

Field Evaluation of Sugarcane Orange Rust for First Clonal Stage of the CP Cultivar Development Program

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Abstract: Consistent development of high-yielding sugarcane (a complex hybrid of *Saccharum* spp.) cultivars with resistance or tolerance to biotic and abiotic stresses is critical to commercial sugarcane production. Currently, orange rust (caused by *Puccinia kuehnii* E.J. Butler) is a big challenge for the sugarcane production in Florida, USA. A better understanding of sugarcane genotypic variability in response to orange rust disease will help optimize breeding and selection strategies for disease resistance. Orange rust ratings, scaled from non-infection (0) to severe infection (4) with intervals of 0.5, were recorded from genotypes at the first clonal selection stage (Stage I) of the Canal Point sugarcane breeding and cultivar development program in Florida. Data were collected from all 14,272 and 12,661 genotypes and four replicated reference cultivars, CP 78-1628, CP 80-1743, CP 88-1762 and CP 89-2143, in July-August 2012 and 2013, respectively. Mean rust rating, % of rust infection and rust severity in each family (i.e., progeny of the cross from a female and male) and female parent and their Coefficients of Variation (CV) within and among families (females) were estimated. Results indicated that considerable variation exists in rust tolerance among families or females. The families or females for their progenies with the high susceptibility or resistance to orange rust were identified and ranked. The findings of this study are useful for evaluating sugarcane crosses and parents for rust disease and can help breeders use desirable parents for crossing and improve genotypic resistance to orange rust in the sugarcane breeding programs.

Keywords: Sugarcane, Canal Point (CP) Sugarcane Breeding and Cultivar Development Program, Orange Rust, Genotype/Parental Evaluation for Rust Resistance

Introduction

Sugarcane (a complex hybrid of *Saccharum* spp.) is an important industrial crop in Florida, USA with an annual economic impact of more than \$677 million (USDA-NASS, 2014). Consistent and continuous development of high-yielding sugarcane cultivars with resistance or tolerance to biotic and abiotic stresses is critical for commercial sugarcane production in South Florida (Zhao *et al.*, 2010). The USDA-ARS Sugarcane Field Station at Canal Point (26.52° N; 80.36° W), Florida was initially established at its present site in 1920 to conduct sugarcane breeding and selection for Louisiana to make crosses and produce true sugarcane seed for the sugarcane industry. Since the 1960s, the Canal Point station has been developing sugarcane cultivars with CP prefixes for Florida under a three-party cooperative

agreement among the USDA-ARS, the University of Florida and the Florida Sugar Cane League, Inc. Also, the Canal Point station still makes crosses for the USDA-ARS in Houma, Louisiana. The CP cultivars now account for more than 90% of the hectareage in Florida up from 14% in 1970. In 2012, the top six major sugarcane cultivars grown in Florida were 'CP 89-2143' (Glaz *et al.*, 2000), 'CP 88-1762' (Tai *et al.*, 1997), 'CP 00-1101' (Gilbert *et al.*, 2008), 'CP 96-1252' (Edme and Tai *et al.*, 2005), 'CL 88-4730' (a cultivar of the United States Sugar Corporation) and 'CP 78-1628' (Tai *et al.*, 1991) and their percent hectares were 20.7, 19.2, 10.2, 8.6, 7.4 and 7.3%, respectively (Rice *et al.*, 2013).

The Canal Point sugarcane breeding and cultivar development program (CP program) consists of six stages, namely Crossing, Seedlings and Stages I, II, III and IV (Zhao *et al.*, 2012). It takes at least eight years to

release a cultivar from the time a cross is made (Tai and Miller, 1989). Cane yield (TCH, Mg ha⁻¹), commercial recoverable sucrose (CRS, kg sucrose Mg⁻¹ cane) and sucrose yield (TSH, Mg ha⁻¹) with disease resistance are the major agronomic traits considered in advancing sugarcane clones during the selection stages. Edme and Miller *et al.* (2005) reported that CRS, TCH and TSH of Florida commercial sugarcane cultivars linearly increased by 26.0, 15.5 and 47.0%, respectively, from 1968 to 2000. Underscoring the critical need for cultivar development for the Florida sugarcane industry, about 69% of the sugar yield gain in Florida came from genetic improvement attributable to the CP program (Edme and Miller *et al.*, 2005), indicating importance of the CP program in sugarcane production in Florida.

Sugarcane orange rust, caused by *P. kuehnii* (W. Krüger) E.J. Butler, resulted in considerable economic loss in Australia (Braithwaite *et al.*, 2009). This disease was recorded in Florida for the first time in 2007 (Comstock *et al.*, 2008). Sugarcane orange rust has caused significant yield losses in Florida because most widely planted commercial cultivars in the region are susceptible to this disease (Raid *et al.*, 2013). Orange rust has also been found in sugarcane growing in the Central American countries of Guatemala (Ovalle *et al.*, 2008), Costa Rica (Chavarría *et al.*, 2009), Nicaragua (Chavarría *et al.*, 2009), Mexico (Flores *et al.*, 2009), El Salvador (Flores *et al.*, 2009), Panama (Flores *et al.*, 2009) and more recently in Brazil (Barbasso *et al.*, 2010) and in Louisiana, USA (Grisham *et al.*, 2013). Zhao *et al.* (2011) determined physiological mechanisms of the orange rust-induced reductions in sugarcane growth and yield by quantifying effects of this disease on leaf relative chlorophyll level, photosynthetic rate, dark respiration rate, photosynthetic radiation use efficiency, carbon use efficiency and the relationships between these leaf photosynthetic components and rust disease ratings.

Currently, leaf orange rust and brown rust (caused by *Puccinia melanocephala* H. & P. Sydow) diseases are great challenges for sugarcane production in Florida. Most dominant commercial cultivars in Florida are susceptible to one or both rusts. Growers use fungicides to control the negative effects of rusts on yields, but the cost of three split applications of fungicides (at a hectare level) during a growing season is equivalent to 3 tons of cane yield lost per hectare. The frequent applications of fungicides and low net profits preclude their use for controlling sugarcane brown rust (Jiang, 1985) and orange rust (Staier *et al.*, 2003), making cultivar resistance and cultural practices are the most viable alternatives. Therefore, development of rust resistant varieties is the first priority for sustaining sugarcane production in Florida. Scientists in the CP program and in industry are using multiple approaches to develop new cultivars with rust resistance and high yields. Cultivars developed in the CP program not only are used in the United States, but also many Central American sugarcane industries use them for either

breeding or commercial production (Machado, 2013). Therefore, evaluation and screening of genotypes for resistance to rusts in the CP program are critical for sustainable sugarcane production in USA and other countries. Although Sood *et al.* (2009) developed a whorl inoculation method to more accurately and efficiently test sugarcane genotype resistance in brown rust (Sood *et al.*, 2009) and orange rust (Zhao *et al.*, 2011), it is still difficult to use the artificial inoculation test in Stage I of the CP program because of a large number (12,000-15,000) of genotypes in this stage due to limited resources (Zhao *et al.*, 2012). Therefore, natural infection has been the primary means of assessing rust resistance in the Stage-I sugarcane in the CP program.

The selection of which sugarcane clones to be used as crossing parents is a crucial decision for breeders. Knowledge and better understanding of variability in rust infection and severity among genotypes may provide useful information for genotype advancement and for efficient use of parents in future crossing efforts for developing orange rust resistance. Thus, a study was conducted in the Stage I fields of the CP program at the USDA-ARS Sugarcane Field Station, Canal Point, Florida. Objectives of this study were to determine variability in orange rust ratings among crosses based on data collected from the Stage I clones of the CP program in 2012 and 2013 and to use the information for consideration of parental selection and cross appraisal. Overall, orange rust in the 2012 and 2013 sugarcane growing seasons in south Florida were the most severe in the last five years due to favorable environment conditions for rust development. The data of orange ratings in Stage I may be useful for achieving the specific breeding objectives for rust resistance.

Materials and Methods

Plant Culture

Individual stalks of 14,272 genotypes in 2012 and 12,661 genotypes in 2013 were visually selected from fields of the 2011 and 2012 Seedling stages and planted in single-row plots in the Stage I fields of the CP program in January of 2012 and 2013, respectively. All the visually selected genotypes in the Seedling stages did not show clear orange rust disease symptoms due to late transplanting (Mid May) in the CP program. To facilitate stalk transport and planting, two to five stalks (each stalk came from a true seed plant) in the Seedling fields were bundled and labeled by family (i.e., cross) prior to advancing them to Stage I (Zhao *et al.*, 2012). These bundles were randomly distributed in the Stage I fields. One stalk was placed in each plot and cut into two sections (each approximately 0.9 m long). The two sections were placed in the center of the plot as double pieces of seedcane. The plot length was 2.4 m, with 1.5-m between-row spacing. There was a 1.5-m gap between adjacent clones within a row to allow scientists to

recognize individual clones during disease rating, growth vigor evaluation and selection and to easily distinguish individuals at selection time. Four commercial cultivars, CP 78-1628, CP 80-1743 (Deren *et al.*, 1991), CP 88-1762 and CP 89-2143, were used as checks each year and one check plot was randomly planted in approximately every 100 plots. There were approximately 40 replicated plots for each check cultivar each year. There was a 4.5 m alley every eight rows to facilitate field maintenance and genotype selection.

Evaluation of Orange Rust

During the grand growth (July-August) in 2012 and 2013, orange rust ratings were recorded in all plots of Stage I under natural infection using a scale from 0 (no rust infection) to 4 (most severe rust infection) with intervals of 0.5. The 0 to 4 scale levels were defined as: 0 = no rust, 1 = one to a few pustules, 2 = patching presented, 3 = patching widespread up into the upper canopy with some lower leaf death and 4 = massive amounts of rust pustules with heavy lower leaf death. In general, the plants with rust ratings of 0 to 1 were considered resistant or tolerant, with rating of 2 were considered moderately susceptible and with ratings of 3 and 4 were considered susceptible and highly susceptible.

Additionally, all clones were visually evaluated for other diseases [brown rust, leaf scald (*Xanthomonas albilineans*), smut (*Sporisorium scitamineum*) and mosaic] and plant vigor and agronomic traits (Zhao *et al.*, 2012) at the same time and in early September. A subjective plant vigor rating was determined for individual clones using a scale from 1 (worst) to 9 (best). All clones with a vigor rating ≥ 6 or better than that of check cultivars, acceptable rust resistance (rust rating ≤ 1) and no other disease symptoms were further assessed for Brix (an indicator of sucrose content) in early November. Approximately 1,500 best clones with the largest vigor \times Brix products and proper disease resistance were advanced to Stage II. Recently, Zhao *et al.* (2012) reported the details of vigor rating, Brix and the Stage-I selection strategies in the CP program. Therefore, we mainly focused on orange rust on the basis of families and female parents in this study. Three variables of mean orange rust rating, percent of rust infection and rust severity were used to determine variation of rust diseases among families or females based on their progenies. These variables were defined and estimated using the following formulas:

$$\text{Mean orange rust rating} = \frac{\sum (\text{rust rating})}{\text{total number of clones (including clones with 0 rating)}} \quad (1)$$

$$\text{Percent of infection} = \frac{(\text{the number of clones infected})}{\text{total number of clones}} \times 100 \quad (2)$$

$$\text{Severity} = \frac{\sum (\text{rust rating})}{\text{the number of clones infected}} \quad (3)$$

Data Analysis

For the four check cultivars, replicated plots were completely randomized in the Stage-I field both years. The MIXED procedure of SAS (SAS Institute Inc., Cary, NC) was used to test differences of cultivar, year and their interaction for the mean rating of orange rust and for rust severity. If the hypothesis of equal means between the check cultivars was rejected by the F test, the trait means were separated with the LSD at $P = 0.05$. The LSD values were calculated with the SE values generated by the Diff option in the SAS MIXED procedure.

For the Stage I clones, their parental combinations in the Crossing stage varied annually. Thus, the Stage I data were analyzed separately for each year. Orange rust distributions were determined by pooling data across all clones within a year. Data of orange rust were analyzed for each family and female parent. Data of male parents were not analyzed because many of the progeny in the 2012 and 2013 Stage I were developed from Poly crosses (i.e., where a female tassel received pollen from several different male tassels and their male parents could be any one of these males). Thus, the specific males were unknown for all the Poly crosses. For families and female parents that had ≥ 15 progeny clones planted in Stage I, the mean ratings and Coefficients of Variation (CVs) were determined for orange rust to assess variability. The means and CVs were calculated using PROC MEANS of SAS. Then, the CVs of among-families (females) were obtained based on their means and the CVs of within-families (-females) were estimated by averaged CVs of individual families (females). Coefficients of variation for the rust in each family were calculated from the individual clonal values of rust rating from all clones within a family or female according to Zhao *et al.* (2012). The within-family or within-female CV for each parameter was estimated by calculating the overall mean CV of all individual family (female) CVs for that trait. The among-family CVs were estimated using the mean (rather than individual clone) values of each family (female). For example, to calculate the among-family CV of 20 families for orange rust, we would have calculated the CV based on the standard deviation and overall mean from the 20 mean rust rating values of each of the 20 families. The variability among- and within-families was described using respective CVs. The top 20 families that were most susceptible or most tolerant to orange rust in each year were further determined based on ranking their mean rust ratings. The top 20 females in which their progenies were most susceptible or most tolerant to the rust in each year were also determined based on mean ratings of orange rust.

Results

Orange Rusts of Check Cultivars

There were significant differences among check cultivars and between years in mean ratings of orange

rust and the cultivar × year interactions were also highly significant (Table 1). The mean rating of orange rust in 2012 was lower than that in 2013. Averaged across the four cultivars, mean ratings of orange rust in 2012 and 2013 were 1.0 and 2.0, respectively. CP 78-1628 had significantly lower mean rating of orange rust than other three cultivars in both years.

The differences in % infection of orange rust among cultivars or between years were similar to those in the mean rust ratings. On the other hand, the differences in severity were relatively less among cultivars or between years compared with % infection (Table 1). Orange rust severity differed significantly among cultivars, but did not differ between years. There was no interaction of cultivar × year on orange rust severity (Table 1). Averaged across years, the orange rust severity values of CP 78-1628, CP 80-1743, CP 88-1762 and CP 89-2143 were 1.8, 2.3, 2.2 and 2.1, respectively.

Distributions of Orange Rust Ratings

Numbers of clones used for orange rust ratings in Stage I of the CP program were 14,272 in 2012 and 12,661 in 2013 (Fig. 1). These did not include 158 and 166 replicated check plots in 2012 and 2013,

respectively. The % infections of orange rust in 2012 and 2013 were 18.3 and 50.2%, respectively. The distributions of orange rust ratings based on the number of clones at each rating level are given in Fig. 1. Although the peak frequency of the rating distribution for orange rust was at 2 and the overall severity value was also approximately 2 in both years, the peak value of orange rust in 2012 was smaller than that in 2013 (Fig. 1). The CVs of orange rust ratings across the infected clones in 2012 (2,607) and 2013 (6,356) were 19.8 and 28.8%, respectively. Additionally, the % infection and mean rating of orange rust in 2013 were much greater than in 2012. The differences in severity between years were small (Fig. 1).

Variability in Clonal Numbers and Rust Ratings among Families

Total crosses (families) advanced to Stage I from the Seedling stage by individual selection were 576 and 455, respectively, in 2012 and 2013. Clone numbers among families ranged from 1 to 214 with a mean of 25 in 2012 and from 1 to 209 with a mean of 28 in 2013 and their CVs in two years were 113 and 100%, respectively (Table 2).

Table 1. Mean rating, percent of infections and severity of orange rust of four check cultivars tested with sugarcane clones in the Stage I field of the Canal Point (CP) sugarcane cultivar development program in 2012 and 2013

Cultivar	Clone #	Mean rating	% infection	Severity
2012				
CP 78-1628	40	0.13 b	7.5	1.67
CP 80-1743	40	1.46 a	65.0	2.25
CP 88-1762	40	1.24 a	57.5	2.15
CP 89-2143	38	1.18 a	55.3	2.14
Mean	40	1.00	46.3	2.05
2013				
CP 78-1628	40	1.69 b	87.5	1.93
CP 80-1743	40	2.10 a	97.5	2.31
CP 88-1762	43	2.02 a	93.0	2.18
CP 89-2143	43	2.06 a	97.7	2.11
Mean	42	1.97	93.9	2.13
Effect	DF		<i>P>F</i>	
Cultivar	3	<0.0001	---	0.0001
Year	1	<0.0001	---	0.2451
C×Y	3	0.0043	---	0.4625

Table 2. Maximum (Max), Minimum (Min), Mean, Standard Deviation (SD) and Coefficient of Variation (CV) for clone number and mean ratings of Orange Rust (OR) of 576 (in 2012) and 455 (in 2013) sugarcane families with a total of 14,272 and 12,661 clones, respectively, tested in the 2012 and 2013 Stage I fields in the Canal Point (CP) sugarcane cultivar development program

Parameter	2012		2013	
	Clone (No. family ⁻¹)	OR rating	Clone (No. family ⁻¹)	OR rating
Max	214	2.50	209	2.50
Min	1	0.00	1	0.00
Mean	25	0.46	28	0.96
SD	28	0.49	28	0.51
CV (% among families)	113	104.90	100	52.70
CV (% within families)	---	184.20	---	119.10

Table 3. Number of total sugarcane clones planted, mean Orange Rust (OR) rating, % of infection and severity and their parents for the 20 families with the highest mean OR ratings using 295 and 263 families with ≥ 15 clones in Stage I of the Canal Point (CP) sugarcane cultivar development program in 2012 and 2013, respectively

2012						2013							
Family	No. clone	OR rating	% infect	Severity	Parent		Family	No. clone	OR rating	% infect	Severity	Parent	
					Female	Male						Female	Male
X4011A	26	2.23	88.5	2.52	CP00-1101	Ho05-961	X08-0283	37	2.11	86.5	2.44	CP04-1250	CPCL97-0393
X09-1136	75	1.54	16.0	2.31	CP94-2203	Mix09G	X10-0176	16	2.09	100.0	2.09	CP06-2897	CL88-2747
X09-0109	37	1.51	24.3	2.33	CP04-1250	CP04-1844	X10-0362	92	2.02	90.2	2.23	CP94-2203	CP88-1762
X09-0320	21	1.50	66.7	2.25	CP04-1250	Mix09A	X09-0783	40	1.99	90.0	2.21	CP88-1762	Poly09-21
X10-0353	16	1.50	68.8	2.18	CP94-2203	CP06-2042	CP06-2042	55	1.97	81.8	2.41	CP94-2203	CP97-1777
X09-0069	96	1.43	62.5	2.25	CL87-1630	CP04-1844	X10-0089	43	1.97	83.7	2.35	CL94-0150	CP06-3025
X10-0546	18	1.33	61.1	2.18	CP06-2335	Poly10-11	X09-1077	31	1.95	90.3	2.16	CL89-5189	CP94-2059
X10-0360	16	1.31	62.5	2.10	CPCL06-3458	CP88-1762	X09-0793	20	1.95	90.0	2.17	CL87-2282	Poly09-21
X09-0793	49	1.30	59.2	2.19	CL87-2882	Poly09-21	X10-0318	21	1.88	90.5	2.08	CP07-1860	CP97-1777
X09-1144	29	1.28	51.7	2.06	CP99-1896	Mix09G	X11-0471	21	1.81	85.7	2.11	CP04-1844	CPCL02-8021
X10-0269	31	1.24	58.1	2.14	CPCL02-6848	Poly10-05	X10-0800	28	1.79	85.7	2.08	CPCL97-1864	CPCL01-6755
X09-1150	25	1.22	56.0	2.18	CP05-1518	CP88-1762	X10-0895	28	1.77	89.3	1.98	CPCL96-4974	Poly10-19
X10-0535	23	1.22	52.2	2.33	CP02-1554	Poly10-10	X09-0069	51	1.75	76.5	2.29	CL87-1630	CP04-1844
X08-1022	55	1.12	50.9	2.23	CP04-1250	CP04-2166	X09-1136	28	1.75	85.7	2.04	CP94-2203	MaleMix09G
X09-1034	34	1.10	50.0	2.21	CPCL99-2103	CL88-4730	X09-0856	20	1.75	80.0	2.19	CL89-5189	Poly09-22
X10-0273	16	1.09	50.0	2.19	TCP97-4416	Poly10-05	X09-0893	18	1.75	83.3	2.10	CP97-1387	Poly09-24
X09-1302	18	1.08	50.0	2.17	CP03-1401	Mix07S	X10-0562	70	1.73	82.9	2.09	CP94-2203	Poly10-12
X09-0783	44	1.08	50.0	2.16	CP88-1762	Poly09-21	X10-0940	31	1.73	80.6	2.14	CP89-2377	CP80-1743
X10-0540	17	1.00	41.2	2.43	CP84-1198	Poly10-10	X09-1034	18	1.72	77.8	2.21	CPCL99-2103	CL88-4730
X09-0979	77	0.99	46.8	2.13	CP99-1896	Poly09-25	X09-0796	16	1.69	81.3	2.08	CL90-4500	Poly09-21
Mean [†]	36 (42)	1.30 (0.39)	68.8 (35.8)	2.23 (2.0)			Mean	34 (43)	1.86 (0.97)	85.6 (51.6)	2.17 (1.85)		
Max	96 (214)	2.23 (2.23)	82.1 (82.1)	2.52 (3.0)			Max	92 (209)	2.11 (2.11)	100 (100)	2.44 (2.44)		
Min	16 (15)	0.99 (0.00)	52.9 (2.3)	2.06 (1.4)			Min	16 (15)	1.69 (0.11)	76.5 (5.3)	1.98 (1.00)		
CV(A) [‡]	64.4 (71)	21.4 (94.1)	9.8 (44.5)	5.1 (10.5)			CV(A)	58 (66)	7.3 (46.9)	6.4 (39.4)	5.6 (13.5)		
CV(W)		89.7 (255)		15.5 (13.8)			CV(W)		47.4 (118.6)		21.3 (27.3)		

[†]The first values of mean, Maximum (Max), Minimum (Min) and CV are calculated based on the top 20 families and the second values within parentheses are based on all 279 (2012) or 265 (2013) families with ≥ 15 clones planted

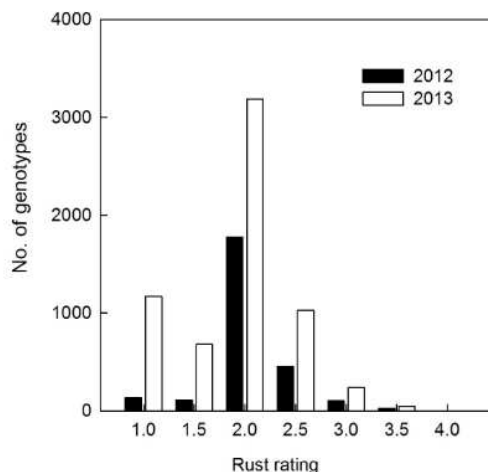
[‡]The CV (A) and CV (W) represent among- and within-family CVs, respectively

Table 4. Number of total sugarcane clones planted, mean Orange Rust (OR) rating, % of infection and severity and their parents for the 20 families with the lowest mean OR ratings using 295 and 263 families with ≥ 15 clones in Stage I of the Canal Point (CP) sugarcane cultivar development program in 2012 and 2013, respectively

2012						2013							
Family	No. clone	OR rating	% infect	Severity	Parent		Family	No. clone	OR rating	% infect	Severity	Parent	
					Female	Male						Female	Male
X09-0698	20	0.00	0	0	CP06-3098	CP92-1167	X10-0382	63	0.32	20.6	1.54	CPCL01-6755	Poly10-09
X09-1242	24	0.00	0	0	CL88-4730	CP04-1252	X10-0159	35	0.31	17.1	1.83	CP06-2400	Poly10-04
X09-1236	44	0.00	0	0	CP03-1160	CP04-1105	X09-1235	32	0.31	15.6	2.00	CP01-2390	CP04-1105
X09-1110	84	0.00	0	0	CL95-5255	Mix09E	X10-0476	24	0.29	20.8	1.40	CP06-2664	CPCL06-3332
X09-1091	24	0.00	0	0	CP78-1628	CP80-1743	X10-0812	24	0.29	16.7	1.75	CPCL02-7386	CP06-2170
X09-1087	21	0.00	0	0	CL88-4730	CP80-1743	X10-0286	30	0.28	13.3	2.13	CPCL02-8021	L01-283
X09-1050	24	0.00	0	0	CP97-1777	CL92-1787	X09-0972	209	0.28	18.2	1.51	CP06-2214	Poly09-25
X09-0888	26	0.00	0	0	CP06-2664	Poly09-24	X11-0405	31	0.27	16.1	1.70	CP92-1167	CP06-2897
X09-0887	26	0.00	0	0	CP06-2657	Poly09-24	X09-0884	37	0.27	18.9	1.43	CPCL01-0271	Poly09-24
X09-0874	33	0.00	0	0	CPCL05-1108	Poly09-23	X09-0995	119	0.27	14.3	1.88	CP92-1167	Poly09-26
X09-0867	20	0.00	0	0	CP02-2281	Poly09-23	X10-0816	21	0.26	19.0	1.38	CPCL01-6755	CP06-2170
X09-0458	45	0.00	0	0	CPCL02-8021	Poly09-14	X10-0206	38	0.25	15.8	1.58	CP05-1451	Mix10-01
X09-0457	23	0.00	0	0	CPCL02-7363	Poly09-14	X10-0165	74	0.23	14.9	1.55	CP03-1026	Poly10-04
X09-0447	21	0.00	0	0	CP01-2459	Poly09-14	X10-0213	49	0.22	14.3	1.57	CP08-1032	CP06-2897
X09-0388	44	0.00	0	0	CP06-2335	Poly09-13	X11-0498	28	0.20	14.3	1.38	CP92-1167	TCP04-4709
X09-0186	21	0.00	0	0	CP96-1252	TCP98-4447	X09-0784	17	0.18	11.8	1.50	CP96-1252	Poly09-21
X09-0178	26	0.00	0	0	CP04-1844	CP04-1844	X10-0445	20	0.18	14.3	1.17	CP05-1451	CP02-2281
X07-1299	24	0.00	0	0	CP00-1751	Mix07S	X10-0833	24	0.17	16.7	1.00	CPCL01-0271	Poly10-16
X07-1284	60	0.00	0	0	CP92-1167	Poly07-16	X11-0497	16	0.13	12.5	1.00	CP97-1777	TCP04-4709
X07-0869	56	0.00	0	0	CPCL05-1102	Poly07-07	X10-0116	19	0.11	5.3	2.00	CP06-2400	CP04-1844
Mean [†]	33 (42)	0.00 (0.39)	0.0 (35.8)	0.00 (2.0)			Mean	46 (43)	0.24 (0.97)	15.5 (51.6)	1.56 (1.85)		
Max	84 (214)	0.00 (2.23)	0.0 (82.1)	0.00 (3.0)			Max	209 (209)	0.32 (2.11)	20.8 (100)	2.13 (2.44)		
Min	20 (15)	0.00 (0.00)	0.0 (2.3)	0.00 (1.4)			Min	16 (15)	0.11 (0.11)	5.3 (5.3)	1.00 (1.00)		
CV(A) [‡]	51.5 (71)	---	---	---			CV(A)	100 (66)	27.8 (46.9)	22.4 (39.4)	19.9 (13.5)		
CV(W)		---	---	---			CV(W)		255.1 (118.6)		28.9 (27.3)		

[†]The first values of mean, Maximum (Max), Minimum (Min) and CV are calculated based on the lowest 20 families and the second values within parentheses are based on all 279 (2012) or 265 (2013) families with ≥ 15 clones planted

[‡]The CV (A) and CV (W) represent among- and within-family CVs, respectively



Variable	2012	2013
Total clones planted	14,272	12,661
No. of clones infected	2,607	6,356
% of infection	18.3	50.2
Severity (mean rating of infected clones)	2.07	1.89
Overall mean rating (including no-infected clones)	0.36	0.95

Fig. 1. Distributions of orange rust on the number of the infected clones at different rust rating levels from 1 to 4 in Stage I of the Canal Point (CP) sugarcane development program in 2012 and 2013. Note: Rust ratings were assessed in July; the rust rating is equal to 0 or no rust infected clones and other important parameters are also listed in the inserted table of the figure

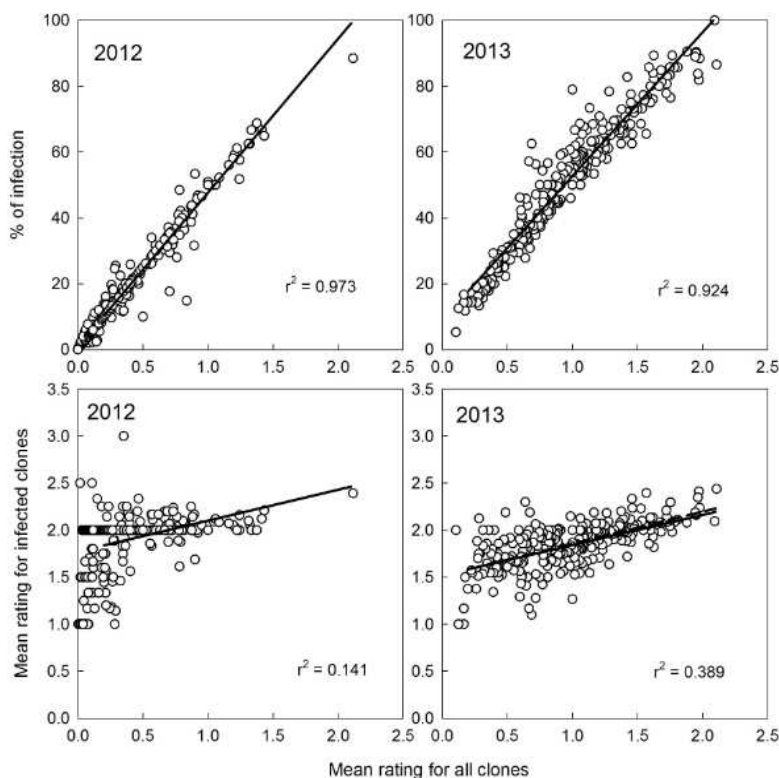


Fig. 2. Relationships between mean rust ratings and % infection or severity (calculated by mean rating of infected clones) of orange rusts for 295 and 263 families with ≥ 15 clones, respectively, in the 2012 and 2013 Stage I of the Canal Point (CP) sugarcane breeding and cultivar development program

Table 5. Number of total sugarcane clones planted and mean Orange Rust (OR) rating for the 20 females with the highest mean OR ratings using 135 and 113 females with ≥ 15 progeny clones in Stage I of the Canal Point (CP) sugarcane cultivar development program in 2012 and 2013, respectively

2012			2013		
Female	No. clone	Mean OR rating	Female	No. clone	Mean OR rating
CP00-1101	26	2.23	CL94-0150	43	1.97
CP94-2203	146	1.31	CL87-2282	20	1.95
CP02-1554	23	1.22	CL87-1630	61	1.83
CP04-1250	135	1.17	CPCL96-4974	28	1.77
CL87-1630	150	1.12	CP97-1387	18	1.75
CL87-2882	83	1.12	CP88-1762	96	1.69
HoCP04-856	16	1.09	CPCL02-6334	33	1.68
CP88-1762	44	1.08	CP57-0614	28	1.63
CP05-1518	56	1.04	CP08-2003	25	1.62
CP99-1896	178	1.03	CPCL99-2574	28	1.59
CP02-1143	54	1.02	CP94-2203	404	1.58
CP84-1198	17	1.00	CP00-2188	37	1.55
CPCL99-2103	65	0.95	CP06-2897	51	1.49
CPCL05-1009	18	0.92	CPCL99-2103	36	1.49
CP80-1743	20	0.90	CP99-1896	48	1.48
L07-057	17	0.88	CL90-4643	58	1.42
CPCL02-8017	33	0.86	CP01-2365	128	1.42
CPCL00-4027	30	0.85	CP05-1730	92	1.41
CP06-2874	28	0.84	CP04-1250	162	1.40
CP00-2188	65	0.84	CPCL02-6848	19	1.39
Mean [†]	60 (102)	1.07 (0.44)	Mean [†]	71 (108)	1.60 (1.02)
Max	178 (639)	2.23 (2.23)	Max	404 (630)	1.97 (1.97)
Min	16 (15)	0.84 (0.00)	Min	18 (16)	1.39 (0.22)
CV(A) [‡]	85.3 (108.7)	28.3 (78.0)	CV(A)	123.5 (109.3)	11.1 (39.0)
CV(W)		109.8 (262.9)	CV(W)		60.0 (108.7)

[†]The first values of mean, Maximum (Max), Minimum (Min) and CV are calculated based on the top 20 female parents based on the mean rating of orange rust and the second values within parentheses are based on all 136 (2012) or 113 (2013) female parents with ≥ 15 clones planted

[‡]The CV (A) and CV (W) represent among- and within-family CVs, respectively

This substantial variability in the number of clones among families was probably associated with differences in the number of viable seeds per cross in the Crossing stage and/or low selection intensity in the Seedling stage for some families that had poor performance in seedling fields. On a family basis, mean rust ratings ranged from 0.0 to 2.5 in both years. Among-family CV values of orange rust were 104.9% in 2012 and 52.7% in 2013. Within-family CVs were greater than their among-family CVs.

Large variability in the number of clones and in rust ratings (Table 2) among families in Stage I of the CP program suggested that Stage I data can be used to identify useful parental combinations and individual parents for their progenies to be resistant/tolerant to orange rust in the test years. The greater CV values for rust rating within families than among families suggested that placing more emphasis on both individual clonal evaluation and family-based evaluation in Stage I of the CP program may help improve our knowledge and ability to select genotypes with potential for eliminating rust effects.

Correlations of Mean Rust Ratings with % Infection and Severity

In 576 (2012) and 455 (2013) families planted in Stage I, 295 and 263 families, respectively, had ≥ 15 clones. From these families with ≥ 15 clones, the mean ratings, % infections and severity values of orange rusts for each family were calculated (Fig. 2). Overall, the mean ratings of orange rust ranged from 0.0 to 2.1; the values of % infection ranged from 2.3 to 100.0% and the values of severity (i.e., mean ratings calculated based on infected clones) ranged from 1.0 to 3.0. Averaged across years, the CV values of the mean rating, % infection and severity were 69.8, 41.8 and 11.8%, respectively among these families. The mean ratings of orange rust highly correlated with % infection ($r^2 = 0.92-0.97$), but the relationships between the mean ratings and severity values were poor ($r^2 = 0.14-0.39$) (Fig. 2). Consideration of Variability (CV) and correlation between the three rust variables, the rust severity might not be a good parameter to distinguish family differences in response to orange rust in the early selection stage of sugarcane breeding program.

Table 6. Number of total sugarcane clones planted and mean Orange Rust (OR) rating for the 20 females with the lowest mean OR ratings using 135 and 113 females with ≥ 15 progeny clones in Stage I of the Canal Point (CP) sugarcane cultivar development program in 2012 and 2013, respectively

2012			2013		
Female	No. clone	Mean OR rating	Female	No. clone	Mean OR rating
CP02-1985	103	0.12	CPCL08-2274	36	0.61
CP96-1252	192	0.11	TCP04-4688	18	0.61
CPCL97-0393	87	0.10	CP08-1965	128	0.61
CP01-2459	88	0.10	CP00-1301	28	0.61
CP06-2657	40	0.10	CL88-4730	91	0.57
CPCL02-2273	39	0.09	CPCL96-2061	24	0.54
CP00-2180	68	0.09	CP05-1451	374	0.53
CL88-4730	332	0.08	CP07-2301	17	0.53
CPCL02-8001	61	0.07	CP01-2390	204	0.51
CP01-2390	259	0.06	CPCL97-0393	38	0.46
CP00-2164	51	0.06	CP96-1252	243	0.44
CP03-1160	72	0.06	CPCL01-6755	190	0.42
CPCL02-7386	73	0.05	CP03-1026	261	0.41
CPCL05-1201	106	0.04	TCP04-4685	26	0.38
CP92-1167	496	0.03	CP08-2398	36	0.36
CP01-1178	38	0.03	CP92-1167	285	0.34
CPCL00-1373	58	0.03	CP06-2214	252	0.34
CP89-2143	115	0.02	CP06-3098	16	0.25
CP00-1751	24	0.00	CP08-1032	49	0.22
CP06-3098	20	0.00	CP06-2400	63	0.22
Mean [†]	116 (102)	1.07 (0.44)	Mean [†]	119 (108)	0.45 (1.02)
Max	496 (639)	2.23 (2.23)	Max	374 (630)	0.61 (1.97)
Min	20 (15)	0.84 (0.00)	Min	16 (16)	0.22 (0.22)
CV(A) [‡]	102.9 (108.7)	28.3 (78.0)	CV(A)	95.4 (109.3)	29.1 (39.0)
CV(W)		109.8 (262.9)	CV(W)		182.7 (108.7)

[†]The first values of mean, Maximum (Max), Minimum (Min) and CV are calculated based on the lowest 20 female parents based on the mean rating of orange rust and the second values within parentheses are based on all 136 (2012) or 113 (2013) female parents with ≥ 15 clones planted

[‡]The CV (A) and CV (W) represent among- and within-family CVs, respectively

Evaluation of Families Based on Mean Rust Ratings

The families with ≥ 15 clones were also used to evaluate family tolerance to orange rust. The 295 and 263 families that had ≥ 15 clones planted in Stage I in 2012 and 2013, respectively, were ranked based on their mean ratings of orange rust (Tables 3 and 4). The mean orange rust ratings across families were 0.4 (ranged from 0.0 to 2.2) in 2012 and 1.0 (ranged from 0.1 to 2.1) in 2013 with the CV values of 94 and 47%, respectively. Top 20 families with the highest (Table 3) and 20 families with the lowest (Table 4) mean rust ratings in each year were identified. The % infection and severity of orange rust for the families and their parents are also listed in Tables 3 and 4. Orange rust had greater variability (CV) in the mean rating and in severity within the family than among families and the CV values of the mean orange rust rating were greater than those of orange rust severity. Parental information of these families might help breeders select proper parents for crossing to develop genotypes with resistance to orange rust. The results of orange rust suggest that using CP 94-2203, CP 04-1250 and CP 99-1896 as female parents will increase probability to produce progenies with high susceptibility to orange rust (Table 3). In contrast, using CP

06-2664, CP 92-1167 and CL 88-4730 as female parents may improve orange rust resistance of progeny (Table 4).

Evaluation of Females Based on Mean Rust Ratings of Their Progenies

When data were analyzed by female parents regardless of families and males, there were 204 and 157 females used for generating the Stage I clones, respectively, in 2012 and 2013. Of these females, 135 in 2012 and 113 in 2013 females had at least 15 progenies planted in the Stage I fields. The progenies of these females were sorted by the rust mean ratings. The 20 females with their progenies having the highest (Table 5) and 20 females with progenies having the lowest (Table 6) mean rating of orange rust were further identified and ranked.

Orange rust is one of the most devastated sugarcane diseases for sugarcane production in Florida. Using proper parents with resistance to orange rust to make crosses is the priority for the CP program. There was great variation in orange rust ratings among families (Tables 3 and 4) in Stage I of the CP program. The great variation in mean ratings of orange rust among female parents for their progenies was also detected (Tables 5 and 6). Clearly, using clones listed in Table 5 as female parents increased the risk to get progenies with high orange rust ratings. In both years, progenies from

females CP 94-2203, CP 04-1250, CL 87-1630, CP 88-1762, CPCL 99-2103 and CP 00-2188 had high mean rating of orange rust and ranked in the top 20 (Table 5). These female parents should be limited in the CP program to develop genotypes with resistance to orange rust. Overall, the progenies developed from female parents listed in Table 6 had the lowest mean ratings of orange rust. Increase in proportion of these female clones for crossing might improve resistance to orange rust of progenies in Stage I of the CP program.

Discussion

Sugarcane orange rust is a devastating disease for sugarcane production in the United States because most commercial cultivars are susceptible to this disease and it caused substantial yield loss of susceptible varieties (Raid *et al.*, 2013). Multiple approaches have been and are being used for eliminating rust negative effect on yield of sugarcane. These include disease monitoring, adjustment of management practices, fungicide applications (Raid *et al.*, 2011) and development of new cultivars with resistance to orange rust by intensive screening in the breeding program (Zhao *et al.*, 2013). The increase in frequent applications of fungicides results in high input cost and low net profits of sugarcane production (Jiang, 1985; Staier *et al.*, 2003). Therefore, development of rust resistant varieties is important for sustaining sugarcane production and for improving production and profitability.

In the present study, we investigated orange rust disease in the first clonal stage of the CP program based on three parameters of mean rust rating, percentage of infection and severity and found that great variability (CV) existed in both mean rust ratings and % infection across large numbers of clones and families. Therefore, these two parameters can be used to evaluate genotypes for orange rust resistance. The rust severity might not a good parameter to distinguish family differences in response to orange rust in the early selection stage of sugarcane breeding program because of relatively smaller CVs than the mean rust rating or % infection.

It is important for sugarcane breeders to develop a data base of parental clones using agronomic and physiological traits and molecular markers for disease resistance and for yield and profit improvement. Virtudazo *et al.* (2001) conducted phylogenetic analysis of sugarcane rusts, including orange rust, based on rDNA sequences. Sugarcane brown rust resistance gene (*Bru1*) has been used as a marker to identify if sugarcane genotypes are potentially resistant to brown rust (Asnaghi *et al.*, 2004). This technology was also utilized in the CP program to direct breeding strategies for brown rust resistance (Glynn *et al.*, 2013). Scientists are working on developing a reference genomic map and identifying the markers linked to sugarcane orange rust

resistance genes (Yang *et al.*, 2014). However, the molecular markers for orange rust resistance are not commercially available yet. Traditional breeding approaches for rust resistance have involved a better understanding of inheritance of resistance in seedling populations (Ramdoyal *et al.*, 2000) and selection and advancement of genotypes that are free from visual disease symptoms. To evaluate performance of sugarcane families in some specific traits, Wang *et al.* (2008) suggested that at least 10 to 20 clones per family were required. Therefore, the families that had ≥ 15 clones and females that had ≥ 15 progeny clones were used in the present study to assess orange rust resistance at the family and female parental levels based on the disease data collected from individual clones. Our results indicated that orange rust ratings collected in the first clonal stage can be used not only for advancing elite clones to next stage of the CP program as one of selecting criteria, but also for evaluating families and their parents for orange rust resistance. These findings could be useful for breeders to design cross combinations and to improve selection efficiency of new cultivars for rust resistance.

Shanthi *et al.* (2008) suggested that parents producing progeny with a high frequency of transgressive recombination for agronomic traits should provide the best opportunity for sugarcane breeders to select clones superior to their parents. In addition to directly evaluating parental lines for disease resistant traits, therefore, parental evaluation based on their progeny performance for rust resistance and other agronomic traits in the early clonal stage of a sugarcane breeding program with large numbers of clones might help optimize parental selection and crossing combination. Quantitative analyses of sugarcane clonal data of rusts in Stage I of the CP program could help us evaluate not only crosses (families), but also their parents as described above. Studies have suggested that family selection is effective in improving sugarcane populations in early selection stages (Chang and Milligan, 1992; Cox and Hogarth, 1993; McRae *et al.*, 1993; Shanthi *et al.*, 2008) because it can identify those families that harbor the highest proportion of desirable clones and makes it possible to focus selection for superior clones (Shanthi *et al.*, 2008). Availability of family data in rust diseases helps sugarcane breeders improve crossing combinations for developing genotypes with resistance to diseases and high yields.

Conclusion

Early stage selection methods and specific selection strategies of sugarcane cultivar development programs are dependent on environmental conditions and the unique goals of each selection program. Analyses of orange rust data collected from large numbers of individual clones in Stage I of the CP program in 2012

and 2013 revealed that there was great variation in orange rust ratings among genotypes and among families. Our results indicated that using the rust rating data along with individual selection data on plant vigor and stalk juice Brix (Zhao *et al.*, 2012) for making comparisons of family performance and among- and within-family variability would improve our parental selection and optimize crosses among selected parents, which should then improve the progenies for rust resistance and yield potential in the sugarcane breeding programs.

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Author's Contributions

Duli Zhao: Contributed to conception and design of the experiment, analyzed and interpreted data, wrote the manuscript and integrated all ideas and comments from other authors and peer reviewers.

R. Wayne Davidson: Coordinated the work of data acquisition, analysis and interpretation.

Miguel Baltazar: Coordinated the work of data acquisition and analysis.

Jack C. Comstock: Made contributions to the original ideas of the experiment and disease data quality.

Ethics

The authors wish to state that this article conforms to the ethical standards specified by the American Journal of Agricultural and Biological Sciences.

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