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Evaluation of new hybrid brachiaria lines in Thailand. 2. Seed production

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Abstract

Forty-three new hybrid bracharia lines bred at CIAT, Colombia, were evaluated for seed production in Northeast Thailand between 2006 and 2010 in 2 experiments at 2 sites, Ubon Ratchathani and Amnart Charoen. These lines were compared with Mulato II hybrid brachiaria. From the BR02 collection, 4 lines, BR02/1718, BR02/1752, BR02/1794 and BR02/0465, were granted Plant Variety Rights in 2011. BR02/1794 produced more seed than Mulato II on most occasions, including both harvests at Ubon Ratchathani and 2 of 3 harvests at Amnart Charoen. The next best yielding lines were BR02/1718 and BR02/0465, which produced more seed than Mulato II in 1 of 2 harvests at Ubon Ratchathani and 2 of 3 harvests at Amnart Charoen. Seed-set (percentage of cleaned seed to spikelets) was generally very low in all hybrid lines (1–12%). The reasons for low seed-set in hybrid brachiaria grasses are discussed, including: being a common defect in newly formed apomictic forage hybrids; previous selection for seed yield not being rigorous enough; and insufficient selection at latitudes and sites where commercial brachiaria seed production is practiced.

Resumen

En el período 2006–11 en 2 sitios del noreste de Tailandia (Ubon Ratchathani y Amnart Charoen) fueron evaluadas por su producción de semilla 43 líneas nuevas de híbridos de *Brachiaria*, incluyendo el cultivar (cv.) Mulato II como testigo, procedentes del CIAT. La línea BR02/1794 produjo más semilla que cv. Mulato II en 2 cosechas realizadas en Ubon Ratchathani y en 2 de las 3 cosechas en Amnart Charoen. Otras líneas con buenos rendimientos de semilla fueron BR02/1718 y BR02/0465 que produjeron más semilla que cv. Mulato II en una de las 2 cosechas en Ubon Ratchathani y 2 de 3 cosechas en Amnart Charoen. La formación de semilla (porcentaje del número de semillas limpias en relación con el número de espiguillas formadas) fue, en general, muy baja en todas las líneas, con un valor entre 1 y 12%. Se analizan las posibles razones de este bajo porcentaje en los híbridos de *Brachiaria* evaluados, entre ellas, un defecto común en híbridos apomícticos recién formados, falta de rigor en las etapas previas de selección para producción de semilla, y fallas en la selección de las latitudes y los sitios de producción comercial de semilla de *Brachiaria*. Las líneas BR02/1718, BR02/1752, BR02/1794 y BR02/0465 alcanzaron la protección de obtención vegetal (*Plant Variety Rights*) en 2011.

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Introduction

Mulato II [*Brachiaria ruziziensis* (now *Urochloa ruziziensis*) x *B. decumbens* (now *U. decumbens*) x *B. brizantha* (now *U. brizantha*)] was the second hybrid brachiaria cultivar released from the hybridization programs begun in 1988 at the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia (Argel et al. 2007). Even though Mulato II produced 60% higher seed yields than Mulato (*Brachiaria ruziziensis* x *B. brizantha*), which was the first hybrid brachiaria released (Hare et al. 2007a), Mulato II seed yields of 232–258 kg/ha were still very low compared with yields from other commercial brachiaria cultivars (not hybrids) elsewhere. In order to compete in price internationally with commercial brachiaria cultivars from Brazil and Australia, commercial seed yields from hybrid brachiarias must be at least 600–700 kg/ha. Commercial seed yields average 650–700 kg/ha in Brazil for cv. Marandu (*B. brizantha*) and cv. Basilisk (*B. decumbens*) (Souza 1999). In Australia, seed yields of Basilisk have reached 1,000 kg/ha (Hopkinson and Clifford 1993). Seed of these commercial brachiaria species is almost half the price of hybrid brachiaria seed. The high price of hybrid brachiaria seed is a reflection of low seed yields and represents a significant barrier to farmer uptake.

From 2006 to 2011, studies were conducted in Thailand on hybrid brachiaria collections from CIAT. The first paper of these studies reported on forage production and quality (Hare et al. 2015), while this paper focuses on seed production.

Materials and Methods

Two experiments were conducted with the aim of selecting lines that had higher seed yields than Mulato II.

Experiment 1. BR02 and MX02 collections

The first experiment was conducted at Ubon Ratchathani University, Thailand, (15° N, 104° E; 130 masl) during 2006 and 2007 alongside a forage biomass experiment. The site was on an upland sandy low humic gley soil that was acid (pH 4.6) and low in organic matter (1.1%), N (0.04%), P (3.5 ppm) and K (27.4 ppm). The mean rainfall was 1,620 mm, with a dominant dry season from November to April (Figure 1). The site is further described in the first paper on forage production (Hare et al. 2015). Thirteen hybrid brachiaria lines from the BR02 collection and 2 from the MX02 collection (Hare et al. 2015) were planted in a randomized complete block design with 3 replicates in June 2006. Seedlings were grown in a nursery and transplanted into the field plots using 50 x 50 cm spacings (48 plants per plot). Details of field crop management are presented in Table 1. Two seed harvests were conducted in 2006 and 2007.

Experiment 2. BR06 collection

This experiment was conducted at one site at the Amnart Charoen Livestock Development Centre, Amnart Charoen province, Northeast Thailand (15.5° N, 104.4° E; 168 masl) from 2008 to 2010 (3 harvests of each plot) alongside the forage trial. The site was on an upland sandy reddish brown earth with a mean rainfall of 1,640 mm, and a dominant dry season from November to April (Figure 2). Soil samples taken at planting in July 2008 showed that the soil was acid (pH 4.6), sandy (75%), and low in organic matter (0.4%), N (0.04%), and K (31 ppm), and adequate for P (25.2 ppm). The site is described further in the first paper on forage production (Hare et al. 2015).

Table 1. Field crop management of hybrid brachiaria lines during evaluation in Ubon Ratchathani, Thailand (Experiment 1).

Field cultivation	Plowing x 2, disking x 1, harrowing x 1
Plot size	3 m x 4 m with 50 cm walkway around plots and 1 m between replications
Sowing date	1–3 Jun 2006
Cleaning and closing cuts	2006: 3 Aug
	2007: 27 Apr & 24 Jul
	All plots cut to 5 cm above ground level
Fertilizer	2006: 3 Aug 200 kg/ha NPK (15:15:15); 7 Sep & 3 Oct 46 kg N/ha as urea
	2007: 24 Jul 46 kg N/ha as urea; 28 Aug urea (46 kg N/ha), double superphosphate (18 kg P/ha), potash (52 kg K/ha), gypsum (17 kg S/ha); 5 Oct urea (46 kg N/ha)

Twenty-eight hybrid brachiaria lines from the BR06 collection (Hare et al. 2015), 4 from the BR02 collection, Mulato II, Toledo (*B. brizantha*) and Marandu (*B. brizantha*) were planted in July 2008 in a randomized complete block design with 4 replications. Seedlings were grown in a nursery and transplanted during 26–28 July 2008 into the field plots in 80 x 50 cm spacings (32 plants per plot). Seed harvests were conducted in 2008, 2009 and 2010. Details of field crop management are presented in Table 2.

For both experiments all inflorescences in 3 m of the middle 2 rows were counted once a week. Twenty inflorescences were taken from just outside this area for reproductive analysis at peak anthesis (Table 3). All racemes were counted on each inflorescence and spikelets were counted on 3 racemes per inflorescence, selected from the top, middle and bottom of each inflorescence. At peak anthesis, nylon bags were tied over each seed head of 10 plants (5 plants/row in the above middle 2 rows) to collect the seed. The seed was allowed to fall naturally into the bags and collected once at the end of the season and cleaned through hand screens and a small seed blower to 99% pure seed. Settings were adjusted according to seed weights of each line. Following cleaning, seed yields were corrected to 10% seed moisture content. One thousand seed weights (TSW) were calculated by drying 4 lots of 100 seeds per plot and correcting to 10% seed moisture.

Data from the experiments were subjected to analysis of variance, using the IRRISTAT program from the International Rice Research Institute (IRRI). Entry means were compared using Fisher's protected LSD ($P \leq 0.05$) procedure.

Results

Rainfall

Experiment 1. BR02 and MX02 collections. Rainfall for the experimental period is shown in Figure 1. The critical period of rainfall for seed production in Thailand is the period from July to October, when the plants establish, develop, and initiate and elongate inflorescences and seed is set and matures. The medium-term mean (13 years) rainfall at Ubon Ratchathani for this period is 917 mm, and in 2007 rainfall closely approximated the mean, but in 2006, rainfall during this critical period was 17% higher. October and November are important months for seed maturity and harvest. In 2006 and 2007, rainfall during these months exceeded the mean, by a factor of 1 in 2006 and 0.5 in 2007.

Table 2. Field crop management of hybrid brachiaria lines during evaluation in Amnart Charoen, Thailand (Experiment 2).

Field cultivation	Plowing x 2, disking x 1, harrowing x 1	
Plot size	3.2 m x 4 m with 50 cm walkway around plots and 1 m between replications	
Sowing date	26–28 Jul 2008	
Cleaning cuts	2008: No cuts before harvest	
	2009: 13 Jan & 13 May	
	2010: 28 Apr & 16 Jun	
	All plots cut to 5 cm above ground level	
Closing cuts[1]	2008 & 2009:	No closing cuts from sowing
		2 Jul (first group[2]), 28 Jul (second group), 8 Sep (third group)
	2010:	20 Jul (first group), 10 Aug (second group)
Fertilizer (amounts of fertilizer applied based on experience of soils in the region)	2008: 8 Sep NPK (15:15:15) 200 kg/ha	
	2009: 13 May, 2 Jul (first group), 28 Jul (second group), 8 Sep (third group) NPK (15:15:15) 200 kg/ha	
	13 Aug (first group), 28 Sep(second group), 19 Oct (third group) 46 kg N/ha as urea	
	2010: 28 Apr, 16 Jun, 20 Jul (first group), 10 Aug (second group) NPK (15:15:15) 200 kg/ha	
	31 Aug (first group), 21 Sep (second group) 46 kg N/ha as urea	

[1]Closing cuts, 5 cm above ground level, were implemented about 90 days before peak anthesis (recorded in the first year in 2008) to avoid seed head lodging prior to anthesis.
[2]Groups are recorded in Table 3.

Table 3. Dates for peak anthesis for hybrid brachiaria lines in Ubon Ratchathani (Experiment 1) and Amnart Charoen (Experiment 2), Thailand.

Experiment	Peak anthesis date and hybrid line/cultivar
Experiment 1	
2006	Oct 24: BR02/0779; MX02/1423;
	Nov 11: BR02/1372, 1794;
	Nov 20: BR02/1484, 1718, 1728, 1747, MX02/1263;
	Dec 12: BR02/0465, 0768, 0771, 1245, 1452, 1752, Mulato II
2007	Oct 10–17: BR02/0779, 1372, 1728, 1794, MX02/1423;
	Oct 24: BR02/0465, 1718, 1752;
	Nov 1: BR02/0768, 0771, 1452, 1485, 1747, 1752;
	Nov 8: MX02/1263, Mulato II;
	No flowering: BR02/1245
Experiment 2	
2008	Sep 25–Oct 3: BR06/0405, 1366, 1388, 1433, 1454, BR02/1372;
	Oct 24: BR06/0206, 0387, 0423, 1000, 1132, 1175, 1415, 1696, 1832, 2058, BR02/0465, 1718, 1794;
	Nov 1–8: Mulato II, Marandu;
	Nov 14–21: BR06/0012, 0204, 0584, 0850, 1278, 1348, 1567, 1922, 1932, 2020, 2204;
	Dec 4: Toledo;
	No flowering: BR06/0531, 1254
2009	[1]*Sep 15: BR06/0405, 1366, 1388, 1433, 1454, 1922, BR02/1794, 1372;
	**Sep 28–Oct 8: BR06/0206, 0387, 0423, 0531, 0850, 1175, 1278, 1415;
	**Oct 19: BR06/0012, 1000, 1132, 1567, 1696, 1832, 1932, 2020, 2058, BR02/0465, 1718;
	**Nov 15: BR06/0204, 1348, 1584, 2204, Mulato II, Marandu;
	No flowering: **BR06/1254, ***Toledo
2010	*Sep 21–28: BR06/0206, 0405, 0850, 1132, 1175, 1278, 1366, 1388, 1415, 1433, 1454, 1922, 1932, 2020, BR02/1794, 1372;
	**Oct 19: BR06/0204, 1000, 1348, 1696, 1832, 2058, 2204, BR02/0465, 1718;
	**Nov 2: BR06/0012, 0387, 0423, 0531, 1567;
	**Nov 23: BR06/0584, Mulato II, Marandu;
	**Dec 20: Toledo;
	No flowering: **BR06/1254

[1]Groups for closing cuts and fertilizer application: * First group, ** Second group, *** Third group.

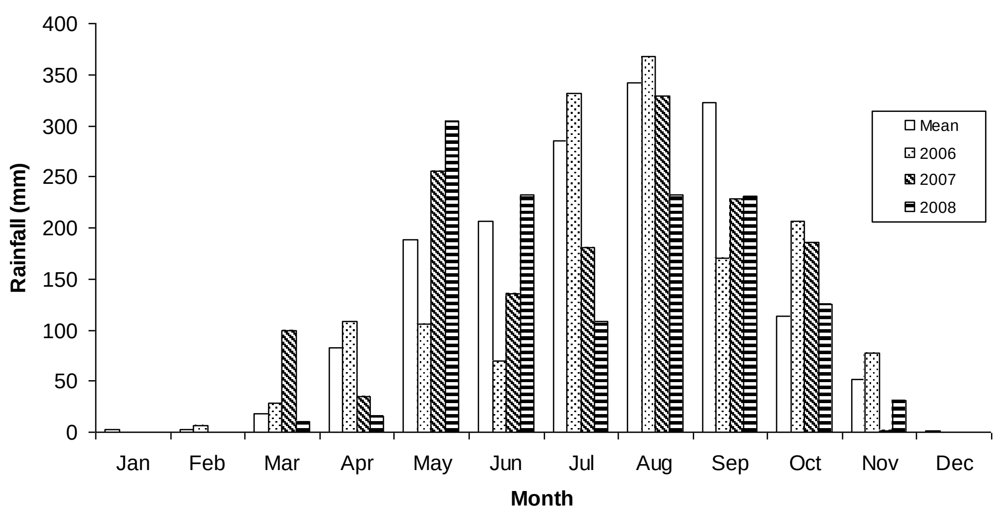

Figure 1. Rainfall at the Ubon Ratchathani University meteorological station, 1 km from the research site, during the experiment and the 13-yr mean (2000–2012).

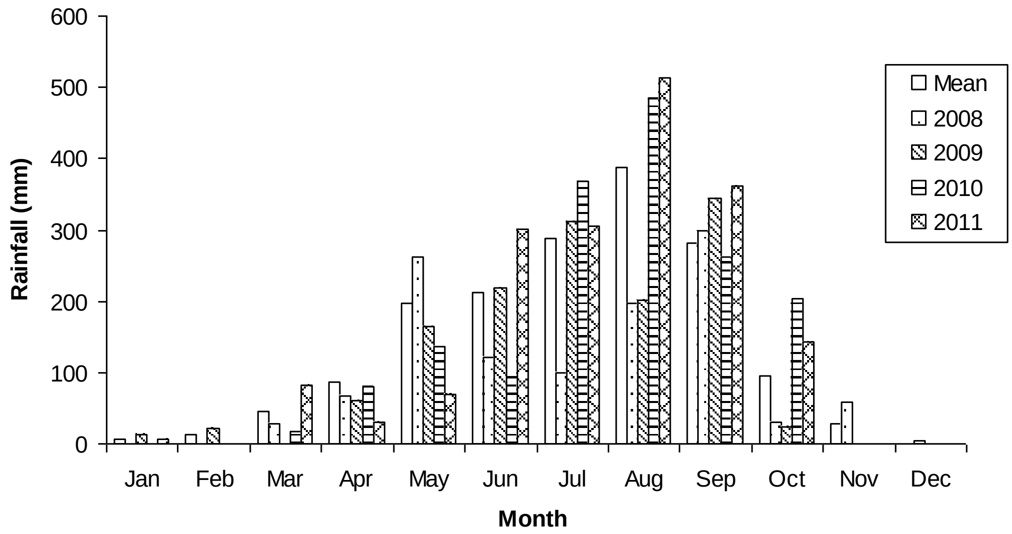

Figure 2. Rainfall at the Amnart Charoen meteorological station, 9 km from the research site, during the experiment and the 13-yr mean (2000–2012).

Experiment 2. BR06 collection. Rainfall for this experiment is shown in Figure 2. Rainfall for the July–September period in 2008 and 2009 was 38 and 22%, respectively, lower than the medium-term mean (13 years), while in 2010, it was 25% higher than the mean. For the October–November period, rainfall in 2008 and 2009 was 40 and 80%, respectively, lower than the medium-term mean. In 2010, rainfall for the same period was 62% higher than the mean but with no rain at all during November.

Seed production

Experiment 1. BR02 and MX02 collections. Seed yields ranged from 12 to 282 kg/ha and one line produced no seed at all in the second year. Hybrid brachiaria line BR06/1794 produced significantly higher seed yields than all other lines in both years, except for BR02/1718 and BR02/0465 in the second year (Table 4). BR02/1718 and BR02/0465 also produced higher seed yields than Mulato II in the second year but not in the first year. One line, BR02/1245, failed to produce any seed in the second year.

Mulato II produced significantly lower numbers of inflorescences per m^2 than many of the other hybrid lines, which were also significantly lower than the overall mean (Table 4). Lower numbers of inflorescences per m^2 were produced in the first year compared with the second year.

Racemes per inflorescence, spikelets per raceme and TSW were lower in the second year than in the first year (Table 4). There was large variability in spikelet numbers among the lines, ranging from 24 to 48. BR02/0465 produced significantly heavier seed (10.3–10.5 g per 1,000 seeds) than all other lines (Table 4). Three lines (BR02/1485, 1747 and 1794) had significantly higher TSW than Mulato II at both harvests.

Experiment 2. BR06 collection. Seed yields ranged from 6 to 659 kg/ha with 2 lines producing no seed in some years and 1 line producing no seed at any harvest (Table 5). In the first year (2008), BR02/1794 and Marandu produced significantly more seed than the other hybrid lines, including Mulato II. The majority of the BR06 lines had lower seed yields in 2008 than Mulato II, Marandu and Toledo and the BR02 lines, except for BR02/1372, which produced low seed yields at every harvest. In the second year (2009), BR02/0465 produced a significantly higher seed yield than the other hybrid lines and cultivars. Seed yields of many of the BR06 lines improved, with BR06/1278 producing similar seed yields to Mulato II, and BR06/0423 and BR06/1000 producing, respectively, 412 and 400 kg/ha. In the third year, seed yields of nearly all cultivars and lines declined significantly, except for BR06/1000 and 2058, which produced a little over 200 kg/ha (Table 5). BR06/1254 failed to produce seed at any harvest.

The majority of the BR06 lines produced significantly higher numbers of inflorescences (300–400/m^2) in the first year than the cultivars and the BR02 lines (Table 5). In the second year, inflorescence numbers increased compared with numbers in the first year for most lines and cultivars, with a similar range (300–600/m^2) for BR02 and BR06 lines and Mulato II. In the third year, there was a substantial decrease in inflorescence numbers for all lines and cultivars, particularly for Mulato II and Marandu. Toledo produced very few inflorescences in Years 1 and 3, and no inflorescences at all in Year 2.

Racemes per inflorescence declined with age, averaging 4.7 in the first year, 3.8 in the second year and 3.4 in the third year (Table 5). Overall, the majority of the BR06 lines produced fewer racemes per inflorescence than Mulato II. Five BR06 lines (0204, 0584, 1132, 1348 and 1696) produced numbers of racemes similar to or higher than Mulato II at each harvest.

Spikelet numbers per raceme were similar in the first and second seed harvests, 38 and 39, respectively, but declined to 34.6 at the third seed harvest (Table 5). Several BR06 lines produced more than 40 spikelets per raceme at each seed harvest, significantly higher than Mulato II and most BR02 lines.

BR02/0465 produced significantly heavier seed than all other lines and cultivars at all harvests, except for Toledo at the first harvest (Table 5). BR02/1794 and BR02/1718 produced significantly heavier seed than Mulato II and all 3 produced significantly heavier seed than all BR06 lines, except for BR06/0531, at the second and third harvests (Table 5).

Table 4. Seed yield and components of seed yield at peak anthesis of hybrid brachiaria lines during 2006 and 2007 in Ubon Ratchathani, Thailand (Experiment 1).

Hybrid line/ cultivar	Seed yield (kg/ha)		Inflorescences[1] (no./m^2)		Racemes/ inflorescence[1] (no.)		Spikelets/ raceme[1] (no.)		TSW[3] (g)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Mulato II	116	166	128	280	5.1	4.3	35.4	32.8	8.2	8.3
BR02/0465	87	244	230	352	5.6	4.4	36.0	26.4	10.3	10.5
BR02/0768	124	121	628	886	3.7	3.2	28.1	20.2	7.1	7.1
BR02/0771	94	58	533	454	4.6	3.5	32.3	24.3	7.4	7.6
BR02/0799	49	130	423	776	3.1	3.3	35.3	32.9	6.8	7.8
BR02/1245	74	-[2]	173	-	4.1	-	31.1	-	9.6	-
BR02/1372	20	23	509	468	3.6	3.1	48.4	36.7	6.7	6.6
BR02/1452	161	65	282	343	3.6	3.3	35.2	24.1	8.3	8.2
BR02/1485	59	50	278	413	4.2	3.1	36.9	25.4	9.3	9.3
BR02/1718	94	249	352	721	5.8	4.0	38.2	39.2	8.9	8.6
BR02/1728	85	73	306	257	4.0	4.5	35.3	32.0	7.8	7.1
BR02/1747	87	89	306	458	5.1	4.8	42.3	27.8	9.3	9.2
BR02/1752	118	155	346	351	3.9	3.4	40.2	30.0	8.7	9.0
BR02/1794	282	272	380	488	4.7	4.2	45.7	30.6	8.9	9.1
MX02/1263	154	12	483	181	4.6	3.4	41.0	27.6	8.8	8.9
MX02/1423	18	30	435	682	3.0	2.9	45.8	33.4	6.7	7.6
Mean	103	109	362	444	4.3	3.5	37.9	27.7	8.3	7.8
LSD (P≤0.05)	74	54	199	133	0.5	0.4	2.5	2.2	0.6	0.5
F ratio	6.94	13.19	3.83	35.03	27.9	81.5	42.7	135.8	35.2	194.3
Probability	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Counted at peak anthesis. [2]Failed to produce inflorescences. [3]One-thousand-seed weight.

Table 5. Seed yield and components of seed yield at peak anthesis of hybrid brachiaria lines during 2008–2010 in Amnart Charoen, Thailand (Experiment 2).

Hybrid line/ cultivar	Seed yield (kg/ha)			Inflorescences[1] (no./m^2)			Racemes/ inflorescence[1] (no.)			Spikelets/ raceme[1] (no.)			TSW[3] (g)		
	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010	2008	2009	2010
Mulato II	256	497	19	111	361	19	5.8	4.5	3.8	37.9	32.7	29.1	8.1	7.9	7.6
BR02/1794	370	206	95	231	348	132	5.7	3.7	3.9	36.7	42.5	36.6	9.4	8.4	8.8
BR02/0465	276	659	140	149	338	128	5.3	4.3	3.7	35.9	37.9	30.5	10.2	11.1	10.4
BR02/1718	309	492	151	221	528	105	6.0	3.1	4.3	37.5	36.7	34.2	8.8	8.3	8.0
BR02/1372	64	11	6	199	463	229	3.9	5.3	3.1	51.5	39.6	32.9	7.6	6.8	7.1
Marandu	344	334	103	94	152	23	4.8	3.9	3.3	47.0	38.3	32.7	9.0	8.4	8.4
Toledo	256	-[2]	36	48	-	23	6.5	-	4.0	35.5	-	29.2	11.4	-	9.6
BR06/0012	47	58	22	263	376	64	3.3	2.7	2.6	53.4	45.7	28.8	8.2	7.4	7.0
BR06/0204	18	67	51	64	185	126	6.7	6.0	3.7	31.8	35.3	53.2	6.7	6.7	7.3
BR06/0206	23	73	19	298	302	135	3.0	2.8	2.6	58.5	49.0	39.2	7.1	7.2	6.9
BR06/0387	176	264	143	407	575	271	4.7	3.1	2.8	41.8	41.7	27.2	6.9	7.1	7.1
BR06/0405	34	29	31	307	475	250	3.5	3.2	3.3	41.1	34.3	33.0	6.1	5.8	6.0
BR06/0423	218	412	58	266	356	41	5.8	4.6	4.2	38.5	37.8	36.3	7.1	7.1	6.6
BR06/0531	-[2]	141	54	-	280	137	-	3.2	2.9	-	38.4	30.6	-	8.2	8.1
BR06/0584	45	32	5	121	121	22	5.4	4.8	3.6	20.3	20.8	18.4	6.8	6.3	6.2
BR06/0850	148	204	42	188	275	95	4.5	3.8	3.0	24.9	48.3	44.6	6.0	5.6	5.2
BR06/1000	126	400	202	220	317	215	4.9	4.3	3.2	30.0	48.8	37.9	7.7	7.8	8.1
BR06/1132	114	220	41	281	421	102	6.6	4.9	4.3	30.6	31.7	31.7	6.8	6.9	6.6
BR06/1175	169	320	64	303	327	195	3.6	3.4	3.0	50.0	46.6	35.7	7.9	7.7	6.8
BR06/1254	-[2]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BR06/1278	137	483	72	223	262	100	3.3	3.2	3.0	57.5	55.0	46.4	7.8	7.6	6.9
BR06/1348	112	88	93	207	456	124	5.8	4.1	4.1	28.3	27.6	33.9	6.0	5.2	6.0
BR06/1366	172	71	65	339	324	216	3.5	3.4	3.1	58.7	50.7	43.8	6.8	6.0	6.3
BR06/1388	76	6	12	468	421	145	3.3	3.2	3.3	42.8	35.0	35.4	5.7	5.4	5.6
BR06/1415	90	134	29	462	416	136	3.7	4.2	4.0	50.3	40.3	35.3	6.7	5.7	5.5
BR06/1433	95	13	11	444	634	272	3.1	2.2	2.2	41.7	31.8	26.1	6.2	5.5	6.1
BR06/1454	164	28	17	367	447	231	3.3	3.2	2.9	53.8	50.6	43.7	6.9	6.4	6.5
BR06/1567	57	71	28	368	359	69	4.0	3.8	3.4	19.3	22.9	20.1	6.6	6.0	6.2
BR06/1696	200	228	106	388	394	265	7.2	5.8	4.2	47.3	37.2	28.6	7.8	7.5	6.7
BR06/1832	121	214	65	336	442	73	5.2	4.2	3.3	34.2	34.6	33.4	7.3	7.1	7.8
BR06/1922	140	59	38	379	316	131	3.7	3.4	3.1	25.4	35.1	33.2	6.6	6.0	6.5
BR06/1932	46	269	39	171	318	122	4.7	2.9	3.0	26.8	50.9	48.2	7.0	7.2	6.8
BR06/2020	58	161	56	220	333	176	4.2	3.6	3.4	31.7	44.5	41.4	7.0	6.9	6.1
BR06/2058	223	315	205	395	532	155	6.0	4.5	3.6	30.9	33.8	32.0	7.1	7.2	7.1
BR06/2204	52	99	85	234	342	117	3.8	3.5	3.0	32.7	33.9	34.5	7.2	6.6	7.6
Mean	135	191	63	251	349	133	4.4	3.6	3.3	37.0	36.9	33.7	7.0	6.6	6.8
LSD (P<0.05)	59	60	32	62.	69	42	0.4	0.3	0.3	2.5	3.6	3.0	0.4	0.3	0.4
F ratio	21.7	66.5	22.2	33.0	32.1	26.6	129.6	113.5	63.1	247.9	89.0	77.4	220.8	270.9	121.6
Probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]Counted at peak anthesis. [2]Failed to produce inflorescences. [3]One-thousand-seed weight.

Discussion

The main aims of the experiments were to identify hybrid brachiaria lines with seed yields higher than Mulato II and equal to or better than commercial seed yields of over 600 kg/ha produced by commercial brachiaria species in Australia and Brazil (Hopkinson and Clifford 1993; Souza 1999). These aims were partially achieved. While BR02/0465 was the only hybrid line that produced in excess of 600 kg/ha (659 kg/ha in the second harvest at Amnart Charoen), a number of lines produced more seed than Mulato II. The best overall seed producer, BR02/1794 (95−370 kg/ha), produced significantly more seed than Mulato II (19−497 kg/ha) in both harvests at Ubon Ratchathani and 2 of 3 harvests at Amnart Charoen. The next best lines (BR02/1718 and BR02/0465) produced more seed than Mulato II in 1 of 2 harvests at Ubon Ratchathani and 2 of 3 harvests at Amnart Charoen. BR02/1794 and BR02/0465 produced heavier seed (TSW) than Mulato II at every harvest.

The 3 lines above all reached peak flowering earlier than Mulato II (Table 3). BR02/1794 usually flowers earliest (late September–early October). Peak flowering of BR02/1718 and BR02/0465 is usually 2 to 3 weeks later in mid-October, while peak flowering of Mulato II is nearly always in the second week of November in Northeast Thailand.

Different flowering times can strongly influence seed production. If late October–early November is particularly dry, late-flowering species can fail to set seed on sandy soils with low soil moisture retention. This appeared to be the case with Mulato II in the third year at Amnart Charoen (2010). Heavy rainfall in the first half of October benefited the earlier-flowering lines, but with no rain falling from late October onwards, Mulato II produced only 19 inflorescences per m^2 and only 19 kg/ha of seed was harvested.

The seed yields from the BR06 lines overall were disappointing. It was only when Mulato II failed to produce a large number of inflorescences at the third seed harvest at Amnart Charoen, that the BR06 lines produced more seed than Mulato II. However, these third-harvest seed yields were also extremely low, averaging only 60 kg/ha.

Our experience with seed production of hybrid brachiaria grasses in Thailand is that seed yields decline with age, even though adequate levels of soil N are maintained by applying fertilizer. At the Ubon Ratchathani site seed yields from the first and second seed harvests were similar but at Amnart Charoen, seed yields were higher at the second harvest than at the first and very low for nearly all lines at the third harvest. The decline in seed yield over years in many tropical grass species is considered to be caused by larger tillers in older stands providing nutritional support for weaker tillers (low-yielding or sterile) to the detriment of their own seed development and the long-term productivity of the stand (Loch et al. 1999), though for the brachiaria lines we have no data to support this hypothesis. Farmers in Thailand have found that seed yields from second-year hybrid brachiaria grass seed crops were less than half those of first-year seed crops. In order to get satisfactory seed yields, (300−400 kg/ha of clean seed), they treat hybrid brachiaria grass seed crops as annuals and replant every year, as they do with all other tropical grass seed crops (Hare 2014).

We consider that cleaned seed yields from commercial operations must be above 600 kg/ha for the seed prices of the hybrid brachiaria cultivars to become competitive with other commercial cultivars of brachiaria species. Some farmers in Thailand can produce more than 600 kg/ha of Mulato II seed by ground-sweeping but the majority produce only about 385 kg/ha (Hare 2014). Farmers in Northern Laos currently average 250 kg/ha of Mulato II seed from hand-knocking seed from seedheads (Hare 2014). In our experiments, we have at times produced 500 kg/ha of clean seed (98–99% purity by weight) (Hare et al. 2007b) by catching the seed in bags tied over the seedheads, but these occasions have been extremely rare. Commercial seed production of Mulato II is still very erratic.

Another factor which adds to the cost of hybrid brachiaria seed production is acid-scarification. This results in a loss of seed weight of 15–20% from scarifying off the glume, lemma and palea around each seed, light and empty seed, and small amounts of viable seed. Even though some viable seed is lost, without acid-scarification, germination of the seed fails to exceed 30% (Hare 2014).

Nearly all hybrid lines produced sufficient numbers of inflorescences, racemes and spikelets to indicate a potential for useful seed yields. In the trials at Ubon Ratchathani, most hybrid lines produced 300–500 inflorescences/m^2 and at Amnart Charoen, the BR06 lines produced 300–500 inflorescences/m^2 in the first and second years. Mulato II produced fewer inflorescences than the new lines at both sites. Inflorescence numbers have nearly always been the main indicator of whether a forage plant has the potential to produce seed. However, with hybrid brachiaria grasses, it appears that seed-set is the most determining factor of seed yields. By seed harvest there seems to be a massive failure of seed-set, caryopsis maturation or both, with the cleaned seed coming from fewer than 10% of spikelets. In other brachiaria species it is not uncommon for abscission to precede maturation in a high proportion of spikelets (Hopkinson et al. 1996), but in the hybrid brachiaria grasses it appears to be an extremely high proportion.

Previous studies have shown seed yields of Mulato II are generally very low, with fewer than 2% of the spikelets formed producing viable seed (Hare et al. 2007a). There is speculation that this low seed-set is caused by pollen sterility, as Risso-Pascotto et al. (2005) found that more than 65% of pollen grains in brachiaria interspecific hybrids (*B. ruziziensis* x *B. brizantha*) were sterile and this sterility was genetic. Miles and Hare (2007) suggested this poor seed-set may be a common defect of newly formed apomictic forage grass hybrids. They referred to failures of buffel grass hybrids, which produced erratic and usually poor seed yields, leading to high seed prices. A hybrid-derived apomictic bahiagrass clone was not released because of concerns of low seed yields (Miles and Hare 2007).

In these current studies, we did not examine the percentage of spikelets that formed a caryopsis to calculate biological seed-set, but rather calculated economical seed-set, which is the ratio between realized and potential seed yield (Elgersma 1985). Mulato II seed-set (percentage of the number of cleaned seed to formed spikelets) ranged from 1.6 to 3.8% at Ubon Ratchathani and from 11.8 to 12.9% at Amnart Charoen. BR02/1794, which had superior seed yields to Mulato II, averaged 4.3% seed-set across both sites and seed-set for BR02/1752 ranged from 1.1 to 3.6% across both sites. Values for BR02/1718 were 1.8–3.1% at Ubon Ratchathani, and 7.0–12.2% at Amnart Charoen. Similarly, BR02/0465 had seed-set of 1.6–3.8% at Ubon Ratchathani, rising to 9.3–10.8% at Amnart Charoen.

The superior seed-set at Amnart Charoen compared with Ubon Ratchathani is interesting. Amnart Charoen is farther north than Ubon Ratchathani (15.5° vs. 15° N) and at a slightly higher elevation (168 vs. 130 masl). Grof (1968) showed that Basilisk signalgrass could set good seed yields and these seed yields were enhanced in drier upland regions in tropical latitudes (Loch et al. 1999). Basilisk seed production in Australia is predominantly on the Atherton Tablelands at lower latitudes but at elevations of 600–900 masl. In Brazil, successful seed production of Basilisk signalgrass and cv. Marandu is in the higher tropical latitudes (20 and 22° S) and at elevations of 700–1,000 masl (Souza 1999). The slightly higher elevation at Amnart Charoen compared with Ubon Ratchathani may have compensated for insufficient latitude and encouraged greater seed-set. Ferguson et al. (1983) showed that, at similar latitudes in South America (15–19° S), the site with the highest elevation (1,000 masl) produced the highest seed yields of signalgrass, even though it had the lowest latitude (15° S).

Under commercial conditions in Thailand, we have produced hybrid brachiaria seed in Ubon Ratchathani, Amnart Charoen, Mukdahan and Roi-et provinces. It is only in the more northerly province, Roi-et (16.8° N; 160 masl), that farmers still continue with Mulato II seed production (Hare 2014). In the other provinces seed yields are too low and erratic to be economical and farmers have ceased production. Roi-et farmers, however, found that BR02/1752 seed yields (100–200 kg/ha) were too low to interest them; thus BR02/1752 seed production is limited to Northern Laos (19–21° N; 700–1,200 masl), where farmers find the seed yields satisfactory (200–300 kg/ha) under their low-input management (Hare 2014). We have also commenced seed production of BR02/1794 in these Northern Laos provinces.

While 43 hybrid Brachiaria lines were evaluated for seed yield between 2005 and 2010, only 3 lines, BR02/1794, BR02/1718 and BR02/0465, displayed a potential for seed yields greater than or equal to Mulato II. BR02/1752 had seed yields similar to or slightly lower than Mulato II, though in another study at Ubon Ratchathani, BR02/1752 and BR02/1794 produced significantly higher seed yields than Mulato II (Bouathong et al. 2011).

In considering the commercial release of hybrid brachiaria lines as named cultivars, forage production and quality (Hare et al. 2015) and seed production were important considerations, together with the waterlogging tolerance of BR02/1752, released as cv. Cayman (Pizarro et al. 2013), and the upright nature of BR02/1794 for cut-and-carry forage, released as cv. Cobra (E. Stern pers. comm.).

Further research is needed to verify the influence of elevation and latitude on flowering and seed-set in hybrid brachiaria grasses. In future breeding of new hybrids, there must be more rigorous selection for seed production characteristics at latitudes and sites typical of where commercial brachiaria seed production occurs.

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