

Report on marker-assisted selection to identify reproductive behavior in Bh22 population

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Accelerated breeding initiative

Summary

Continuing with the fourth cycle of humidicola breeding scheme (*Urochloa humidicola* (Rendle) Morrone & Zuloaga), a half-sibs population was formed through a genetic cross between susexual population BhSx19 and the apomictic tester CIAT/16888. Using standardized protocols, the identification of the reproduction mode was carried out in the population of hybrids obtained (called Bh22) using the molecular marker p779 / p780. A total of 1,914 (38%) hybrids, from 5,024, showed the apomictic marker, 2,900 (58%) unshowed the apomictic marker and, 210 (4%) were uncertain or did not amplify. The variation in the apomictic and sexual relationship between formed families is presented.

Keywords: Marker assisted selection, *Brachiaria humidicola*, Apomictic plants.

Resumen

Continuando con el cuarto ciclo de del esquema de mejoramiento de pasto humidicola (*Urochloa humidicola* (Rendle) Morrone & Zuloaga), se conformó una población de medios hermanos mediante el cruzamiento entre población sexual BhSx19 por el probador apomítico CIAT/16888. Mediante protocolos estandarizados, se realizó la identificación del modo de reproducción en la población de híbridos obtenidos (llamada Bh22) mediante el marcador molecular p779 / p780. De 5,024 híbridos evaluados, 1,914 (38%) mostraron ser apomíticos, 2,900 (58%) sexuales y 210 (4%) dudosos o no amplificaron. Se presenta la variación en la relación entre apomicticos y sexuales entre familias formadas.

Palabras Clave: Selección asistida por marcadores, *Brachiaria humidicola*, Plantas apomicticas

Introduction

Recurrent selection based on specific combining ability (RS-SCA) allows to maximize the genetic gain in a short period of time and is the ideal scheme to take full use of apomixis and heterosis. Urochloa breeding program works with this scheme and actually is running the fourth breeding cycle. Each breeding cycle is conformed by three steps: Firstly (Yr1), sexual genotypes are crossed to a genetically distant apomictic genotype (tester) selected according to its combining ability. Secondly (Yr2), superior sexual parentals are selected based on their progeny performance. In this, sexual parents and apomictic hybrids are selected in tandem as follows: in a first phase, ~7000 apomictic hybrids are evaluated in acid soils, selecting the 100 superior hybrids and their sexual parentals. These are evaluated in a second phase against to spittlebug resistance complex. Thirdly (Yr3), the selected superior sexual parentals females are recombined with the aim to form sexual genotypes for the next cycle of selection (Worthington and Miles, 2015).

During 2020 and 2021 a genetic cross between superior sexual parentals and apomictic tester CIAT/16888 was established to produce a progeny to evaluate their performance. To guarantee that the hybrids evaluated are apomictic, it is necessary to identify their reproduction mode. This report summarizes the results obtained when evaluating the reproduction mode in the Bh22 population using the molecular marker p779 / p780, which allows identifying ASGR – BBML gene sequences present in apomictic genotypes in some Paniceae species (Fig. 1; Worthington et al. 2016).

Materials and Methods

In 2020-2021, a cross between 1 apomictic (CIAT/16888) and 264 sexual genotypes was made. Seed was hand-harvested, planted in sand (Fig. 1A) and transplanted individually in plugs after 30 days (Fig. 1B-1D). In 2022, between August and October, leaf tissue from 5,024 hybrids were sampled (Fig. 1) and the reproduction mode was assessed using the molecular marker p779/p780 (Worthington et al. 2016).



Figure 1. Process to identify apomictic plants.

Results and Discussion

Among each of the 264 families formed, the sexual to apomictic ratio varied widely (Table 1). For the following studies, families with high percentage of apomixis (>85%) were discarded based on the premise that sexual parental really can be apomictic. Very high percentages of apomixis suggest that the genotype used as sexual may be facultative or obligate apomictic. Otherwise, low percentages of apomixis may indicate that there is a low pollination index, a phenomenon that can be influenced by different factors. For example, asynchronous flowering between apomictic and sexual genotypes, low presence of pollinators (*Aphis* sp.) and, scarce wind currents that allows pollen flow.

Table 1. Apomixis percentage expressed in 198 families conformed in a factorial cross among sexual an apomictic genotypes.

Family	Sexual	Apomictic	Uncertain	AP+SX	AP PROPORTION
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BHSX19/0005	4	1	1	5	20.00
BHSX19/0010	2	3	0	5	60.00
BHSX19/0015	7	7	0	14	50.00
BHSX19/0021	76	49	1	125	39.20
BHSX19/0022	30	25	1	55	45.45
BHSX19/0023	1	1	0	2	50.00
BHSX19/0032	6	4	0	10	40.00
BHSX19/0034	3	1	1	4	25.00
BHSX19/0044	3	2	0	5	40.00
BHSX19/0055	7	3	1	10	30.00
BHSX19/0062	11	9	0	20	45.00
BHSX19/0063	2	1	0	3	33.33
BHSX19/0069	15	5	0	20	25.00
BHSX19/0071	27	28	2	55	50.91
BHSX19/0073	60	13	0	73	17.81
BHSX19/0074	1	1	0	2	50.00
BHSX19/0080	2		0	2	0.00
BHSX19/0082	9	11	1	20	55.00
BHSX19/0083	4	2	0	6	33.33
BHSX19/0084	4	2	0	6	33.33
BHSX19/0089	29	20	1	49	40.82
BHSX19/0090	18	9	5	27	33.33
BHSX19/0091	27	18	4	45	40.00
BHSX19/0093	7	6	0	13	46.15
BHSX19/0094	19	3	2	22	13.64
BHSX19/0095	15	1	0	16	6.25
BHSX19/0096	7	7	0	14	50.00
BHSX19/0098	4	6	0	10	60.00
BHSX19/0105	1	1	0	2	50.00
BHSX19/0111	3	5	0	8	62.50
BHSX19/0113	26	17	0	43	39.53
BHSX19/0117	25	24	3	49	48.98
BHSX19/0120	53	35	2	88	39.77
BHSX19/0121	4	4	0	8	50.00
BHSX19/0122	21	17	2	38	44.74
BHSX19/0126	8	9	1	17	52.94
BHSX19/0127	1	2	2	3	66.67
BHSX19/0128	20	19	2	39	48.72
BHSX19/0133	10	9	0	19	47.37
Family	Sexual	Apomicitic	Uncertain	AP+SX	AP PROPORTION
BHSX19/0134	10	4	1	14	28.57
BHSX19/0135	7	3	0	10	30.00
BHSX19/0146	1	5	0	6	83.33

BHSX19/0147		1	0	1	100.00
BHSX19/0155	21	12	3	33	36.36
BHSX19/0157	8		1	8	0.00
BHSX19/0162	1		0	1	0.00
BHSX19/0171	1	1	0	2	50.00
BHSX19/0172	39	32	3	71	45.07
BHSX19/0176	1		0	1	0.00
BHSX19/0180	2		0	2	0.00
BHSX19/0187		1	0	1	100.00
BHSX19/0196	2	1	0	3	33.33
BHSX19/0197	1	1	0	2	50.00
BHSX19/0199	13	9	0	22	40.91
BHSX19/0201	20	11	1	31	35.48
BHSX19/0202	2	1	0	3	33.33
BHSX19/0203	2	3	0	5	60.00
BHSX19/0205	4	8	0	12	66.67
BHSX19/0206	1		1	1	0.00
BHSX19/0215	1		0	1	0.00
BHSX19/0216	4	1	0	5	20.00
BHSX19/0221	6	3	0	9	33.33
BHSX19/0225	1	2	0	3	66.67
BHSX19/0230	2	1	0	3	33.33
BHSX19/0231	6	2	0	8	25.00
BHSX19/0232	51	41	3	92	44.57
BHSX19/0233	12	5	2	17	29.41
BHSX19/0240	2	1	0	3	33.33
BHSX19/0242	1		0	1	0.00
BHSX19/0243	4	1	0	5	20.00
BHSX19/0245	1		0	1	0.00
BHSX19/0251	2	3	0	5	60.00
BHSX19/0253	4	10	0	14	71.43
BHSX19/0254	16	12	0	28	42.86
BHSX19/0258	1		0	1	0.00
BHSX19/0261	15	4	0	19	21.05
BHSX19/0262	1	2	1	3	66.67
BHSX19/0265	2		0	2	0.00
BHSX19/0266	8	6	1	14	42.86
BHSX19/0267	5		1	5	0.00
BHSX19/0268	15	3	1	18	16.67
Family	Sexual	Apomictic	Uncertain	AP+SX	AP PROPORTION
BHSX19/0271	3	4	0	7	57.14
BHSX19/0275	1		0	1	0.00
BHSX19/0276		2	0	2	100.00

BHSX19/0279	3	1	0	4	25.00
BHSX19/0286	1	1	1	2	50.00
BHSX19/0288	14	4	0	18	22.22
BHSX19/0299	14	5	1	19	26.32
BHSX19/0303	3		0	3	0.00
BHSX19/0304	2	1	0	3	33.33
BHSX19/0305	1		0	1	0.00
BHSX19/0306	11	7	0	18	38.89
BHSX19/0311	8	4	0	12	33.33
BHSX19/0312	50	11	4	61	18.03
BHSX19/0316	9	2	1	11	18.18
BHSX19/0317	12	7	0	19	36.84
BHSX19/0318	32	21	1	53	39.62
BHSX19/0319	20	26	3	46	56.52
BHSX19/0321	38	17	9	55	30.91
BHSX19/0322	31	29	2	60	48.33
BHSX19/0328		1	0	1	100.00
BHSX19/0329	2	2	0	4	50.00
BHSX19/0333	9	7	2	16	43.75
BHSX19/0335	6	9	4	15	60.00
BHSX19/0337	2		1	2	0.00
BHSX19/0338	1	1	0	2	50.00
BHSX19/0339	4	2	0	6	33.33
BHSX19/0340	17	4	0	21	19.05
BHSX19/0343	2	3	0	5	60.00
BHSX19/0345	14	4	0	18	22.22
BHSX19/0346	1		0	1	0.00
BHSX19/0347	50	44	1	94	46.81
BHSX19/0358	7	6	0	13	46.15
BHSX19/0360	2	1	0	3	33.33
BHSX19/0367	2	1	0	3	33.33
BHSX19/0379	3	2	0	5	40.00
BHSX19/0380	1		0	1	0.00
BHSX19/0382	2	1	0	3	33.33
BHSX19/0386	27	9	0	36	25.00
BHSX19/0389		1	0	1	100.00
BHSX19/0392	20	23	1	43	53.49
BHSX19/0394	2	2	0	4	50.00
BHSX19/0396	3	2	1	5	40.00
Family	Sexual	Apomicitic	Uncertain	AP+SX	AP PROPORTION
BHSX19/0397	4	3	0	7	42.86
BHSX19/0399	32	22	2	54	40.74
BHSX19/0404		2	0	2	100.00

BHSX19/0408	2		0	2	0.00
BHSX19/0410		2	0	2	100.00
BHSX19/0412	2		1	2	0.00
BHSX19/0413	37	11	2	48	22.92
BHSX19/0414		2	0	2	100.00
BHSX19/0417	9	7	0	16	43.75
BHSX19/0418	10	4	0	14	28.57
BHSX19/0420	7	3	0	10	30.00
BHSX19/0422	25	23	1	48	47.92
BHSX19/0425	2	2	0	4	50.00
BHSX19/0430	3		0	3	0.00
BHSX19/0432	6	3	0	9	33.33
BHSX19/0433	6	3	0	9	33.33
BHSX19/0434	6	3	0	9	33.33
BHSX19/0435	14	5	0	19	26.32
BHSX19/0436	1	3	0	4	75.00
BHSX19/0437	4	3	0	7	42.86
BHSX19/0438	13	9	1	22	40.91
BHSX19/0440	4	2	0	6	33.33
BHSX19/0442	3	2	0	5	40.00
BHSX19/0443	2	1	0	3	33.33
BHSX19/0445		1	0	1	100.00
BHSX19/0447	40	20	1	60	33.33
BHSX19/0449	5	2	0	7	28.57
BHSX19/0451	7	14	0	21	66.67
BHSX19/0452	78	47	5	125	37.60
BHSX19/0455	9	8	1	17	47.06
BHSX19/0456	15	12	1	27	44.44
BHSX19/0457	5	3	0	8	37.50
BHSX19/0462	3	2	0	5	40.00
BHSX19/0463	3	2	0	5	40.00
BHSX19/0468	3	2	0	5	40.00
BHSX19/0469	3	4	1	7	57.14
BHSX19/0470	1	1	1	2	50.00
BHSX19/0471	1	5	0	6	83.33
BHSX19/0473	10	5	0	15	33.33
BHSX19/0476	18	8	2	26	30.77
BHSX19/0479	14	12	2	26	46.15
BHSX19/0481	15	7	0	22	31.82
Family	Sexual	Apomicitic	Uncertain	AP+SX	AP PROPORTION
BHSX19/0484	7	7	0	14	50.00
BHSX19/0490	3	5	0	8	62.50
BHSX19/0499	4	5	1	9	55.56

BHSX19/0501	5	3	0	8	37.50
BHSX19/0502	11	3	4	14	21.43
BHSX19/0506	1		0	1	0.00
BHSX19/0516	10	2	0	12	16.67
BHSX19/0517	55	28	4	83	33.73
BHSX19/0518	2	2	0	4	50.00
BHSX19/0521		3	0	3	100.00
BHSX19/0522	1		0	1	0.00
BHSX19/0523	5	10	1	15	66.67
BHSX19/0524	16	7	9	23	30.43
BHSX19/0526	1		0	1	0.00
BHSX19/0527	10	12	2	22	54.55
BHSX19/0531		1	0	1	100.00
BHSX19/0532	3		1	3	0.00
BHSX19/0536	9	2	0	11	18.18
BHSX19/0538	5		0	5	0.00
BHSX19/0540	3	2	0	5	40.00
BHSX19/0543	1		0	1	0.00
BHSX19/0556	3	3	0	6	50.00
BHSX19/0562		1	0	1	100.00
BHSX19/0564	3	3	0	6	50.00
BHSX19/0572	3	4	0	7	57.14
BHSX19/0573	1	1	0	2	50.00
BHSX19/0578	13	12	0	25	48.00
BHSX19/0582	6	10	0	16	62.50
BHSX19/0587	10	2	0	12	16.67
BHSX19/0592	1		0	1	0.00
BHSX19/0594	3	2	1	5	40.00
BHSX19/0605	47	10	4	57	17.54
BHSX19/0606		1	0	1	100.00
BHSX19/0607		2	0	2	100.00
BHSX19/0609	5		0	5	0.00
BHSX19/0613	1	10	1	11	90.91
BHSX19/0616	13	22	9	35	62.86
BHSX19/0618	23	13	5	36	36.11
BHSX19/0619	35	3	4	38	7.89
BHSX19/0621	67	39	15	106	36.79
BHSX19/0628	14	5	14	19	26.32
BHSX19/0629	4		0	4	0.00
Family	Sexual	Apomicitic	Uncertain	AP+SX	AP PROPORTION
BHSX19/0634		1	0	1	100.00
BHSX19/0636		1	1	1	100.00
BHSX19/0638	14	10	1	24	41.67

BHSX19/0639	3	3	0	6	50.00
BHSX19/0642	1	10	0	11	90.91
BHSX19/0643	3	2	0	5	40.00
BHSX19/0646	10	10	1	20	50.00
BHSX19/0650	13	5	0	18	27.78
BHSX19/0651	1		0	1	0.00
BHSX19/0656	2	2	0	4	50.00
BHSX19/0657	1		0	1	0.00
BHSX19/0658	4	2	1	6	33.33
BHSX19/0663	10	4	0	14	28.57
BHSX19/0667	43	28	2	71	39.44
BHSX19/0670	6	6	1	12	50.00
BHSX19/0675	9	5	1	14	35.71
BHSX19/0677	1	2	0	3	66.67
BHSX19/0679	37	25	1	62	40.32
BHSX19/0681	5	9	0	14	64.29
BHSX19/0682		1	0	1	100.00
BHSX19/0684	1		0	1	0.00
BHSX19/0694	20	25	1	45	55.56
BHSX19/0704		1	0	1	100.00
BHSX19/0705	13	5	0	18	27.78
BHSX19/0706	20	29	1	49	59.18
BHSX19/0708	1	2	0	3	66.67
BHSX19/0709	10	5	0	15	33.33
BHSX19/0719	23	11	0	34	32.35
BHSX19/0722	3	4	0	7	57.14
BHSX19/0724	3	1	0	4	25.00
BHSX19/0729	3	3	0	6	50.00
BHSX19/0745	1	1	0	2	50.00
BHSX19/0748	8	5	1	13	38.46
BHSX19/0753	77	25	0	102	24.51
BHSX19/0762	3		0	3	0.00
BHSX19/0765	2	5	0	7	71.43
BHSX19/0766	1		0	1	0.00
BHSX19/0767	30	42	3	72	58.33
BHSX19/0770	63	36	1	99	36.36
BHSX19/0772	1	1	0	2	50.00
BHSX19/0773	18	17	0	35	48.57
BHSX19/0774	5	5	1	10	50.00
Family	Sexual	Apomicitic	Uncertain	AP+SX	AP PROPORTION
BHSX19/0778	12	9	0	21	42.86
BHSX19/0782	37	31	4	68	45.59
BHSX19/0784	29	18	1	47	38.30

BHSX19/0785	21	10	0	31	32.26
BHSX19/0787	2		0	2	0.00
BHSX19/0789		1	0	1	100.00
BHSX19/0791	2	1	0	3	33.33
BHSX19/0792	22	12	0	34	35.29
BHSX19/0799	28	6	1	34	17.65
BHSX19/0804	52	56	0	108	51.85
BHSX19/0807	40	31	2	71	43.66
BHSX19/0808	10	1	0	11	9.09
BHSX19/0814	3		1	3	0.00
BHSX19/0815	32	33	4	65	50.77
BHSX19/0819	17	14	1	31	45.16

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