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# BREEDING ARROWLEAF CLOVER FOR RESISTANCE TO BEAN YELLOW MOSAIC VIRUS

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## SUMMARY

Three cycles of evaluation and selection of arrowleaf clover (Trifolium vesiculosum Savi.) for resistance/tolerance to bean yellow mosaic virus (BYMV) have been conducted. 'Yuchi' arrowleaf is susceptible to BYMV with mortality ranging from 23 to 29 percent. Each cycle of selection improved tolerance to BYMV and plants approaching resistance have been identified in cycle 3. Mortality rate dropped to near zero after one cycle of selection. Germplasm superior to Yuchi in tolerance to BYMV has been identified.

## INTRODUCTION

Arrowleaf clover (Trifolium vesiculosum Savi.) is an important forage crop in East Texas and across the U.S. southern region. Bean yellow mosaic virus (BYMV) is one of several virus diseases that have the potential to severely reduce forage and seed yield of arrowleaf clover. In 1985, a breeding program was initiated at the Texas A&M University Agricultural Research and Extension Center at Overton with the objective to develop virus resistant clover germplasm. Multiple virus resistance was the eventual goal with BYMV resistance as the first component.

## PROCEDURE

### General culture and inoculation

Arrowleaf clover seed was pre-germinated on moist germination pads in petri dishes, then transferred to plastic trays with individual pots (5.5 in<sup>3</sup>) or cone-tainers (10 in<sup>3</sup>). Growth media was prepared as shown in Table 1. All studies were conducted in a greenhouse.

Alsike clover (T. hybridum L.), infected with BYMV strain 204-1, was obtained from O. W. Barnett, Clemson University, SC, and maintained as the inoculum source. Inoculum was prepared by grinding BYMV infected tissue (1 leaf/1 ml buffer) in sodium phosphate buffer (4.5 g diethyldithiocarbamic acid per liter 0.03 M Na<sub>2</sub>HPO<sub>4</sub>) with

mortar and pestle. Plants were mechanically inoculated by rubbing carborundum-dusted (600 mesh) leaves with a cotton swab saturated with inoculum. The youngest and second youngest fully expanded leaves were inoculated on consecutive days, respectively.

#### Germplasm and selection procedures

Three cycles of evaluation and selection have been completed to date. In cycle 1, 78 half-sib breeding lines of arrowleaf and the variety 'Yuchi' were inoculated with BYMV at 32 days of age (n=17 per line). At 73 days, the plants were rated from 1 to 4 (mild to severe) based on symptom severity. Mild symptoms included slight mosaic or chlorosis and/or little leaf distortion. Severe symptoms included plant death, severe mosaic and chlorosis, leaf distortion and stunting. Nineteen plants exhibiting the least severe symptoms were selected and 95 hand crosses made. Thirteen hundred and six F1's from cycle 1 crosses were evaluated in cycle 2 (1986). Plants were inoculated at 40 and 41 days of age. Seventy-five days after inoculation, notes were made on symptom severity and top growth was harvested for dry matter yield. Twenty-nine plants with the mildest symptoms were selected and 196 crosses made. BYMV infection of selections was confirmed using ELISA procedures by Dr. M. R. McLaughlin, USDA-ARS, Mississippi State, MS. Eleven hundred and eighty-three F1's from crosses in cycle 2 were evaluated in cycle 3 (1987). Plants were inoculated at 41 and 42 days of age and evaluated for symptom severity at 100 days.

#### RESULTS

The performance of the 78 half-sib lines (original population) in cycle 1 is shown in Table 2. Line ratings ranged from 2.6 to 4.4 for lines 1 and 29, respectively. Yuchi arrowleaf rated 3.8 with a mortality of 29 percent. The ratings improved in cycle 2 ranging from 1.8 to 3.1 for crosses 36 and 10, respectively (Table 3). Dry matter production ranged from 0.40 to 0.21 g DM/plant for crosses 26 and 11, respectively (Table 4). Yuchi produced 0.27 g DM/plant on 20 surviving plants.

Figure 1 gives a summary of three cycles of selection in arrowleaf clover for resistance/tolerance to BYMV. We are making

progress toward BYMV resistance using the selection procedures and germplasm described here. In cycle 3, fourteen plants have been identified with either no or very mild virus disease symptoms. Confirmation of BYMV infection of these selections is in progress.

TABLE 1. GREENHOUSE MEDIA FOR CLOVER

Component	Quantity	Notes
	---bu---	
Coarse peat	0.50	screen through 1.3 cm mesh
Coarse vermiculite	0.25	
Coarse perlite	0.25	
Coarse washed sand and fine gravel mix	<u>0.25</u>	not builders sand
Total	1.25	
Fertilizer <sup>†</sup>	---g---	
0-0-60	7.50	Peters soluble
9-45-15	14.75	Peters soluble
Micronutrient mix <sup>†</sup>		Peters soluble (STEM)
Dolomitic lime	125.00	pass a #20 sieve
Gypsum	125.00	pass a #20 sieve

<sup>†</sup> Maintenance fertilizer (69.5 g  $\text{KH}_2\text{PO}_4$ /20L of water; 100 ml/6 in pot) was applied at 60 day intervals.

<sup>†</sup> When placed in containers, the mix was watered to capacity with 1 tsp Peters soluble trace element mix per 20L of water.

TABLE 2. REACTION OF ARROWLEAF CLOVER LINES TO MECHANICAL INOCULATION WITH BYMV

Line <sup>s</sup>	Rating <sup>†</sup>	Mortality -----%
1	2.6 a <sup>†</sup>	6
24	2.7 a	0
64	2.8 a	12
21	3.2 a	12
71	3.5 ab	18
Yuchi (check)	3.8 bcd	29
3	3.8 bcd	6
4	3.8 bcd	12
17	3.8 bcd	18
36	4.1 cd	35
29	4.4 d	47

<sup>†</sup>Symptom severity rating: 1 = mild symptoms, 4 = severe symptoms, 5 = dead plant

<sup>†</sup>Means followed by the same letters are not significantly different according to Fisher's LSD,  $\underline{p} = 0.05$

<sup>s</sup>Five most tolerant and five most susceptible lines from a total of 78 inoculated. Seventeen plants tested per line.

TABLE 3. VIRUS DISEASE SYMPTOM SEVERITY OF ARROWLEAF CLOVER CROSSES  
INOCULATED WITH BYMV 204-1

Top 20%			Bottom 20%		
Cross	n	Score <sup>†</sup>	Cross	n	Score <sup>†</sup>
36	12	1.83	22	18	2.66
62	12	1.83	18	16	2.68
79	9	1.88	29	17	2.70
67	15	1.93	40	18	2.72
37	16	1.94	9	20	2.75
65	6	2.00	55	17	2.76
66	11	2.00	42	14	2.78
84	3	2.00	yuchi <sup>†</sup>	20	2.85
74	6	2.00	28	7	2.85
20	19	2.05	12	14	2.85
31	19	2.05	86	7	2.85
23	15	2.06	11	18	2.88
57	11	2.09	24	18	2.88
83	10	2.10	6	20	3.00
21	18	2.11	1	13	3.07
8	17	2.11	10	7	3.14

C.V. = 28.8%

LSD (0.05) = 0.626

<sup>†</sup> Score on scale of 1 to 4, with 4 exhibiting the most severe virus symptoms and 1 the least severe.

<sup>†</sup> Mean score of 20 surviving plants. Twenty six Yuchi plants were inoculated and six died (death rate = 23.1%).

TABLE 4. DRY MATTER PRODUCTION OF ARROWLEAF CLOVER CROSSES INOCULATED WITH BYMV 204-1

Top 20%			Bottom 20%		
Cross	n	Yield	Cross	n	Yield
		g DM plant <sup>-1</sup>			g DM plant <sup>-1</sup>
26	18	0.402	24	18	0.273
74	6	0.396	64	5	0.272
78	10	0.395	Yuchi <sup>†</sup>	20	0.269
83	10	0.391	22	18	0.266
31	19	0.384	18	16	0.265
3	6	0.383	47	17	0.262
34	20	0.382	13	9	0.258
65	6	0.381	56	18	0.258
66	11	0.374	40	18	0.255
62	12	0.373	53	13	0.255
67	15	0.364	55	17	0.244
8	17	0.363	12	14	0.228
43	20	0.359	10	7	0.215
44	12	0.356	6	20	0.211
21	18	0.355	85	4	0.210
27	18	0.352	11	18	0.208
C.V. = 35.8			LSD (0.05) = 0.092		

<sup>†</sup>This is the mean of 20 surviving plants. Twenty-six Yuchi plants were inoculated and six died (death rate = 23.1%). Mean DM yield including dead plants is 0.207 for Yuchi. Uninoculated Yuchi produced 0.802 g DM plant<sup>-1</sup> (n=10).

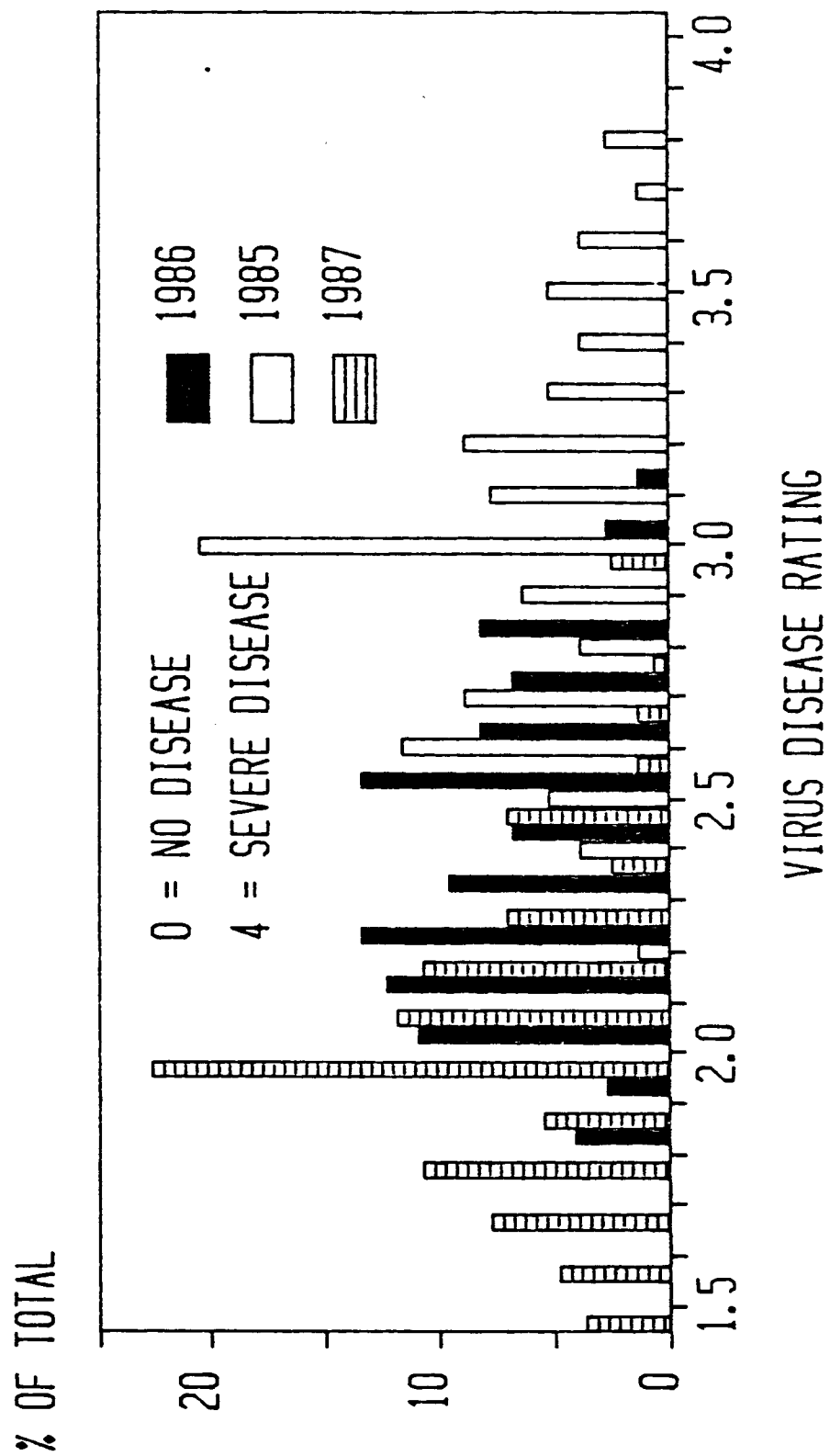


FIG. 1. Virus disease rating of three arrowleaf clover populations infected with BYMV 204-1