

Generation	Parental phenotype	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean value in percent	Chi square for ratio	
		100	95	75	50	25	5	0			3:1†	1:2:1‡
					percent							
S_1 (pollen parent selfed)	5				15.4	57.7	26.9		26	23.4		
F_1 (pistillate × monoecious)	100 × 5		36.0	62.0	2.0				50	81.7		
S_2 (11 S_1 plants selfed)					17.3	56.1	26.5		98	24.0		
F_2	75(1)	19.6	44.3	13.4	16.5	3.1	3.1		97	80.9	1.24	2.84
	75(2)	27.5	27.5	14.5	18.8	11.6			69	76.9	.12	1.88
	75(3)	27.6	34.5	22.4	8.6	5.2	1.7		58	82.8	.09	2.80
	75(4)	16.5	30.8	28.6	16.5	3.3	4.4		91	76.4	3.29	4.25
	75(5)	27.0	32.0	15.0	23.0	3.0			100	80.9	.12	.38
	75(6)	27.5	25.0	22.5	20.0	2.5	2.5		40	78.9	.03	.15
	95(1)	26.4	32.1	15.1	17.0	5.7	3.8		53	78.3	.01	.16
	95(2)	23.1	28.2	17.9	23.1	2.6	5.1		39	75.8	.01	.70
	95(3)	20.1	24.0	28.0	22.0	2.0	4.0		50	75.5	.43	.72
Total (F_2)		23.4	32.2	19.3	18.3	4.4	2.5		597	78.8	.68	.82
F_3	5(1)				37.5	37.5	25.0		8	29.4		
	25(1)				17.6	29.4	52.9		34	18.8		
	25(2)	23.7	3.5	17.5	12.3	22.8	20.2		114	53.0	.01	60.93**
	50(1)	25.0	15.6	15.6	9.4	15.6	18.8		32	62.3	0.00	6.75*
	75(1)	22.0	8.0	24.0	16.0	18.0	12.0		50	60.7	.11	9.20*
	75(2)	30.2	3.2	19.0	11.1	17.5	19.0		63	58.3	.64	27.15**
	75(3)	25.0	9.4	9.4	21.9	15.6	18.0		32	56.7	0.00	18.75**

† 3 monoecious: 1 pistillate.

‡ 1 monoecious (50, 25 or 5 percent female): 2 monoecious (95 or 75 percent female): 1 pistillate (100 percent female).

* Significant at the .05 level.

** Significant at the .01 level.

F_1 plants (scored as 95 or 75 percent female) and the other class was phenotypically indistinguishable from the S_1 and S_2 segregates (scored as 50, 25, or 5 percent female). The two classes appeared in a ratio of 2 to 1, and the F_2 ratio of 3 monoecious to 1 pistillate could be broken down into a ratio of 1 monoecious (50, 25, or 5 percent female) to 2 monoecious (95 or 75 percent female) to 1 pistillate (100 percent female). It was hypothesized that the highly pistillate monoecious plants (scored as 95 or 75 percent female) in the F_2 segregation were heterozygous for some factor controlling monoecism, and would segregate into monoecious and pistillate plants when selfed, while the more highly staminate monoecious plants (scored as 50, 25 or 5 percent female) were homozygous for this factor and when selfed would breed true for the monoecious condition. Of the seven F_2 selections that were selfed, two selections (scored as 5 and 25 percent female) were found to be true-breeding for the monoecious character and gave progenies restricted to the 50, 25, and 5 percent female classes. Five other selfed selections, scored as 25, 50, 75, 75, and 75 percent female, gave progeny that segregated into a ratio of 3 monoecious to 1 pistillate. The segregating F_3 lines, however, did not fit the ratio of 1 monoecious (50, 25, or 5 percent female) to 2 monoecious (95 or 75 percent female) to 1 pistillate (100 percent female). This lack of fit may be explained as an environmental effect, since the high greenhouse temperatures under which these plants were grown bring about a shift towards maleness (JANICK and STEVENSON in press).

These results may be explained on the assumption that a single incompletely dominant gene, M, is responsible for the monoecious character. The precise relationship between the M gene and the XY factors cannot be established from these data. The results obtained can be explained by assuming that the M gene is independent of the XY factors and that the monoecious plants under consideration are genetically XX, then:

$XXMM$ = true-breeding monoecious

$XXMm$ = monoecious, more highly pistillate than $XXMM$ and segregating

$XXmm$ = pistillate

The assumption that the gene M is allelic to the XY factor would give identical results. Using the symbol X^m as the gene responsible for the monoecious character, then:

X^mX^m = true-breeding monoecious

X^mX = monoecious (segregating)

$X\,X$ = pistillate

An analysis of true-breeding monoecious × staminate crosses was carried out in order to determine the relationship between the factor responsible for the monoecious character and the XY factor pair.

The difficulty of analyzing monoecious × staminate crosses was overcome by the use of a dominant marker gene for red stem in the pollen parent. Nine white-stemmed monoecious plants derived from 73A0,0,7 (from line derived from plant classified as 75 percent female, table 2) were crossed with a single red-stemmed staminate plant from the variety Long Standing Bloomsdale. At the same time, two other sister monoecious selections were selfed. The true-breeding, white-stemmed monoecious × red-stemmed staminate crosses segregated red-stemmed progenies in a ratio of 1 monoecious to 1 staminate; while the two selfed monoecious selections gave only monoecious progenies. The monoecious plants derived from the monoecious × staminate crosses were generally more highly pistillate than those monoecious plants obtained from the selfed monoecious plants as shown in table 4, indicating that the staminate plant used was recessive for the M gene, i.e., $XYmm$ or XY.

These results are compatible with either of the two hypotheses concerning the nature of the M gene. If the M gene is independent of the XY factors, and assuming the staminate plant from Long Standing Bloomsdale to be homozygous recessive

TABLE 4

Analysis of true-breeding monoecious × staminate crosses (I). Distribution of progenies from monecious selections derived from 73A0,0,7, when selfed and crossed with a staminate plant

Generation	Parental phenotype	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean		Chi square for 1:1 ratio†
		100	95	75	50	25	5	0		Entire progeny	Monoecious progeny	
		percent										
Monoecious plants selfed	50			6.2	34.4	37.5	21.9		32	32.3	32.3	
	25			10.5	42.1	36.8	10.5		19	38.7	38.7	
F_1 (monoecious × staminate)	25 × 0		10.0	24.3	11.4	5.7		48.6	70	34.9	67.8	.02
	50 × 0		5.9	35.3	13.7			45.1	51	38.9	70.9	.32
	50 × 0			29.4	11.8			58.8	17	27.9	67.8	.24
	50 × 0		13.3	26.7	13.3			46.7	15	40.7	76.2	0.00
	50 × 0		3.1	12.5	18.8	9.4		56.2	32	24.1	55.0	.28
	50 × 0			40.4	7.7	1.9		50.0	52	34.6	69.2	0.00
	75 × 0		7.4	21.4	17.8			53.6	28	31.8	68.5	.04
	75 × 0			28.0	16.0			56.0	25	29.0	65.9	.16
	75 × 0		10.3	31.0	10.3	1.7		46.6	58	38.7	72.4	.16
Total (F_1)......................			6.3	28.7	12.6	2.6		50.0	348	34.2	68.5	0.00

† 1 monoecious: 1 staminate.

for this gene ($XYmm$) and that Y is epistatic to M, the true-breeding monoecious × staminate cross should yield progenies in a ratio of 1 monoecious to 1 staminate, namely,

$$XXMM \times XYmm \rightarrow 1\ XXMm : 1\ XY\underline{M}m$$

1 monoecious : 1 staminate

If the M gene is allelic to the XY factors and the staminate plant was genotypically XY, the same results would be expected:

$$X^mX^m \times XY \rightarrow 1\ X^mX : 1\ X^mY$$

1 monoecious : 1 staminate

Under either hypothesis, the true-breeding monoecious × staminate cross should yield monoecious plants that are heterozygous, which when selfed, should give progeny in a ratio of 3 monoecious to 1 pistillate. The staminate plants, however, from the true-breeding monoecious × staminate cross when crossed on pistillate plants would be expected to give one of two distinct types of segregation, depending on which of the above hypotheses is correct. If the M gene is independent of the XY factor pair, the staminate plants ($XYMm$) from the above cross, when crossed on pistillate plants, should give progenies which segregate 1 monoecious to 1 pistillate to 2 staminate, namely,

$$XXmm \times XYMm \rightarrow 1\ XXMm : 1\ XXmm : 1\ XYMm : 1\ XYmm$$

1 monoecious : 1 pistillate : 2 staminate

TABLE 5

Analysis of true-breeding monoecious × staminate crosses (II)

A. Distribution of progenies of pistillate × staminate plants where staminate plants are the progeny of true breeding monecious × staminate crosses (table 4, F_1).

B. Distribution of progeny of selfed monoecious plant derived from the true-breeding monoecious × staminate cross (table 4, F_1).

Parental phenotype	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean value of monoecious plants
	100	95	75	50	25	5	0		
				percent					
A. 100 × 0		3.9	16.3	15.7	11.8	2.8	49.4	178	53.2
100 × 0	.8	10.4	10.4	17.6	3.2	.8	56.8	125	51.5
100 × 0		5.6	14.4	14.4	13.3	4.4	52.2	98	65.1
95 × 0‡	.6	3.4	12.3	12.8	16.2	9.5	45.2	179	43.7
B. 75 selfed	28.4	11.0	13.8	12.8	19.3	13.8	.9	109	46.3

‡ Pistillate plant reclassified as 95 percent female after crossing.

If the *M* gene is allelic to the *XY* factors, however, the staminate plant (X^mY) from the above cross, when crossed on pistillate plants, would give progeny which segregate 1 monoecious to 1 staminate, namely,

$$XX \times X^mY \rightarrow 1\ X^mX : 1\ XY$$

1 monoecious : 1 staminate

In order to distinguish between these two hypotheses, four staminate plants from the progenies of the true-breeding monoecious × staminate crosses were crossed on pistillate plants from the variety Long Standing Bloomsdale, and one monoecious plant from this cross was selfed as shown in table 5. All of these four crosses gave progenies which segregated in a ratio of 1 monoecious to 1 staminate, indicating that the *M* gene is allelic to the *XY* factor pair. Although the monoecious progenies were extremely variable and contained highly staminate monoecious plants, this may have been due to an environmental shift toward maleness. The pistillate plant recorded in two of the progenies is probably due to misclassification. The progeny from the selfed monoecious selection segregated in a ratio of 3 monoecious to 1 pistillate as would be expected under either hypothesis.

If the *M* gene is allelic to the *XY* pair, the staminate segregates from the pistillate × F_1 staminate crosses should be genetically *XY*, and when crossed on pistillate plants, should give progenies segregating staminate and pistillate plants in a 1:1 ratio. To test this, six of the staminate progenies from the pistillate × F_1 staminate crosses were crossed on pistillate plants from the variety Long Standing Bloomsdale. However, one plant that was believed to be staminate was reclassified later as monoecious and scored as 50 percent female on the basis of the seed set. This is a further indication that the highly staminate monoecious plants in the pistillate × F_1 staminate cross represents an environmental shift toward maleness.

The five pistillate × staminate crosses segregated progenies in a ratio of 1 staminate to 1 pistillate as shown in table 6, although in progenies of two of the crosses some

TABLE 6

Analysis of true-breeding monoecious × staminate crosses (III)

A. Distribution of progenies of a monoecious selection when selfed and crossed with a pistillate plant.
B. Distribution of progenies of pistillate × staminate crosses.

(The monoecious and staminate plants are derived from the progenies of pistillate × staminate crosses (table 5, A.) where staminate plants are derived from the progenies of true-breeding monoecious × staminate crosses (table 4, F_1).)

Parental phenotype	Frequency distribution of phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants
	100	95	75	50	25	5	0	
				percent				
A. 50 selfed	29.2	31.2	17.7	16.7	4.2		1.0	96
100 × 50	50.0	35.6	7.1	1.9			2.9	104
B. 100 × 0	54.3						45.7	46
100 × 0	37.4	8.7	3.5	.9			50.4	115
100 × 0	34.3	21.9	1.0				49.8	105
100 × 0	49.5						50.5	109
100 × 0	45.1						54.9	102

of the pistillate plants contained some perfect or staminate flowers and were classified as monoecious (95 and 75 percent female). The monoecious phenotype of some of the progenies from these two lines might have been due to the presence of modifying genes in the pistillate parent from Long Standing Bloomsdale. The progeny obtained from selfing the monoecious plant that was first classified as staminate segregated in a ratio of 3 monoecious to 1 pistillate and, when crossed on a pistillate plant, gave progeny which segregated in a ratio of 1 monoecious to 1 pistillate as expected.

DISCUSSION

Sex determination in spinach appears to be controlled by a "switch" mechanism that acts as if it were a single gene with three alleles, Y, X^m and X. X^m is incompletely dominant to X, because plants that are heterozygous, X^mX, although phenotypically monoecious, contain a higher proportion of pistillate flowers than X^mX^m. The allele Y is completely dominant to X^m and X, because X^mY and XY types are staminate.

In addition to this major factor conditioning monoecism, there are also apparently many modifying genes quantitatively inherited affecting the monoecious character, for it has been possible by selection to obtain true-breeding monoecious lines that have high and low values of femaleness, as measured by the proportion of pistillate to staminate flowers per plant. In three generations of inbreeding, it was found possible to select plants whose progeny mean values were as high as 70 to 80 percent female and as low as 5 percent female (Janick 1954).

If we assume that these factors determining sex expression in spinach actually represent three alleles at a single locus, and that the monoecious condition in spinach is more primitive than the dioecious, as is generally believed in flowering plants (Lewis 1942), it is possible to suggest methods of gene action of these alleles from their dominance relationships. Their allelism suggests that they control a single physiological function. Crosses involving diploids and tetraploids have shown that a single dose of Y causes the plant to be staminate even in combination with three doses of X (JANICK and STEVENSON 1954b and in press). Thus the Y allele appears to be definitely male determining as found in the Melandrium studies (WARMKE and BLAKESLEE 1940) and in contrast to the Drosophila pattern (BRIDGES 1939). The X allele can be described as an amorphic gene, because its action represents a loss of a "Y" function. This would explain why only a single dose of Y is needed to produce the staminate condition regardless of the number of X alleles present. The X^m allele permitting functioning of male- and female-producing substances appears to function in a similar manner as the Y allele but not as efficiently. X^m acts as a hypomorphic gene in contrast to the Y allele. This would explain why an increase in the dose of X^m results in a shift toward maleness, i.e., X^mX^m types are more highly staminate than X^mX types, as well as why X^mY types are staminate. Apparently, plants of the genotype X^mX are physiologically unstable and may be readily shifted by environmental conditions. Modifying genes affecting the proportion of staminate to pistillate flowers may be presumed to control other reactions affecting the same physiological end product.

Some of the progeny segregations reported by BEMIS and WILSON (1953) indicate that there is a gene independent of the XY factors that results in the formation of pistillate flowers on plants containing a Y gene. While this gene has not been observed in the lines investigated in this study, the existence of such a factor appears perfectly reasonable. It has been shown that staminate plants, genetically XY, may produce hermaphroditic flowers (JANICK and STEVENSON 1954a).

While this model of gene action is only suggestive, it appears credible and conforms to the experimental data. It does not seem unreasonable to assume that the X and Y alleles arose from the X^m gene by mutation, namely,

$$X \leftarrow X^m \rightarrow Y$$

The selective advantage of either mutation in the evolutionary process might come about by assuring cross pollination with a subsequent increase in vigor and variability. Furthermore, the assumption that the sex determining mechanism in spinach consists of a single gene with a number of alleles rather than a complex of genes as suggested by STOREY (1953) for *Papaya carica* and WESTERGAARD (1948) for *Melandrium album* helps explain the lack of heteromorphic sex chromosomes in spinach, which presumably only arise via the selective advantage of a crossover suppressor system.

SUMMARY

The monoecious character in spinach appears to be controlled by one major gene, X^m, which was found to be allelic to the XY factor pair. X^m is incompletely dominant to X. The Y allele is dominant to X and X^m. Methods of gene action of these alleles were hypothesized from their dominance relationships.

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