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Downy Mildew Disease Resistance in F1 of C30-5-1 × 'Chardonnay'

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Downy mildew, caused by the fungal pathogen [*Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni], is one of the most damaging diseases worldwide in grapes (*Vitis* L.). It not only adversely affects grapevine ecological characteristics but also lowers the fruit yield, berry quality, wine-making property, and consumer consumption appearance. This study evaluated downy mildew disease resistance in 182 F1 plants of a cross combination C30-5-1 × 'Chardonnay' based on a scale of 0–5 with 0 score: clear leaves, no sign of any kind of disease symptoms; 1: few single spores on a single leaf; 2: more than 10 single separated incidents of spores on a single leaf; 3: clustered incidents of spores covered more than 50% of a single leaf area; 4: incidents of spores covered more than 75% of a single leaf area; 5: over 75% of a single leaf area was covered by spores. Both parents were used as control. The results indicated that downy mildew resistance varied in F1 offsprings of C30-5-1 × 'Chardonnay', which gives us hope for selection of downy mildew resistant grape cultivars or germplasm in our disease resistant grape breeding program.

Downy mildew (DM) is a destructive grapevine disease worldwide (Gessler et al., 2011). It is caused by the fungal pathogen *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni. The pathogen *P. viticola* is an obligate biotroph and thus requires the genus *Vitis* to remain alive in order to complete its life cycle (Burruano, 2000; Rumbou and Gessler, 2004). *Plasmopara viticola* attacks most species of wild and cultivated grapes (Boso and Kassemeyer, 2008; Brown et al., 1999). Primary infection of grapevines begins with the overwintering oospore of *P. viticola* on infected leaves or plant litter in the soil that germinates in the spring and produces a sporangium. When plant parts are covered with a film of moisture from rain or irrigation, the sporangium releases small swimming spores (zoospores) that are then spread by splashing water. The spores can germinate by producing a germ tube that enters the green tissue, including leaves, inflorescences, bunches, and young berries, through the stomates (Rossi and Cafti, 2007). Secondary infection, which is the major source of disease spread, produces spores that may be mobilized by wind and rain to establish new infection sites (Gobbin et al., 2005; Madden et al., 1994). The infection ends with the sexual production of overwintering oospores (Kortekamp, 2005). When inoculating *P. viticola* onto the abaxial surface of a susceptible grape leaf, zoospores germinate to form a germ tube that grows through a stoma. Then hyphae develop with some branches and numerous haustoria, which could penetrate cell walls of the mesophyll (Dai et al., 1995). At last, sporangiophores emerge through the stomata where they expand into tree-shaped structures carrying the sporangia (Keifer et al., 2002). In resistant species, the infection progress is slowed down, inhibited, or completely stopped (Wan et al., 2007; Wu et al., 2010).

Symptoms of DM are usually first noticed on leaves as yellowish and later oily lesions on the leaf's upper surface with a "downy" mass observed on the corresponding underside of the leaf (Fisher et al., 2007). It can also cause deformation of shoots, tendrils, inflorescences, and clusters of young berries, which can easily cause 50% to 75% crop losses in one season if no control measures are taken (Gessler et al., 2010). It is even more so in areas like Florida with warm and humid climate conditions during the growing season, which facilitate the development of DM diseases and cause disastrous economic and environmental consequences (Wu et al., 2010).

Many measures have been taken to avoid DM damage (Chen et al., 2007; Reuveni et al., 2001). Since the first achieved protection with copper-based fungicides (Bordeaux mixture), the control of DM is accomplished with specific compounds such as azoxystrobine, trifloxystrobin, pyraclostrobin, mefenoxam, fluopicolide, and mancozeb (Lebeda et al., 2008). Control of DM is largely dependent on the use of preventive fungicides each season. The grapevine DM disease can only be effectively controlled by properly timed and effective fungicide application, and control programs usually focus on controlling primary infections in the pre-bloom and early post-bloom periods and on limiting secondary spread during the summer. This is the most daunting measure, which frustrates those who wish to farm in a more sustainable, if not organic, manner (Doazan, 1980). In organic viticulture, grapevine protection strongly depends on copper, which is known as one of the trace elements with the most deleterious effects on living organisms in soil (Renella et al., 2002). Moreover, management of *P. viticola* by fungicides is tenuous, because the pathogen has repeatedly overcome a broad range of previously effective fungicides (Gisi et al., 2007).

The constant search for ecologically harmless approaches to reduce the disease impact is part of the preoccupation of the industry worldwide (Hvarleva et al., 2009; Venuti et al., 2013). Developing resistant grape cultivars to the disease appears to be the most effective alternative to address those concerns (Hoffmann et al., 2007). Screening of grape germplasm is one of the

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primary steps of that process that have been done in many other programs (Staudt and Kassemeyer, 1995). Over the years, many valuable grapevine varieties have been developed by combining the good quality of European cultivars (*Vitis vinifera*) with the fungal resistance from American and Chinese wild *Vitis* species (Fisher et al., 2007; Wu et al., 2010). These varieties can be treated with reduced levels of fungicides, and therefore lead to a more ecologically friendly and cost-efficient viticulture (Batovska et al., 2009). The breeding program of Florida Agricultural and Mechanical University is part of this ambition. With the germplasm collection of the experimental vineyard there are very interesting opportunities to develop new DM resistant cultivars, thus allowing alleviation of the burden related to chemical use.

Unlike several hybrid bunch grape cultivars, such as 'Blanc du Bois', 'Suwannee', 'Stover', 'Miss Blanc', and 'Conquistador', and European grape *V. vinifera*, which is generally more susceptible to *P. viticola* than native American species (Sprague, 1980), the hybrid C30-5-1 is very resistant to DM and has very good wine-making quality but also vigorously growing habit. During the process of improving the vegetative growth habit, we have made a cross between C30-5-1 and 'Chardonnay'. Field evaluation of more than 180 F1 hybrid seedlings reviewed that diversified variation in their agricultural characteristics. The objectives of this research are to screen their DM disease resistance and to identify promising grape germplasm for use as parents in our grape cultivar improvement and breeding program.

Material and Methods

Two to three years of 182 F1 hybrid seedlings from cross combination C30-5-1 × 'Chardonnay' were used for DM evaluation based on a scale of 0–5 with 0 score: clear leaves, no sign of any kind of disease symptoms; 1: few single spores on a single leaf; 2: more than 10 single separated incidents of spores on a single leaf; 3: clustered incident of spores covered more than 50% of a single leaf area; 4: incidents of spores covered more than 75% of a single leaf area; and 5: over 75% of a single leaf area was covered by spores. Both parents were used as control. They were grown at the vineyard of the Center for Viticulture and Small Fruit Research, Florida A&M University, Tallahassee.

The evaluation took place in the last week of June and Sept. 2012. Five leaves were randomly picked up from each F1 hybrid seedling and the numbers of leaves infected with the *P. viticola* were individually recorded. Identification of the DM disease was visually observed. Percentage of leaves infected with *P. viticola* was calculated based on the total leaves observed.

Results and Discussion

Genotypic analysis indicated that the 182 F1 hybrids were a diversified population (Fig. 1). They were different from either of their parents: C30-5-1 or 'Chardonnay'. Of the 182 F1 hybrid seedling field survey for downy mildew in June 2012, all of them showed DM symptoms ranging from 1 to 2 (Fig. 2). No single seedling was immunized to the fungal pathogen *P. viticola*; 53.3% of the F1 seedlings scored 2 on a scale of 5 and 44.51% scored 1, which was relatively low in score and counted as resistant to DM. The rest were dead seedlings. Apparently, the DM disease resistance in C30-5-1 did have some degree of inheritance to its offspring.

Occurrence of DM disease in F1 hybrids in September varied from 0 to 5 (Fig. 3). Most of them (30.22%) showed few spores of fungal pathogen *P. viticola* in a single leaf (score 1), and 9.22% seedlings had no symptoms. Apparently, the 9.22% seedlings

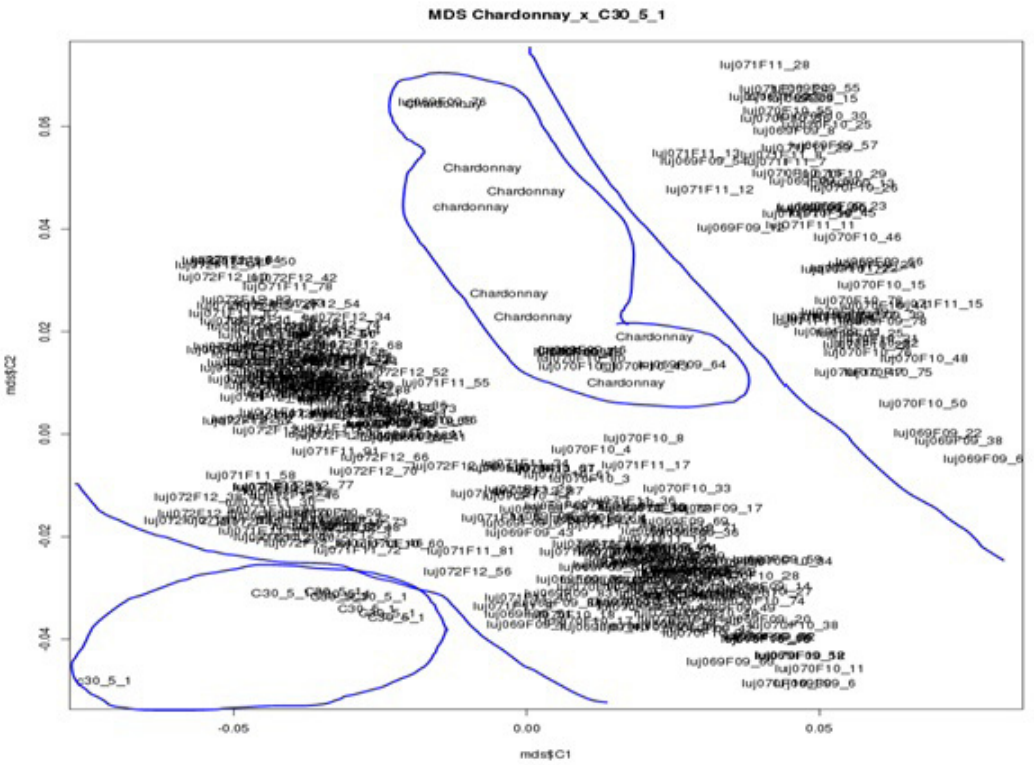

Fig. 1. Genotypic analysis of F1 hybrids: C30-5-1 × 'Chardonnay'.

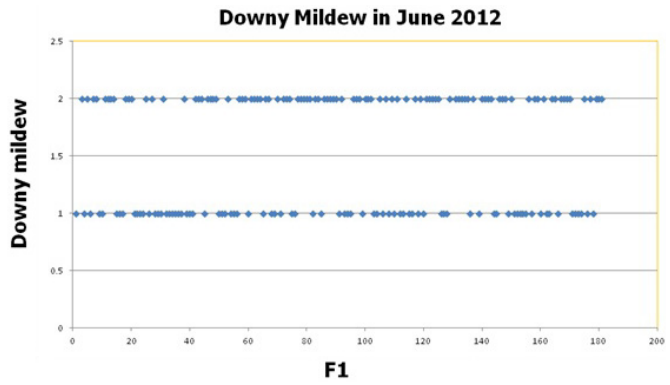

Fig. 2. Field survey of F1 downy mildew resistance in June 2012.

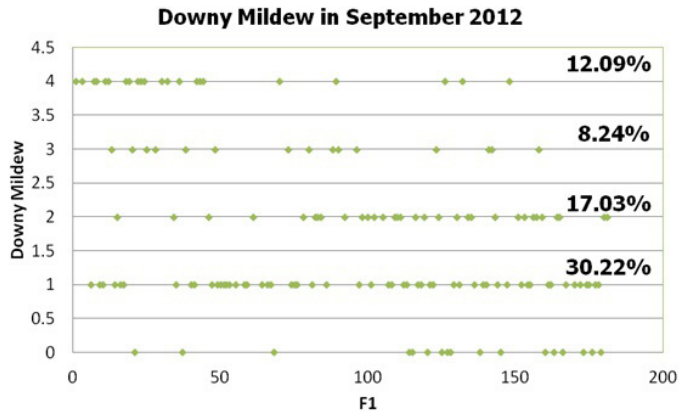

Fig. 3. Field survey of F1 downy mildew resistance in Sept. 2012.

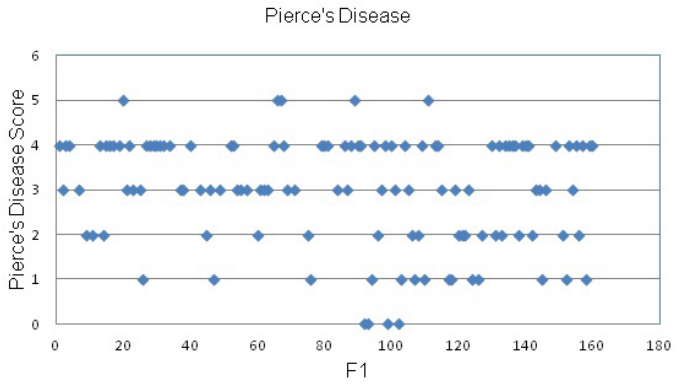

Fig. 4. Relative resistance of F1 to Pierce's disease in June 2012.

with no symptoms were the new growth, with old leaves fallen off the vines or dead because of Pierce's disease or anthracnose (Figs. 4 and 5). The occurrence of Pierce's disease or anthracnose diseases certainly complicated the survey of DM incidence in the late growing season. Their value in our DM breeding program needs further study.

In addition, 12.09% F1 hybrid seedlings scored 4 and 8.24% scored 3 on the DM September field survey, which was considered

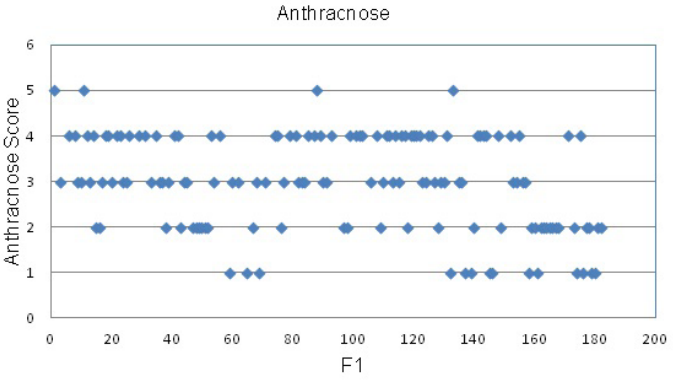

Fig. 5. Relative resistance of F1 to anthracnose in June 2012.

highly susceptible or moderately susceptible to fungal pathogen *P. viticola*. These F1 hybrid seedlings have no practical value in our disease breeding program.

Evaluation of DM disease in the field is a very complex process. Very often, more than one disease attacks the grapevines, especially during the late growing season. Once the first pathogen attacks, the other pathogen diseases easily invade the weakened vines. To further our study, or have more conclusive evidence for DM resistant inheritance in F1 hybrids of C30-5-1 × 'Chardonnay', further laboratory research in sophisticated inoculation, fluorescence microscopic, and electronic microscopic scanning analysis is needed in the future study.

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