

Inheritance of Persistent-Green Color in *Asparagus Officinalis*, L.¹

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INTRODUCTION

This study deals with the inheritance of a presumed spontaneous mutation that occurred in asparagus, in which the mutants, termed "persistent-green", have dark-green stalks and foliage in the autumn when normal plants turn yellow and brown. The persistent-green plants remained green until killed by freezing. The two persistent-green plants discovered in 1959 were from the Viking strain. This type of variant is of extremely low occurrence, in that the authors have surveyed approximately 500,000 plants of commercial Washington fields without finding more than 5 such plants.

Literature reports of the origin and genetics of plant color variations reveal that pigmentation changes usually seem to originate as spontaneous mutations and are conditioned by a single gene (1,3,4,9).⁴ One report indicated that a color variant was controlled by two nonallelic recessive genes (8).

A secondary part of this study was to determine the effect of the color gene or genes on the plant pigment system by means of spectrophotometric analyses. Similar studies have been reported in other crops (5,6,9).

An attempt also was made to identify the persistent-green mutant in the seedling stage, as has been reported in tomato, corn, and castorbean (1,2,7).

MATERIAL AND METHODS

INHERITANCE STUDIES

During the winter of 1962-63, at Rutgers University, New Jersey, a cross was made between the two persistent-green parents, V15 ♀ and V17 ♂. These parents also were crossed with normal plants, RR 27 ♀, RR 29 ♀, and RR 35 ♂. F₁ seeds were planted in the field during the spring of 1963,

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⁴ Italic numbers in parentheses refer to Literature Cited, p. 78.

and the progenies were classified as either persistent-green or normal in the autumn.

From this planting, the following F_1 progenies were selected for further study of the F_2 and reciprocal BC_1 generations: $V15 \times V17$ (F_2 only), $V15 \times RR\ 35$, $RR\ 27 \times V17$, and $RR\ 29 \times V17$. The latter progeny segregated for persistent-green phenotype and both normal and persistent-green plants were selected for study.

During the winter of 1963-64, F_2 crosses and reciprocal BC_1 were made in the greenhouse. In June, 1964, 100 seeds of each cross were planted in the field. A segregation count based on the foliage color was recorded for each cross and backcross during October 1964.

PLANT PIGMENT STUDIES

In July, 1964, 1 g. of fresh plants tissue was collected from a shoot of a persistent-green and normal parent for pigment analysis. These plants were growing under similar field conditions and the stalks were approximately of the same age. Each individual plant sample was treated with methanol for the extraction of the chlorophyll and carotene. This was followed by recording an absorption spectrum curve of the 50-percent diluted extract, using a 1-mm. light path at a wavelength of 700 to 400 $m\mu$.⁵

A second pigment sample was taken in October 1964, when the normal plants had developed a yellow color and the persistent-green plants remained green.

IDENTIFICATION OF PERSISTENT-GREEN PLANTS IN THE SEEDLING STAGE

As a preliminary test, 10 F_1 seeds each of $V15 \times V17$, $RR\ 27 \times V17$, and $RR\ 29 \times V17$ were sown in sand in the greenhouse during the winter of 1963-64. In the summer of 1964, 100 seeds each of F_2 of $V15 \times V17$, as well as F_2 , and reciprocal BC_1 generations of $V15 \times RR\ 35$, also were sown in the greenhouse. Water was supplied daily and a complete nutrient solution was applied twice a week for 4 weeks, after which the nutrient solution was withheld to allow yellowing of the normal plants. After 6 weeks, each seedling was identified as either persistent-green (green foliage) or normal (yellow foliage).

RESULTS AND DISCUSSION

INHERITANCE STUDIES

On the basis of the analyses of the F_1 , F_2 , and reciprocal BC_1 generations, persistent-green color in asparagus apparently is inherited as a single re-

⁵ Beckman, D. B., Spectrophotometer used for absorption spectrum curve.

cessive gene (tables 1,2,3). All the segregating progenies gave X^2 values in which the observed ratios fit similar expected ratios.

It is evident that asparagus plant RR 29 (normal phenotype) is heterozygous for the described character. This might suggest that the persistent-green homozygous recessive mutant can be used for the identification of heterozygous genotypes.

TABLE 1.— F_1 segregation of persistent-green character in asparagus

P ₁ females × P ₁ males	Phenotypes	Tested ratios	Classification of F ₁ progenies		Total	X ²	P
			Number of normal pheno- types	Number of per- sistent- green pheno- types			
V15 × V17	Persistent- green do.	All persist- ent-green	0	30	30	—	—
V15 × RR35	Persistent- green Normal	All normal	30	0	30	—	—
RR27 × V17	Normal Persistent- green	do.	30	0	30	—	—
RR29 × V17	Normal ¹ Persistent- green	1:1	13	17	30	0.534	0.30-0.50

¹ Heterozygous.

PLANT PIGMENT STUDIES

Little difference in quantity of pigments between persistent-green and normal plants was found in July (fig. 1). At this stage, identification of the persistent-green plants would be difficult, if not impossible. Near the end of the growing season (October) both chlorophyll and carotene concentrations were much greater in the persistent-green mutant (fig. 1). In the fall, persistent-green and normal plants can be identified visually or by pigment analysis.

No apparent qualitative differences were observed between persistent-green and normal plant-pigment systems (fig. 1). However, similar studies conducted by Inman *et al.* (5) indicated that differences did exist in *Datura*.

IDENTIFICATION OF PERSISTENT-GREEN PLANTS
IN THE SEEDLING STAGE

The genetic ratios obtained from the greenhouse classification of asparagus seedlings as either persistent-green (green foliage) or normal (yellow foliage), are in accordance with those previously recorded in the field, using the same seed stocks (table 4). Withholding nutrients from

TABLE 2.—*F₂ segregation of persistent-green character in asparagus*

F ₁ generation siblings	Phenotypes	Expected ratios	Classification of F ₂ progenies		Total	X ²	P
			Number of normal phenotypes	Number of persistent-green phenotypes			
(V15 × V17) × (V15 × V17)	Persistent-green	All persistent-green	0	59	59	—	—
(V15 × RR35) × (V15 × RR35)	Normal	3:1	38	6	44	3.031	0.05-0.10
(RR27 × V17) × (RR27 × V17)	do.	3:1	41	12	53	.159	.50-0.70
(RR29 × V17) × (RR29 × V17)	do.	3:1	33	15	48	1.000	.30-0.50
(RR29 × V17) × (RR29 × V17)	Persistent-green	All persistent-green	0	44	44	—	—

seedlings in sand culture in the greenhouse produced color changes similar to those observed in the field in autumn.

SUMMARY

Two mature, dark-green asparagus plants (one female and one male) termed "persistent-green" were selected in a New Jersey asparagus field on November 11, 1959, when the other plants were yellow or brown. The two persistent-green plants were crossed; each of them was crossed also with normal plants for the genetic study of this character.

A secondary part of this study was to determine the effect of the color gene or genes on the plant-pigment system by means of spectrophotometric analyses.

TABLE 3.—Reciprocal BC₁ segregation of persistent-green character in asparagus

P and F ₁ parents	Phenotypes	Expected ratios	Classification of BC ₁ progenies		Total	X ²	
			Number of normal phenotypes	Number of persistent-green phenotypes			
(V15 × RR35) BC RR35	Normal do.	All normal	51	0	51	—	—
V15 BC (V15 × RR35)	Persistent-green Normal	1:1	26	31	57	0.438	0.50-0.70
(RR27 × V17) BC V17	do. Persistent-green	1:1	33	37	70	.228	.50-0.70
RR27 BC (RR27 × V17)	Normal do.	All normal	93	0	93	—	—
(RR29 × V17) BC V17	do. Persistent-green	1:1	20	29	49	1.654	.10-0.20
(RR29 × V17) BC V17	do. do.	All persistent-green	0	46	46	—	—
RR29 BC (RR29 × V17)	Normal ¹ Persistent-green	1:1	18	14	32	.500	.30-0.50
RR29 BC (RR29 × V17)	Normal ¹ do.	3:1	22	12	34	1.921	.10-0.20

¹ Heterozygous.

An attempt also was made to identify the persistent-green mutants in the seedling stage.

The study of the phenotypes of 17 F₁, F₂, and reciprocal BC₁ progenies indicated that persistent-green color in asparagus is inherited as a single recessive gene.

There was a large quantitative difference in chlorophyll and carotene between the persistent-green and normal plant complexes in October, but

not in July. Apparently the persistent-green mutants retain chlorophyll and carotene much later in the season than do the normal plants. No qualitative difference in pigment was found in either July or October.

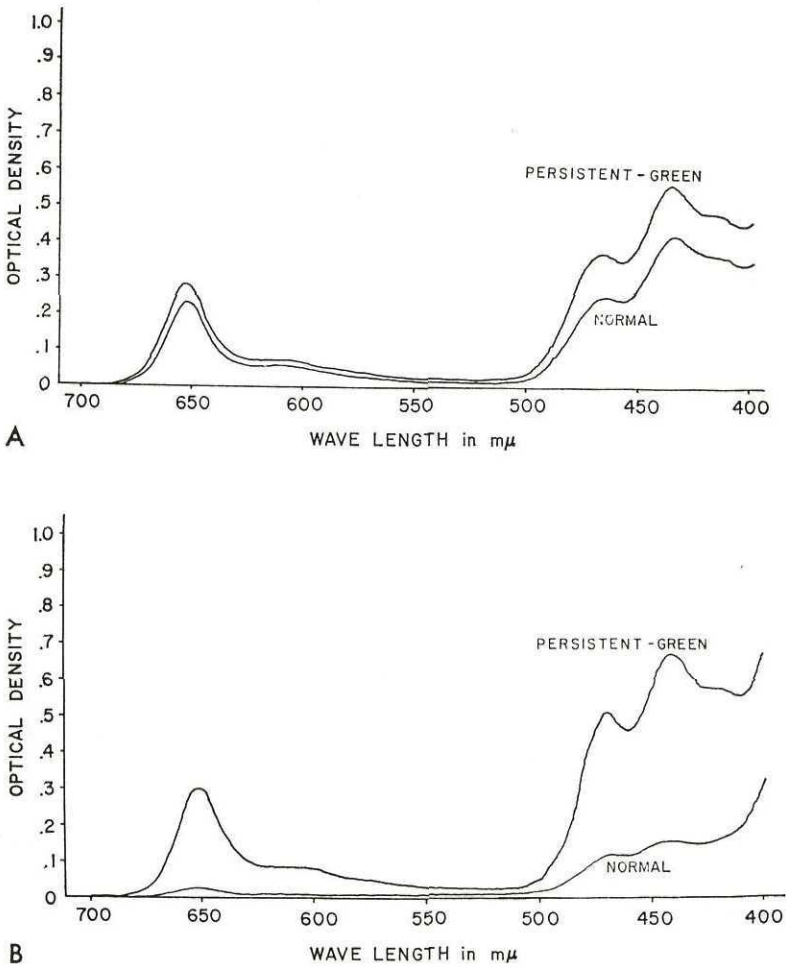


FIG. 1—Absorption spectrum of the 50-percent diluted extracts from a persistent-green and a normal asparagus plant, using 1-mm. light path. A, Plants sampled in July; B, plants sampled in October.

Asparagus seedlings were easily classified as to persistent-green (green foliage) or normal (yellow foliage) in the greenhouse when the plants were 6 weeks old.

RESUMEN

El 11 de noviembre, cuando el follaje de las demás plantas era amarillo o pardo, se seleccionaron en un campo de New Jersey dos plantas de espárrago

TABLE 4.— F_1 , F_2 , and reciprocal BC_1 segregation of persistent-green character in asparagus, grown under greenhouse conditions

P ₁ and F ₁ parents	Phenotypes	Expected ratios	Classification of F ₁ , F ₂ , and BC ₁ progenies		Total	X ²	P
			Number of normal phenotypes	Number of persistent-green phenotypes			
V15 × V17 ¹	Persistent-green do.	All persistent-green	0	10	10	—	—
RR 27 × V17 ¹	Normal Persistent-green	All normal	10	0	10	—	—
RR 29 × V17 ¹	Normal ² Persistent-green	1:1	9	7	16	0.250	0.50-0.70
(V15 × V17) × (V15 × V17) ³	do.	All persistent-green	0	100	100	—	—
(V15 × RR35) × (V15 × RR35) ³	Normal	3:1	56	21	77	.212	.50-0.70
(V15 × RR35) BC RR35 ³	do. do.	All normal	91	0	91	—	—

¹ First experiment, winter 1963-64.

² Heterozygous.

³ Second experiment, summer 1964.

♀ y ♂, cuyo follaje era de un color verde obscuro, el cual se designó como "verde-persistente".

Para estudiar este carácter genético las dos plantas se cruzaron entre sí y luego también se cruzó cada una con otras plantas de color normal.

El estudio tuvo como propósito secundario el determinar mediante el uso del espectrofotómetro, el efecto que el gene o genes responsables del

color puedan tener sobre el sistema de pigmentación de las plantas de espárrago.

También se trató de identificar en plántulas en el invernadero los mutantes de color verde-persistente.

El estudio del fenotipo de 17 progenies F_1 , F_2 y del retrocruce₁, indicó que el color verde-persistente en el follaje de la planta de espárrago se hereda como un gene recesivo simple.

Se observó una gran diferencia cuantitativa de clorofila y carotina entre las plantas de follaje verde-persistente y las de color normal en el mes de octubre, pero no en julio. Aparentemente, los mutantes retienen la clorofila y la carotina hasta más tarde en el otoño que las plantas de color normal. No se observaron diferencias cualitativas entre los pigmentos en ambos tipos de planta durante julio u octubre.

Fue fácil diferenciar entre las plantas de espárrago de color verde-persistente (follaje verde) y las de color normal (follaje amarillo) en el invernadero cuando tenían 6 semanas de nacidas.

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