JOURNAL OF THE AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE
(ISSN 0003-1062)

VOL. 104 NO. 6 NOVEMBER 1979

The *Journal* (ISSN 0003-1062) is published by the American Society for Horticultural Science, 701 North Saint Asaph Street, Alexandria, Virginia 22314, and is issued bimonthly in January, March, May, July, September, and November. The *Journal* supersedes the Society's *Proceedings* which was established in 1903 and published in 93 volumes until 1968. Volume number for the *Journal* is continued without interruption, beginning with Volume 94 in 1969.

Communications in regard to membership in the Society or subscription to the *Journal* or regarding reprints and advertising, should be sent to the Publisher/Business Manager, Cecil Blackwell, Executive Director, American Society for Horticultural Science, 701 North Saint Asaph Street, Alexandria, Virginia 22314, USA.

Manuscripts for publication in the *Journal* and all correspondence **relating to manuscripts should be sent** to the editor, Jules Janick, ASHS Editorial Office, 420 Forest Hill Drive, West Lafayette, Indiana 47906, USA. Because of occasional serious delay in transit, authors and reviewers are urged to send manuscripts and proofs by first class mail.

The nonmember subscription rate is $60.00 per year in the USA, Canada, and Mexico, and $64.00 to other countries. All members of the Society except Associate, Student Associate, Emeritus, and Spouse Members also receive the *Journal* through payment of annual dues (other benefits of membership include receipt of *HortScience*, another bimonthly publication of the Society). For information about membership, subscriptions, or the availability and price of back volumes and issues, write to the Business Office address listed at left. Remittances should be made payable (in U.S. dollars) to: *American Society for Horticultural Science.*

Post Office Entry: Second-class postage is paid at Alexandria, Virginia, and at additional mailing offices.

Postmaster. Please return undeliverable copies and change of address "Form 3579" to the American Society for Horticultural Science, 701 North Saint Asaph Street, Alexandria, Virginia 22314, USA. Return postage guaranteed.

J. Amer. Soc. Hort. Sci. 104(6):721–723. 1979.

Descriptions, Genetics, and Independent Assortment of Red Stem and Pale in Muskmelon (*Cucumis melo* L.)[1]

J. D. McCreight and G. W. Bohn[2]

U.S. Department of Agriculture, Science and Education Administration-Agricultural Research, Imperial Valley Conservation Research Center, 4151 Highway 86, Brawley, CA 92227

Additional index words. pigment, recessive lethal

Abstract. **Red stem, found in PI 157083, is controlled by a single recessive gene, *r*. Red pigment appeared in vascular traces of hypocotyls about 2 weeks after planting. Seed coat color of red-stemmed plants was reddish or tan, in contrast to white or yellow seed coats of green-stemmed plants. Pale, a spontaneous mutant in a second backcross hybrid from 'Campo' x PI 180280, is controlled by a single partially dominant gene, *Pa*, which acts as a recessive lethal: *Pa/Pa* plants die; *Pa/+* are pale; and +/+ are normal. Pale did not affect expression of red stem. Testcross segregations fit the expected ratio for independent assortment of the 2 loci.**

Linkage relationships among the 37 genes of muskmelon listed by Robinson et al. (10) are not well known. Bohn and Principe (3, 4) found independent assortment of bush, glabrous, male steriles 1 and 2, nectarless, powdery mildew resistance and yellow-green. Zink found linkage between yellow virescence and bush (13). Nugent (9) reported independence of halo, glabrous, and yellow-green. Here we report on 2 muskmelon seedling markers, red stem and Pale, and their independent assortment. Brief descriptions of red stem and Pale have appeared previously (1, 2, 10).

Materials and Methods

Red stem. Red stem was first observed in stems of muskmelon populations 90144, 90145, and 90146 seedlings grown in the glasshouse at La Jolla, Calif. in 1961. These populations were derived from plant introduction (PI) 157083, a small-fruited cultivated muskmelon collected by H. L. Crane in Lanchow, China. To our knowledge, this is the only record of a red pigment in stems or leaf tissue of any cucurbit (5). Red-stemmed 90144 (Fig. 1) was crossed with the green-stemmed 'Campo' for genetic analysis of red stem.

Pale. Progeny 22309, derived from a cross of watermelon mosaic-resistant PI 180280 and 'Campo' (Fig. 1), produced a single Pale green plant among 90 dark green sibs. Pale and normal green segregates of 14028 were selfed and crossed with normal plants for genetic analysis.

Linkage study. To test for independent assortment, a red-stemmed segregate of F_2 progeny 44077, derived from the 90144 by 'Campo' cross, was crossed with both Pale and normal segregates of F_3 progeny 45265, which was derived from the Pale 14028 (Fig. 1). Parental, F_1, F_2, and testcross progenies were planted in the greenhouse in pure quartz sand in 7.5 cm^2 peat pots; 1 seed per pot. Plants were watered with Hewitt's (7) standard nutrient solution adapted for greenhouse cucumber culture (12) when cotyledons were expanded. This nutrient solution was further modified by reducing the concentration of the minor elements to one-quarter that in Hewitt's solution since some of our breeding lines were minor element sensitive when grown to fruiting (Dr. A. N. Kishaba, USDA, SEA-AR,

[1]Received for publication March 12, 1979.
The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

[2]Research Horticulturist and Research Geneticist, respectively. The authors thank J. A. Principe and M. B. Marsh for technical assistance.

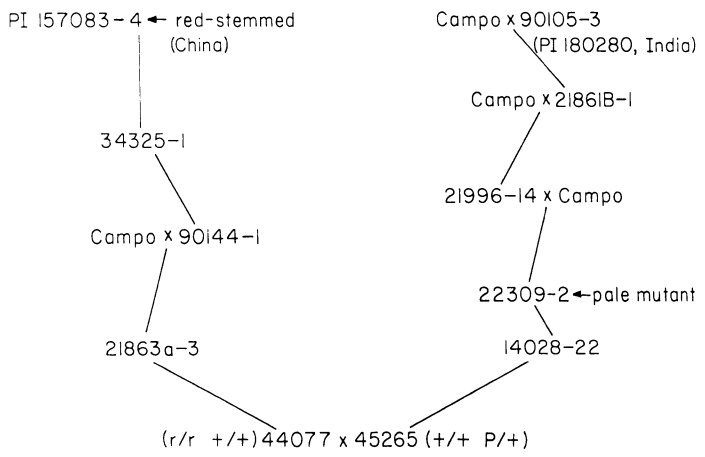

Fig. 1. Pedigrees of red-stemmed 44077 and Pale 45265 progenies crossed to determine the genetic linkage relationship of red and Pale.

Boyden Entomology Lab, Riverside, Calif., personal communication).

Plants were scored for red stem and Pale 2 or 3 times from 14 to 21 days after planting. Seedlings were transplanted into the field and scored again approximately 35 days after transplanting. Seed coat color was scored when fruits were mature or nearly so.

Results and Discussion

Description – Red stem. The red pigment is first apparent in vascular traces of cotyledons as early as 14 days after planting; seedlings can be reliably scored for presence of the pigment at 21 days. Dr. Randolph Wedding (Department of Biochemistry, University of California, Riverside, Calif., personal communication) observed that the pigment was located in walls of thick-walled cells, probably phloem fibers.

In the stem, red pigment develops in the cylinder of thick-walled phloem fibers found between the cortex and the vascular cylinder (6). The pigment is continuous along the ridges formed by the angles of the stem and is interrupted by green areas between these ridges. Pigment also develops in petioles, peduncles, tendrils, and seeds.

Presence and brightness of red pigment are affected by the age of plant tissues. Pigment is not visible in the first 3 to 5 nodes of the main or lateral stems and is reduced or absent in the mature stem portions. The stem portions for several nodes beyond node 5 may, however, be bright red. Pigment is not observed in petioles and tendrils arising from stem portions that have not yet developed pigment but persists in those arising from nodes where the pigment has faded.

Light apparently affects pigment production or accumulation. Wedding (personal communication) observed pigment in plants placed in complete darkness. Pigment was reduced in stems and peduncles directly exposed to sunlight compared to shaded stems and peduncles.

Pigment level follows a diurnal cycle. Stems of selfed progenies of PI 157083 were bright red at 8:00 AM, but faded during the day and color was difficult to detect in some plants. The stems were, however, again bright red the following morning. The earlier report (1) of pigment not persisting in field-grown plants was probably associated with examination late in the day after the pigment faded.

Wedding concluded that the pigment was not an anthocyanin since it was not in the cell vacuole and not directly soluble in (but reacted with) alcohol. Dr. F. P. Zscheile, Jr. (Department of Agronomy and Range Science, University of California, Davis, personal communication) confirmed that the pigment was not an anthocyanin. Zscheile also concluded that the pigment was not a carotenoid form absorption spectra of fractions from a magnesia column, that had been treated with the hexane layer of acetone-hexane extracts and developed with increasing concentrations of acetone. Since the pigment occurred in thick-walled cells of young stems and disappeared in old stems, T. A. Geissman (Department of Chemistry, University of California, Los Angeles, personal communication) suggested that the pigment may be a precursor to lignin.

Pale. A single Pale plant was observed in the hybrid progeny of PI 180280 and 'Campo'. Pale phenotype is characterized by pale green hypocotyl, cotyledons, stems and leaves, and by reduced plant vigor and size. Fruits of Pale mutants are of normal color and under favorable conditions are of comparable size to those of normal plants.

Genetic studies – red stem. The cross of red-stemmed 90144 by 'Campo' produced a green-stemmed F_1 hybrid. Two F_2 populations, 44076 and 44077, segregated 68 green stem: 26 red stem plants, a close fit to the expected 3:1 ratio, χ^2 = 0.35 (probability of fit, 0.50 – 0.70). Thus, the red stem character was dependent on a single, recessive gene designated *r*.

Pale. Pale plants did not occur in the parents or other derivatives of second backcross progeny 22309 suggesting the mutation to Pale was unique (Fig. 2). Progeny 14028, from the first Pale plant, segregated a 3rd phenotype, white lethal. White lethals failed to emerge in soil culture but did germinate on paper towels. Progeny 14028 segregated 8 white lethal: 38 Pale: 19 normal in a 65-plant population from 75 seeds. These results suggest the hypothesis that Pale is controlled by a single partially dominant gene which acts as a recessive lethal (11). Plants homozygous for the mutant gene are white and lethal, dying before or shortly after emergence and heterozygous plants are pale green and viable.

F_1 and F_2 progenies from a cross of a normal segregate of the Pale 14028 by normal were all green. An F_1 progeny of a Pale by normal cross segregated 1 Pale: 1 normal as expected in a cross of a heterozygote with a homozygous recessive (Table 1). F_2 and F_3 progenies did not segregate in the expected 1 white: 2 Pale: 1 normal ratio due to the lethality of the white class, χ^2 = 22.96 and 15.30, respectively. The F_2 data did not fit the 2:1 ratio expected when the white lethal class was omitted, χ^2 = 7.47. The F_3 data did, however, fit the 2:1 ratio when the white lethal class was omitted, χ^2 = 0.11 (Table 1). The gene symbol *Pa* is assigned to the Pale mutant.

Linkage study. Parental progeny 44077 segregated 61 green stem: 24 red stem plants in an approximate 3:1 ratio, χ^2 = 1.12 in the test for independent assortment (Table 2). This agrees with the above data suggesting that a single recessive gene controls red stem. Parental line 45265 segregated 42 Pale: 13 normal, a rough fit to the 2:1 ratio expected after omitting the white lethal class which failed to emerge, χ^2 = 3.24 (Table 2).

F_1 progenies 22970 and 25904 and their reciprocal, 25980, segregated in a 1 Pale: 1 normal ratio, χ^2 = 0.96, 0.17, and zero,

Table 1. Phenotypic segregation in populations derived from Pale crossed with normal plants.

Generation	Parental phenotype	Progeny phenotype			Expected ratio	Probability of fit
		White	Pale	Normal		
F_1	Pale × normal	0	39	35	1:1	0.50 – 0.70
F_2	Pale	21	83	65	1:2:1	< 0.001
					2:1	0.01 – 0.001[z]
F_3	Pale	4	48	22	1:2:1	< 0.001
					2:1	0.90 – 0.95[z]

[z]White lethal class omitted.

Table 2. Phenotypic expression of the parents, F_1, F_2, and testcrosses of red stem and Pale crosses.

Generation	Progeny	Parental phenotype	Progeny characters: Green stem Pale	Green stem Normal	Red stem Pale	Red stem Normal	Expected ratio	Probability of fit
P_1	44077	green stem		61		24	3:1	0.30 – 0.50
P_2	45265	Pale	42	13			2:1	0.05 – 0.10
F_1	22970	$P_1 \times P_2$	10	16			1:1	0.30 – 0.50
F_1	25904	$P_1 \times P_2$	45	49			1:1	0.50 – 0.70
F_1	25980	$P_2 \times P_1$	23	23			1:1	> 0.99
Combined F_1			78	88			1:1	0.30 – 0.50
F_2	49535	Pale 22970	112	55	20	11	6:3:2:1	> 0.00[z]
Combined F_2 from normal 22970[y]				247		80	3:1	0.80 – 0.90
Combined Testcross of normal F_1[x]				399		347	1:1	0.05 – 0.10
Combined Testcross of Pale F_1[w]			245	228	223	203	1:1:1:1	0.20 – 0.30

[z]Disturbed 3:6:3:1:2:1 ratio due to lethality of green stem-white and red stem-white classes.
[y]Homogeneity $\chi^2 = 0.4613$, 1df.
[x]Homogeneity $\chi^2 = 1.4020$, 1df.
[w]Homogeneity $\chi^2 = 9.1315$, 9df.

respectively (Table 2). All F_1 progenies were green-stemmed. Pale segregated in the F_1 as expected from a cross of a heterozygote with a recessive homozygote.

F_2 progenies 49532 and 49544, derived from green segregates of progeny 22970, segregated 3 green stem: 1 red stem, $\chi^2 = 0.05$. F_2 progeny 49535, derived from a Pale segregate of F_1 22970, segregated 6 green stem-Pale: 3 green stem-normal: 2 red stem-Pale: 1 red stem-normal when the white lethal classes were omitted from the expected 3:6:3:1:2:1 ratio (Table 2). A chi-square value of 0.08 was calculated for F_2 progeny 49535 using the procedure outlined by Mather for disturbed segregations (8). The disturbed F_2 ratio in this case was due to the lethal classes, green stem-white and red stem-white.

Reciprocal testcrosses 25919 and 25922, derived from green stem-normal F_1 segregates crossed with the double recessive parent 44077, segregated 1:1 for stem color, $\chi^2 = 3.62$ (Table 2). This segregation is expected if the Pale locus is homozygous for the normal allele and red stem is controlled by a single recessive gene.

Testcross progeny 25923 and its reciprocal testcross progenies 25954, 25955 and 25957, derived from Pale F_1 segregates crossed with the double recessive parent 44077, segregated 1:1:1:1 for the 2 traits as expected in the absence of linkage between these loci, $\chi^2 = 3.99$ (Table 2). Pigment developed between 14 and 21 days in both Pale and normal seedlings, and pigment was present in stems and peduncles of mature Pale plants in the field indicating pale did not affect expression of red stem.

Seed coat color. Seed coat color of the F_2 progeny 44077 from which the red stem parent was selected, segregated 58 white: 20 red, a close fit to the expected 3:1 ratio, $\chi^2 = 0.02$. Seed coat color of the Pale parent 45265 and of the F_1 hybrids was white. Seed coat color and presence of red pigment in the stem were highly correlated in the F_2 progenies, 49532 and 49544, from a normal F_1 plant ($r = 1.00$) and in the progenies of both Pale and normal F_1 plants crossed with the double recessive parent 44077 ($r = 0.99$) (Table 2). The correlation coefficient of 0.99 for the testcrosses resulted from errors in scoring seed coat color. Red seed coats scored in fruits of green-stemmed plants and white seed coats in fruits of red-stemmed plants were attributed to the effect of fruit maturity and to masking of seed coat color by flesh and placenta color. We concluded that gene *r* conditions red pigment in both vegetative portions and seed coats of muskmelon.

Red stem, controlled by a single recessive gene *r*, is a potentially useful seedling marker for mature plant and fruit characters that are linked to red stem. It is readily observed, can be reliably scored 21 days after planting and is stable in the greenhouse and field. Pale, controlled by the single partially dominant gene *Pa*, which acts as a recessive lethal (13), has limited potential as a seedling marker for mature plant and fruit characters that are linked to Pale. Since red stem and Pale were not genetically linked, they could be used as markers for two different mature plant characters involved in a particular cross.

Literature Cited

1. Bohn, G. W. 1968. A red stem pigment in muskmelon. *Veg. Improvement Newsletter* 10:7.
2. _____. 1969. *Pale* muskmelon, a dominant mutant phenotype lethal in homozygotes. *HortScience* 4:159. (Abstr.)
3. _____ and J. A. Principe. 1964. A second male-sterility gene in the muskmelon. *J. Heredity* 55:211-215.
4. _____ and _____. 1968. Independent assortment of young plant characters in muskmelon, *Cucumis melo* L. *HortScience* 3:95. (Abstr.)
5. Chester, K. S. 1951. Selected writings of N. I. Vavilov. The origin, variation, immunity and breeding of cultivated plants. *Chronica Botanica* 13:1-6.
6. Esau, K. 1953. Plant anatomy. Wiley, New York.
7. Hewitt, E. J. 1966. Sand and water culture methods used in the study of plant nutrition, 2nd Ed. *Commonwealth Agr. Bur. Tech. Commun.* 22.
8. Mather, K. 1938. The measurement of linkage in heredity. Wiley, New York.
9. Nugent, P. E. 1978. Independence of halo, glabrous, and yellow-green mutants in muskmelon. *HortScience* 13:287-288.
10. Robinson, R. W., H. M. Munger, T. W. Whitaker, and G. W. Bohn. 1976. Genes of the Cucurbitaceae. *HortScience* 11:554-568.
11. Strickberger, M. W. 1968. Genetics. MacMillan, New York.
12. Ward, G. M. 1973. Calcium deficiency symptoms in greenhouse cucumbers. *Can. J. Plant Sci.* 53:849-856.
13. Zink, F. W. 1977. Linkage of virescent foliage and plant growth habit in muskmelon. *J. Amer. Soc. Hort. Sci.* 102:613-615.