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COMPARISON OF POWDERY MILDEW (Erysiphe cichoracearum) INOCULATION METHODS IN Cucurbita moschata[1]

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ABSTRACT

Powdery mildew (Erysiphe cichoracearum) limits pumpkin (Cucurbita moschata) production in Puerto Rico and other areas of the Caribbean. In order to effectively use half-sib family recurrent selection to develop polygenic mildew resistance we tested various inoculation methods to determine which method can best be used to quantify small differences in resistance. Dusting leaves using infected plant tissue was a fast and effective method that would be useful in a screening program where many genotypes need t4o be evaluated but where resistance need not be quantified. Spraying with a 1 x 10^6 spores/cc triton suspension was an efficient method for a recurrent selection program where small differences in resistance among families must be determined.

INTRODUCTION

Cucurbita moschata is a pumpkin-like vegetable consumed in Puerto Rico and throughout the Caribbean. Because pumpkin is a traditional rather than export crop little research has been carried out on its breeding and genetics. Its large size (vines up to 15 m in length) makes it a difficult breeding subject because population size must be severely restricted in the field. Disease resistance evaluation of greenhouse seedlings allows for a much larger number of genotypes to be sampled than would be possible in the field where a one hectare nursery only accomodates about 100 plants.

Powdery mildew (Erysiphe cichoracearum) often limits pumpkin production in Puerto Rico and in other areas both tropical and temperate. Winter weather conditions in Puerto Rico are favorable for powdery mildew development. Use of single gene resistance has been the main approach in programs breeding for powdery mildew resistance (Adeniji and Coyne, 1983; Rhodes, 1964). This approach can result in very high levels of resistance but of a type that can breakdown in the presence of virulent races. An alternate approach would be to use recurrent selection to accumulate into one population those alleles that contribute to polygenic

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resistance and increase their frequency in that population. In order to effectively use recurrent selection for developing polygenic resistance, an effective method of quantifying that resistance needs to be developed.

Our objective was to compare several powdery mildew inoculation methods for their usefulness in quantifying resistance of C. moschata families in a half-sib recurrent selection program as well as for rapid screening of many genotypes.

MATERIALS AND METHODS

Seven inoculation methods and three controls were applied to each of two susceptible pumpkin varieties (Ponca and PR0009) planted in pots in the greenhouse in a randomized complete block design with five replicates. The second true leaf was inoculated when fully extended (2 to 3 weeks after germination). Inoculation methods included (1) dusting leaf with infected tissue, (2) applying egg white adherent followed by dusting, (3) applying Triton 100 followed by dusting, (4) attaching piece of infected tissue to leaf, (5) attaching piece of infected tissue to leaf using egg white as an adherent, (6) attaching piece of infected tissue of leaf using Triton 100, and (7) spraying with a 1 x 10^4 spores/cc Triton spore suspension. Controls were inoculating with egg white, inoculating with Triton 100, and uninoculated. The experiment was repeated two times. A second experiment was conducted using only Ponca and treatments 1, 3, 4, 6, and 7 (see above) and an uninoculated control. Treatment 7 (Triton spore suspension) was modified from experiment 1 by increasing the spore concentration to 1 x 10^6 spores/cc. In both experiments number of lesions were counted after 10 days (evaluation method 1). Two other evaluation scales were also used: a 0 to 5 scale where 0 = no damage, 1 = 0-2 lesions, 2 = 3-5 lesions, 3 = 6-7 lesions, 4 = 8-9 lesions, and 5 = more than 9 lesions (evaluation method 2). A second 0 to 5 scale (evaluation method 3) was as follows: 0 = no damage, 1 = chlorosis, 2 = chlorosis and sporulation on lower leaf surface, 3 = chlorosis and sporulation on both leaf surfaces, 4 = necrotic spots, and 5 = complete necrosis.

RESULTS AND DISCUSSION

Means presented in tables 1 and 2 are means over genotypes since there was no inoculation method x genotype interaction. Single degree of freedom comparisons ($p=0.05$) found no difference in number of lesions resulting from inoculation by dusting host with infected tissue vs. attaching infected tissue to host. Using no adherent resulted in as many lesions as using Triton. Egg white as an adherent resulted in fewer lesions than using either Triton or no adherent. In the first set of experiments spraying with a Triton spore suspension (1 x 10^4 spores/cc) was not an effective inoculation method. In the 2nd experiment a 1 x 10^6

Table 1. Mean score produced by seven inoculation methods (1-3 refers to evaluation methods 1-3 as defined in materials and methods) (Experiment 1)

Method	1	2	3
1. Dusting with infected tissue	10.9	3.8	2.3
2. Same as 1 with egg white as adherent	7.7	2.3	2.1
3. Same as 1 with triton adherent	9.0	3.4	2.2
4. Attaching piece of tissue	10.3	4.3	2.9
5. Attaching piece of tissue with egg white	4.6	2.0	2.1
6. Attaching piece of tissue with triton	10.2	3.7	2.7
7. Triton suspension (1×10^4)	5.1	0.6	1.1

Table 2. Mean score produced by six inoculation methods (1-3 refers to evaluation methods 1-3 as defined in materials and methods) (Experiment 2)

Method	1	2	3
1. Dusting with infected tissue	4.7	1.9	1.8
3. Same as 1 with triton adherent	4.5	1.7	3.2
4. Attaching piece of tissue	6.6	3.4	3.6
6. Attaching piece of tissue with triton	6.6	3.4	4.9
7. Triton suspension (1×10^6)	6.8	2.9	2.6
10. Uninoculated	0.0	0.0	0.0

spores/cc suspension was used and spores were collected by agitating pieces of infected tissue in Triton rather than scraping them off tissue. This resulted in good production of lesions and is a method that can be more easily quantified than dusting spores or attaching pieces of infected tissue.

All evaluation methods gave similar results. Coefficients of variation were lowest for evaluation method 3 but differences between genotypes were greatest using number of lesions (evaluation method 1) (data not shown).

For a recurrent selection program where many families need to be quantitatively evaluated for disease reaction, application of a spore suspension would be the preferred method. Dusting spores onto leaves could be considered as a faster alternative method, although spore concentration cannot be controlled. This latter method is useful for rapid screening where a quantitative measure of resistance is not needed.

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