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A New Chlorotic Mutant of Muskmelon

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Abstract. **A mutant seedling with retarded growth and interveinal chlorosis of leaves characteristic of iron deficiency was discovered in a group of 'Edisto' muskmelon transplants. The seedling responded to supplemental Fe, suggesting that the mutant affects Fe uptake or use. The F_1 phenotypes from crosses of the mutant with 'Edisto' and 'Mainstream' were normal. F_2, F_3, and testcross data indicate that the chlorotic phenotype is controlled by a single recessive gene, which is not allelic or linked to *virescent*.**

Nine chlorophyll mutants of muskmelon have been reported. Whitaker (17) reported the discovery of *yellow green (yg)* mutant, which affected the ratio of chlorophylls *a* and *b*. Hoffman and Nugent (7) described *virescent (v)* mutant, characterized by yellow cotyledons that slowly turned green and subsequently exhibited normal growth. These *virescent* plants also had yellow-centered white flowers with orange stigmas, and unique yellow-fleshed fruits. The mature plant was normal green, except for yellowish growing points and whitish tendrils. Nugent and Hoffman (12) described *halo (h)*, which causes the cotyledons to be yellow with green margins. Plants that have both *halo* and *virescent* genes appear to have no chlorophyll (10). Zink (18) described *virescent-2 (v-2)*. This mutant exhibited pale yellow cotyledons and yellow green leaves, stems, and tendrils and was linked to bush plant habit. McCreight and Bohn (9) discussed a dominant, pale-green mutant that caused the heterozygous plants to be pale green and the homozygous dominant plants to be white and lethal. Dyutin (4) reported a yellow-green coloration in young melon leaves. Pitrat et al. (14) described a chlorophyll-deficient mutant, *flava (f)*, which produced uniformly yellow plants. Cox and Harding (3) reported relationships of the *light green (lg)* mutant with several other seedling mutants and determined that *light green* was allelic to *yellow green*.

In 1984, a mutant seedling having yellow true leaves with green veins was discovered in a population of 'Edisto' muskmelon transplants. The seedling exhibited interveinal chlorosis of leaves characteristic of Fe deficiency (8, 15, 16). Its foliar appearance was similar to the Fe-deficient (fe) tomato T3238 discovered by Brown et al. (2) in a B-deficient line discovered by Andrus et al. (1). Because the chlorotic 'Edisto' seedling also might be Fe-deficient, efforts were made to preserve this germplasm for genetic and physiological studies of factors controlling chlorophyll deficiency and nutrient availability. Here we report on the inheritance of this chlorophyll mutant.

This chlorotic seedling was transferred to a greenhouse bench containing a peatmoss and sand mix and fertilized with a complete soluble fertilizer mix (NUTRILEAF 60; Miller Chemical and Fertilizer Corp., Hanover, Pa.) In addition, the seedling received 250ml/week of iron chelate solution containing 2g·liter^{-1} of Sequestrene 330. The plant slowly turned green, remained stunted, and produced seven staminate flowers before it died.

In order to save the gene, a mentor pollen technique (5) was used to cross it with *virescent* marker line C879-J2 (11). Pollen from

Table 1. Segregation ratios for the cross 'Mainstream' × chlorotic 'Edisto'.

Generation/pedigree	Seedling classes: Normal	Seedling classes: Chlorotic[z]	Expected ratio	χ^2	*P*
P_1 'Mainstream'	375				
P_2 Chlorotic 'Edisto'		0[y]			
F_1 (P_1 × P_2)	317				
F_2	64	24	3:1	0.242	0.62
F_2	191	59	3:1	0.261	0.61
F_2	219	76	3:1	0.092	0.76
F_2 Pooled	474	159	3:1	0.005	0.94
F_3 Segregating	34	11	3:1	0.007	0.93
	79	24	3:1	0.159	0.69
	164	57	3:1	0.074	0.79
F_3 Pooled	277	92	3:1	0.001	0.97
F_3 Normal	125				
	92				
	167				
F_3 Chlorotic		194			
		231			
BC F_1 × chlorotic	143	137	1:1	0.129	0.72

[z]Two types of muskmelon seedlings: yellow leaves with green veins; and white leaves.
[y]There was only one original chlorotic plant that died before seed was produced.

Fig. 1. Hybrid and *virescent* muskmelon seedlings.

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Table 2. Segregation ratios for crosses first and second of *virescent* x chlorotic 'Edisto'.

Generation/pedigree	Seedling types: Normal	Chlorotic[z]	*Virescent*	*Virescent* chlorotic[z]	Expected ratio	χ^2	*P*
P_3 C879-J2 *virescent*			472				
P_2 Chlorotic 'Edisto'		0[y]					
F_1 (P_3 × P_2)	329						
F_2	50	16	15	4	9:3:3:1	0.576	0.90
	48	20	19	5	9:3:3:1	0.984	0.80
	29	10	9	2	9:3:3:1	0.474	0.92
F_2 Pooled	127	46	43	11	9:3:3:1	1.000	0.80
F_3 F_2 normals selfed	295						
	124						
	242	75			3:1	0.304	0.58
	215	67			3:1	0.232	0.63
F_3 F_2 pooled	457	142			3:1	0.535	0.46
	177		62		3:1	0.113	0.74
	213	67	71	21	9:3:3:1	0.416	0.94
F_3 F_2 Chlorotic selfed		178					
		244					
		129		47	3:1	0.273	0.60
		188		60	3:1	0.086	0.77
F_3 F_2 pooled segregating types		317		107	3:1	0.084	0.77
F_3 F_2 *virescent* selfed			269				
			211				
			173	54	3:1	0.178	0.67
			138	49	3:1	0.144	0.70
F_3 F_2 pooled segregating types			311	103	3:1	0.003	0.96
F_3 F_2 *virescent* chlorotic				246			
				193			
BC F_1 × F_2 *virescent*	76	71	69	72	1:1:1:1	0.361	0.94
chlorotic	45	47	42	41	1:1:1:1	0.317	0.96
Pooled	121	118	111	113	1:1:1:1	0.542	0.91

[z]Two types of muskmelon seedlings: yellow leaves with green veins; and white leaves.
[y]There was only one original chlorotic plant that died before seed was produced.

four male flowers was used in two crosses with the marker plant along with mentor pollen from the marker line. Three flowers were used to make a cross without mentor pollen on 'Mainstream' muskmelon (13). One fruit, developed from each of the three crosses, grew well and produced many seed.

After germination in sand, two types of seedlings were observed in the populations using mentor pollen: *virescent* produced by *virescent* pollen and normal produced by deficient 'Edisto' pollen (Fig. 1). Seeds resulting from self-pollination of the F_1 plants were germinated in a sand bed in a greenhouse and fertilized with a modified Hoagland's nutrient solution (6) lacking iron to enhance the expression of the deficient segregates.

All *virescent* types were marked in the cotyledon stage and the chlorotic types at the first true leaf stage. The chlorotic *virescent* seedlings looked like the other *virescent* seedlings until the first true leaf started to expand and became chlorotic with green veins. Some chlorotic F_2 plants had leaves that were white (Fig. 2), looked very different from the other chlorotic plants, and were slower to turn green in response to Fe application. After seedlings were classified, some of each type were transferred to greenhouse benches for self-pollination and testcrosses. The chlorotic seedlings were fed 250 ml/plant per week of Fe solution containing 2 g·liter^{-1} of Sequestrene 330 until they turned green. The resulting plants were vigorous, grew well, and produced large fruit.

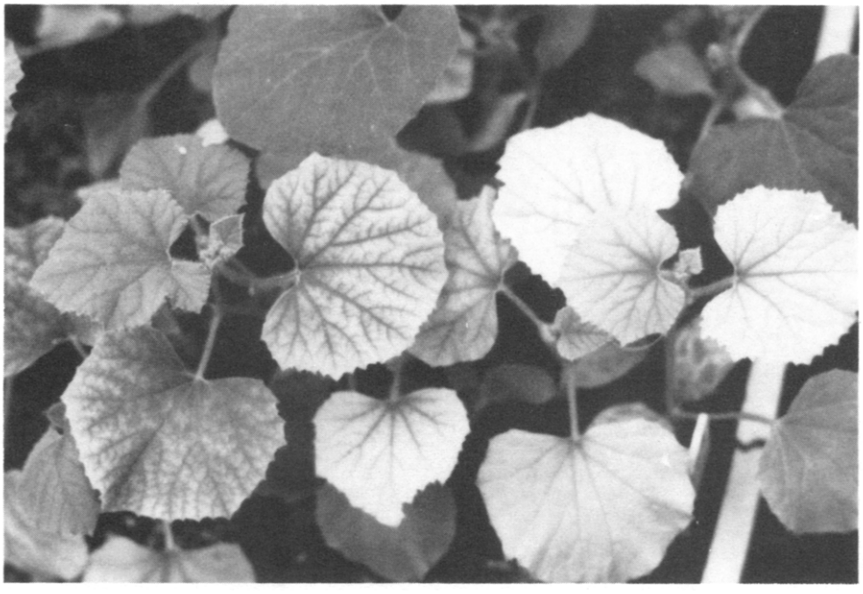

Fig. 2. Muskmelon seedlings: normal leaf (top), yellow leaves with green veins (left), and white leaves (right).

The cross 'Mainstream' x chlorotic 'Edisto' produced a ratio of 3 normal : 1 chlorotic plant in the F_2 (Table 1). The testcross of chlorotic plants with the F_1 'Mainstream' x deficient 'Edisto' plants segregated 1 normal : 1 chlorotic seedling.

When seeds from the *virescent* x chlorotic 'Edisto' crosses were germinated, two types of easily separable seedlings occurred as expected (Fig. 1). About 80% of these seedlings were *virescent* (selfed progeny) and the remaining 20% were normal (F_1) seedlings in one cross. In the second cross, three male flowers of the chlorotic plant and two male flowers of the *virescent* were used for pollen and produced 30% *virescent* (selfed) and 70% normal (F_1) seedlings. Seedlings in the F_2 populations from the F_1 plants segregated 9 normal : 3 chlorotic : 3 *virescent* : 1 chlorotic *virescent*. Because of difficulty distin-

guishing white-leaved from yellow-leaved seedlings, data for them were combined. The testcross of chlorotic *virescent* plants with F_1 plants produced normal, chlorotic, *virescent*, and chlorotic *virescent* seedlings in equal numbers.

To verify the inheritance of this mutant further and to look at the white-leaved plants, several F_2 plants were self-pollinated or crossed with yellow-leaved plants. The resulting F_3 populations segregated in ratios typical of two independent genes (Table 2). When plants having yellow leaves with green veins were crossed with white-leaved plants, there was no clear evidence of segregation.

From this study, we conclude that the expression of the 'Edisto' chlorotic muskmelon mutant is controlled by a single recessive gene that is not allelic or linked to *virescent*.

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Malate Dehydrogenase Isozyme Patterns in Seven *Prunus* Species

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Abstract. **Cultivars from three cherry species, sour cherry (*Prunus cerasus* L.), sweet cherry (*P. avium* L.), and ground cherry (*P. fruticosa* Pall.), and open-pollinated progenies of *P. mahaleb* L., *P. incisa* Thunb., *P. canescens* Bois., and *P. subhirtella* Miq., and the sour cherry cultivars Cigany Meggy and Pitic de Iasi were characterized electrophoretically for malate dehydrogenase (MDH) in leaf tissue. No intraspecific variability for MDH isozymes was detected within the sour cherry, sweet cherry, or *P. fruticosa* cultivars evaluated. The codominant expression of both the sweet cherry and *P. fruticosa* bands in sour cherry supports the hypothesis that sour cherry arose by interspecific hybridization. A single isozyme band was associated with the mitochondrial subcellular leaf extract from sour cherry. Sweet and sour cherry pollen had activity for a cathodal MDH locus that was not detected in the leaf tissue. Open-pollinated populations of *P. mahaleb* and *P. canescens* each exhibited one banding pattern; however, similar progeny from *P. subhirtella, P. incisa*, and the sour cherry cultivars Pitic de Iasi and Cigany Meggy each segregated for two zymogram patterns. Sufficient polymorphism at the MDH locus has been identified to permit its use as a biochemical genetic marker in interspecific hybridizations.**

The tetraploid sour cherry (2n = 32), is considered to have originated through hybridization of sweet cherry (2n = 16), and the cold tolerant ground cherry (2n = 32), which grows wild in Russia (6). Maximum genetic diversity in sour cherry is found in Eastern Europe, where it coexists with sweet cherry and *P. fruticosa*. The major germplasm collections have been made in those countries where sour cherry diversity is highest. Other cherry species that might be useful for the genetic improvement of sour cherry include the diploid species *P. mahaleb*, of Western Asian and European origin, and *P. incisa, P. canescens*, and *P. subhirtella*, of Japanese and Chinese origins.

Isozyme gene markers have been used widely for the identification of cultivars (12), species (1), and interspecific hybrids (8) and for the measurement of genetic divergence between and within populations for ecological, systemic, and phylogenetic study (3). In a breeding program, isozyme gene markers are advantageous because they can be detected at the seedling stage, permitting early selection of desirable individuals. However, no studies on variability for isozyme loci have been reported in cherry species to the best of our knowledge.

Polymorphism for malate dehydrogenase (MDH) has been studied in numerous crop species; however, its inheritance is complicated by overlapping MDH isozymes, which are compartmentalized in the cytosol, microbodies, and mitochondria (5). In *Prunus*, polymorphism for MDH has been described in peach with the observation of three distinct banding patterns (2). The F_2 seedlings segregated in a 1:2:1 ratio for the banding patterns, which is consistent with codominant alleles at a single locus. However, because segregation was not observed for four of the bands, it was not possible to determine the number of loci involved in the control of MDH isozymes in peach. MDH is reported to be a dimer in corn (11, 13) and celery (7) and a multimer in peach (2). In peach, haploid plants derived from heterozygous parents for the MDH allozymes exhibited only homodimeric banding patterns, suggesting that, in peach, heterodimers are formed between the two enzyme products. We present additional studies of MDH in *Prunus* and

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