Performance of Zucchini Yellow Mosaic Virus Resistant 'Golden Delicious' Type Pumpkin Hybrids

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The *Cucurbita maxima* 'Golden Delicious' (GD) type is the preferred pumpkin grown for processing and culinary seed production in the Willamette Valley of Oregon. Processors value their large size (five – 10 kg), thin, red-orange skin (which will not blemish processed product) and high quality flesh (7% soluble solids and 10% total solids). Culinary seed producers prefer GD for its large, plump and attractive white seeds. Over half the GD pumpkin production in the Willamette Valley is for culinary seed, which is most often exported to Pacific Rim markets.

Epidemics of zucchini yellow mosaic virus (ZYMV) occur in the valley every few years. Epidemiology of the virus in the Pacific Northwest is poorly understood. Alternate hosts have not been identified, especially ones that would allow the virus to overwinter from year to year. In an outbreak year, the virus is found first along the Columbia River, and then moves southwards into the Willamette Valley (3). ZYMV epidemics are a problem for pumpkin growers and processors because virus infection of the fruit reduces quality and can cause rejection of fields with too many virus-infected fruit. Fruit symptoms include reduced size, abnormal shape and green patches of skin that are visible on mature fruit. The latter symptom is particularly problematic to processors because the flesh beneath green areas will not ripen, thus not meeting processor specifications for soluble and total solids, and the green skin is a visible contaminant in the processed product.

Immature ovaries and developing fruits of GD are yellow as is typical of pumpkins possessing the *B* gene (4), but fruits ripen to a red-orange color probably conditioned by *Rd* (2). The B^{max} of *C. maxima* is at a different locus from *C. moschata* and *C. pepo* (6), and has been described as completely dominant in contrast to the bicolor pattern of heterozygotes of *C. moschata* and *C. pepo* (5). At maturity, GD fruit exhibit a faint green ring surrounding the stylar scar at the blossom end. When fruit becomes virus infected, the green pigment suppressing effect of *B* and *Rd* is reversed, allowing the expression of a larger green patch at the blossom end, and streaks and patches elsewhere on the fruit (Figure 1).

In 1998, we initiated a program to introgress ZYMV resistance from *C. equadorensis* into *C. maxima* (1). Virus resistance is quantitative, but appears to be controlled by major gene(s). From 1998 to 2001, a cyclical scheme of backcrossing resistant lines to GD followed by intercrossing was conducted for five generations (1). Six generations of selfing followed, resulting in 45 virus resistant inbred lines. Most lines had orange fruit (ranging from yellow-orange to pink, to red-orange), but a few were dark green. The orange-fruited types typically had small fruit size whereas the green skinned types had the more desirable large fruit size. Because inbreds were intended for use in an F1 hybrid production program, we established a yield trial to evaluate hybrid horticultural potential. We tested two sets of materials: crosses of resistant inbreds to GD and crosses between green- and red-orange fruited, virus resistant inbreds. One hypothesis was that virus resistance would be intermediate in crosses between resistant inbreds and susceptible GD. A second hypothesis was that crosses between large green-fruited virus-resistant inbreds and small orange-fruited virus-resistant inbreds would produce medium to large orange-fruited virus-resistant hybrids. We report here the results of a field trial where these hypotheses were tested.

Materials and Methods

Plant materials: Open-pollinated 'Golden Delicious', maintained in our breeding program, was used as the check. Twenty-one virus-resistant inbreds (Table 1) were crossed to GD and to each other to produce 24 unique cross combinations. GD was crossed to a selection of orange-fruited inbreds (Table 2) without regard to which line was the maternal parent. In a second set of materials, one of three X1- inbreds with green skin color were crossed to orange-fruited inbreds. Like the GD crosses, the X1- inbreds were used as the female in some and the male in other crosses.

Trial conditions: Plants were started from seed in the greenhouse on May 13, 2008 in 7.6 cm (3 in) pots using SB40 (Sun Gro Horticulture, Bellview, WA) potting mix supplemented with Apex 14-14-14 slow release fertilizer. Plants were transplanted to the field (Chehalis silt loam) at the Lewis Brown Research Farm, Corvallis, OR on June 6 with five plants per plot. Space between rows was 3.38 m (11 ft) with 92 cm (3 ft) between plants. Plots were arranged in a randomized complete block with three replications. Transplants received 450 lb a^{-1} (504kg ha^{-1}) 12-29-10-4 (N-P-K-S) fertilizer banded into the row just prior to planting. Transplants were irrigated immediately after planting, and the trial received weekly irrigation of approximately 25 mm. Plots were harvested on Oct. 22 and fruit were counted and weighed on an individual plot basis.

Virus inoculation: The ZYMV isolate was originally obtained from Phil Hamm, Hermiston Research and Extension Center, Hermiston, OR. It was stored in frozen (-80C) tissue of 'Honey Boat' (*C. pepo*) Delicata winter squash until use. Virus inoculum was prepared by grinding approximately 10 g of frozen tissue in 100 ml potassium phosphate buffer (2.6 mM monobasic potassium phosphate, 0.047 mM dibasic potassium phosphate, pH 8.5) with 250 mg carborundum powder with a mortar and pestle for one minute. Two-week old 'Honey Boat' plant primary leaves were rub-inoculated using the pestle dipped in inoculum solution. Plants were grown for one month and monitored for symptom expression prior to being used for field inoculation.

Susceptible spreader rows of 'Honey Boat' were direct seeded at the time of transplanting of the GD trial. Spreader rows were planted on the outside of the yield trial and every two rows within the trial. Inoculum for the spreader rows was prepared from greenhouse-infected plants. A Waring blender was loosely packed with symptomatic leaves and about 750 ml of phosphate buffer stored on ice was added and the mixture was blended on the high setting for three minutes. The solution was filtered through three layers of cheesecloth, and was then decanted into an electric paint sprayer modified for large scale virus inoculation. Plants in the spreader rows were inoculated with the paint sprayer when they had at least one expanded primary leaf. Inoculation was considered effective when the paint sprayer left a water-soaked area on the inoculated leaf. We relied on natural aphid transmission to move the virus from the spreader rows into the yield trial. At the time of our first reading on July 10, ½ to ¾ of the GD plants were infected, and by one month later, all GD plants showed virus symptoms (data not shown).

Statistical analysis: Data were analyzed using PROC GLM of SAS (Cary, NC) and means were separated using Fisher's protected least significant difference (LSD). To determine whether differences in virus infection was observed when hybrids had one vs. two parents contributing resistance, LS means were calculated, and the null hypothesis that all means were equal was tested.

Results and Discussion

Yields of the hybrids were generally high, with net yield ranging up to 51 MT ha^{-1} (Table 2). Trials from additional environments would be needed to validate these yields. Fruit weight was generally satisfactory with most hybrids achieving an average fruit weight of 4.5 kg, the minimum sought by processors. Generally, the GD x inbred crosses produced smaller fruit than the inbred x inbred crosses. The GD check was heavily infected with virus, which greatly reduced marketable fruit number and weight (Table 2). Most fruit from this cultivar exhibited typical symptoms of the virus infection, including green patches, misshapen and warty fruit (Figure 1). Symptoms were less severe to nonexistent in the experimental hybrids. Experimental hybrids generally had significantly higher marketable yields under disease pressure compared to GD, however, some experimental lines did have up to 50% of total fruit weight in culls. Culls were considered to be immature fruit, and fruit with a high percentage of the skin with green color. The green fruit color was the result of either virus infection (predominantly in GD and GD crosses), and/or by incomplete dominance of the genes controlling fruit color in green x orange skinned crosses (Figure 2). Partially green fruit color was also observed in green x orange hybrids grown in the absence of the virus (data not shown). *Rd* is epistatic and partially dominant to other fruit colors (2, 4) and is probably the gene responsible for the large green blossom ends observed in the green x orange crosses. We did not expect GD x orange hybrids to show any green at the blossom end since it was thought that both parents were homozygous for *B* and *Rd*. One possibility is that *Rd* had been lost from some inbreds, but the red-orange skin color of all inbreds used in this study does not support this idea. GD crosses had 10 – 28% (mean = 21%) cull fruit whereas green x orange crosses ranged from 14 – 49% (mean = 33%). We attribute the higher cull frequency in hybrids that are heterozygous at the *Rd* locus to greater sensitivity to environmental stresses causing more greening of the fruit. Interaction between virus infection and genes controlling fruit color may account for other cases of greening around the blossom end.

Clear differences between groups were observed for classic virus symptoms as shown by the AUDPC scores in table 3. GD (susceptible) had the highest level

of infection (148.3), followed by the GD crosses (susceptible x resistant; mean = 71.2), and then by the resistant x resistant crosses (mean = 1.9), and these differences were statistically significant ($P < 0.0003$).

We conclude that green x orange fruit color crosses produce F1 hybrids that would not be acceptable to the processing industry because fruit, while mostly orange in color, have significantly more green around the blossom end. Unexpectedly some orange x orange crosses produced hybrid progeny with significant amounts of green at the blossom end, a result that suggests an interaction between color genes and ZYMV symptom expression. Resistance to ZYMV shows partial dominance, with resistant x susceptible crosses being intermediate to GD and resistant x resistant crosses. To achieve the highest levels of resistance with the desired skin color, it will be necessary for both inbreds to be orange-skinned and resistant. The current focus of our program is to backcross orange skin into the large green-fruited virus-resistant inbreds.

Literature cited

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Table 1. Inbreds and OP cultivar used to produce F_1 hybrids evaluated in a trial planted at Corvallis, OR in 2008.

No.	Inbred	Skin color	No.	Inbred	Skin color
1	X1-1-2-1-1	Dark-green	12	X15-1-2-2-6	Red-orange
2	X1-1-2-1-2	Dark-green	13	X15-1-2-2-9	Red-orange
3	X1-1-2-1-3	Dark-green	14	X33-1-9-2-1	Red-orange
4	X7-2-1-3-1	Red-orange	15	X33-1-9-2-5	Red-orange
5	X7-2-1-3-4	Red-orange	16	X41-1-1-1-2	Red-orange
6	X7-2-1-4-2	Red-orange	17	X41-1-1-1-3	Red-orange
7	X7-2-3-1-2	Red-orange	18	X41-1-1-3-2	Red-orange
8	X7-2-3-1-4	Red-orange	19	X41-1-1-4-1	Red-orange
9	X7-2-3-3-5	Red-orange	20	X41-2-1-2-3	Red-orange
10	X15-1-2-2-3	Red-orange	21	X41-2-1-4-4	Red-orange
11	X15-1-2-2-5	Red-orange		Golden Delicious	Red-orange

Table 2. Yield of Golden Delicious derived squash hybrids grown under severe ZYMV infection at Corvallis, Oregon, 2008

	Marketable Fruit				Culls[z]	
Pedigree	No. ha^{-1}	MT ha^{-1}	Average Fruit Weight (kg)	Largest Fruit Weight (kg)	No. ha^{-1}	MT ha^{-1}
Golden Delicious	149	3.8	3.6	7.4	743	16.2
GD x X7-2-1-3-1	475	12.5	4.6	7.0	178	4.9
GD x X7-2-1-3-4	624	22.7	5.7	10.0	178	4.4
GD x X7-2-1-4-2	594	26.8	6.2	9.6	297	6.9
GD x X7-2-3-1-2	713	25.2	5.5	7.7	356	9.7
X15-1-2-2-3 x GD	921	21.6	3.5	7.4	297	6.0
GD x X15-1-2-2-6	743	22.3	4.6	7.5	238	5.3
X33-1-9-2-1 x GD	594	22.5	5.9	8.4	238	5.4
GD x X33-1-9-2-5	772	19.7	4.4	7.0	119	2.2
X41-1-1-1-2 x GD	1069	35.3	5.3	8.8	267	8.7
X41-1-1-3-2 x GD	683	20.7	3.9	6.8	535	7.9
GD x X41-1-1-4-1	832	24.9	4.5	8.6	267	5.7
X41-2-1-2-3 x GD	564	23.0	6.1	12.7	238	6.4
X1-1-2-1-2 x X7-2-1-3-1	416	25.7	9.7	17.3	446	24.4
X7-2-1-4-2 x X1-1-2-1-1	653	41.0	9.2	13.2	386	17.9
X1-1-2-1-2 x X7-2-3-1-4	564	37.8	9.4	13.2	446	20.2
X7-2-3-3-5 x X1-1-2-1-3	446	33.7	10.0	16.6	505	25.8
X1-1-2-1-2 x X15-1-2-2-5	446	24.7	7.9	11.9	416	16.8
X15-1-2-2-9 x X1-1-2-1-3	653	31.4	7.3	11.8	653	26.7
X33-1-9-2-5 x X1-1-2-1-3	1040	51.4	8.0	11.4	267	12.3
X41-1-1-1-3 x X1-1-2-1-2	1247	44.9	5.6	9.2	327	7.3
X41-1-1-3-2 x X1-1-2-1-1	594	27.3	7.1	12.2	356	13.7
X41-1-1-4-1 x X1-1-2-1-2	921	39.0	5.8	9.3	386	6.4
X41-2-1-2-3 x X1-1-2-1-1	653	35.3	7.7	11.8	802	33.2
X1-1-2-1-3 x X41-2-1-4-4	861	35.4	6.2	15.2	416	12.4
$LSD_{0.05}$	345	15.9	1.8		321	11.9

[z]Culls included immature and virus symptomatic fruit.

Table 3. Field Notes and Infection Scores for Golden Delicious Derived Winter Squash Lines, Corvallis, Oregon, 2008

Pedigree	Habit	Fruit Color	Green Blossom Ends	AUDPC Scores[z]	Powdery Mildew[y]
Golden Delicious	vine	red orange	none to slight	148.3	2.3
GD x X7-2-1-3-1	semi-bush	red orange	none to slight	112.3	5.3
GD x X7-2-1-3-4	semi-bush	pale red orange	none to slight	106.3	6.0
GD x X7-2-1-4-2	vine/semi-vine	red orange	none to slight	16.0	5.7
GD x X7-2-3-1-2	semi-bush	red orange	slight	54.7	5.7
X15-1-2-2-3 x GD	vine	red orange	slight	63.7	3.0
GD x X15-1-2-2-6	vine/semi-vine	red orange	large	82.7	3.0
X33-1-9-2-1 x GD	vine/semi-vine	red orange	none to slight	91.0	3.3
GD x X33-1-9-2-5	vine	red orange	none to slight	117.0	3.0
X41-1-1-1-2 x GD	vine/semi-vine	red orange	none to slight	32.0	4.3
X41-1-1-3-2 x GD	vine	red orange	none to slight	10.7	5.0
GD x X41-1-1-4-1	vine	red orange	none to slight	99.3	4.0
X41-2-1-2-3 x GD	vine	red orange	slight	68.3	5.0
X1-1-2-1-2 x X7-2-1-3-1	bush/semi-bush	pink orange	none to slight	8.3	5.3
X7-2-1-4-2 x X1-1-2-1-1	bush	pink orange	large	6.0	5.7
X1-1-2-1-2 x X7-2-3-1-4	bush/semi-bush	red orange	large	1.1	6.7
X7-2-3-3-5 x X1-1-2-1-3	bush/semi-bush	red orange	large	0.0	6.0
X1-1-2-1-2 x X15-1-2-2-5	semi-bush	red orange	large	0.0	3.7
X15-1-2-2-9 x X1-1-2-1-3	semi-bush	red orange	large	0.0	3.0
X33-1-9-2-5 x X1-1-2-1-3	vine	red orange	large	0.0	2.0
X41-1-1-1-3 x X1-1-2-1-2	vine	red orange	none to slight	2.3	5.0
X41-1-1-3-2 x X1-1-2-1-1	vine	red orange	none to slight	0.0	5.7
X41-1-1-4-1 x X1-1-2-1-2	vine	red orange	slight	0.0	5.7
X41-2-1-2-3 x X1-1-2-1-1	vine	red orange	large	5.3	5.3
X1-1-2-1-3 x X41-2-1-4-4	vine/semi-vine	red orange	none to slight	0.0	2.7
$LSD_{0.05}$				42.9	3.0

[z]Area Under the Disease Progression Curve score calculated by visually rating the plots three times with reading taken two weeks apart. Original data taken on a 1-5 scale where the number is number of plants in the plot that showed visual virus symptoms in either leaves or fruit. Maximum possible AUDPC score is 160. [y]Scale of 1-9; 9 = severe.

Figure 1. 'Golden Delicious' (*C. maxima*) fruit from a field trial conducted at the Lewis Brown Farm in Corvallis, OR in 2008 showing symptoms of zucchini yellow mosaic virus.

Figure 2. *C. maxima* hybrids X1-1-2-1-2 x X7-2-3-1-4 (green x orange, left) and GD x X7-2-1-2 (orange x orange, right) from a ZYMV infected field trial in Corvallis, OR in 2008 showing differences in the size of the green blossom end of the fruit. Golden Delicious (not shown) has a faint green ring around the stylar scar.