Effect of nitrogen rates on plant growth, seed yield and seed quality of three lines of brachiaria hybrid grass

Charouvanh Bouathong[1, 2], Michael Hare[2*], Manas Losirikul[2] and Kitti Wongpichet[2]

ABSTRACT: Improved grasses planted for forage are increasing in demand throughout Thailand due to the increasing number of cattle. New varieties are being introduced in response to new demands. Brachiaria grasses, especially the hybrids, are gaining popularity because of their good yields and high quality. However, seed production of the brachiaria hybrids is difficult. The objectives of this experiment were to find out the appropriate rate (0, 20, 40, or 80 kg/ha N) of N fertilizer (46-0-0) for growth, seed yield and quality of three lines of brachiaria hybrid (Mulato II, BR02/1752, and BR02/1794). This experiment composed of a field trial and laboratory tests and all were conducted at the Faculty of Agriculture, Ubon Ratchathani University, Thailand from May 2009 to June 2010. The results showed that N fertilizer at 40 kg/ha N was an optimum rate for seed production and growth (tiller number) for all three brachiaria hybrids. BR02/1752 and BR02/1794 produced significantly ($P<0.05$) higher seed yields than Mulato II. The quality of seeds produced in terms of purity and moisture content was not affected by N fertilization. Dormancy in new seeds was common, but declined over time. However, this seed dormancy could be broken by soaking in sulfuric acid for 10 minutes or passing through an accelerated ageing technique for about three days.
Keywords: brachiaia hybrid grass, nitrogen, plant growth, seed yield, seed quality

Introduction

Planting improved grasses for forage using seed has increased in popularity in Thailand due to the increasing number of cattle (สายัณห์, 2548). The higher demand for forages has been due to the decrease in natural grass supply from both public and private lands (สมศักดิ์ และคณะ, 2542 and Hare and Horne, 2004). Government departments and private organizations have been trying to improve forage production. Common practices to improve production include fertilizer management, especially nitrogen, which is a major limitation to grass production (Hare et al., 1999a). Cutting and grazing managements also play important roles as well (Hare et al., 2001; and Hare et al., 2004). Another relatively easy and quick method is by introducing new forage varieties.

Grasses such as Purple guinea grass, ruzi grass, and Mulato II grass are well known grasses at present in Thailand. It is interesting to see the new hybrid grass Mulato II (*Brachiaria ruziziensis* x *B. decumbens* x *B. brizanta* - released by the International Center of Tropical Agriculture in 1989) has been quickly sought after due to high yields and high quality (CIAT, 2004). For forage production, Mulato II has adapted well to the conditions in northeast Thailand and is very drought tolerant (Hare et al., 2009). However, this brachiaria hybrid does not produce high seed yields (Hare et al., 2007) and seed dormancy is a problem (Hare et al., 2011).

[1] Faculty of Agriculture, National University of Laos, Laos PDR
[2] Faculty of Agriculture, Ubon Ratchathani University
* Corresponding author: michaelhareubu@hotmail.com

New lines of brachiaria hybrid are being evaluated at Ubon Ratchathani University, with BR02/1752 and BR02/1794 showing promise (Hare et al., 2011). Therefore the objectives of this research were to find out the appropriate nitrogen (N) rate for plant growth, seed production and seed quality of three lines of brachiaria hybrid (Mulato II, BR02/1752, and BR02/1794), and study methods of breaking seed dormancy.

Materials and Methods

This experiment composed of two parts; a field trial and laboratory tests. All trials were conducted at the Faculty of Agriculture, Ubon Ratchathani University, Thailand from May 2009 to June 2010.

Field trial (May - December, 2009) A 4 x 3 factorial experimental in a randomized complete block design with two factors was used. Factor A composed of four rates of N fertilizer (urea 46-0-0) and were 0, 20, 40, and 80 kg/ha N; and factor B composed of three lines of brachiaria hybrid which were Mulato II, BR02/1752, and BR02/1794. There were four replications.

Before the trial began, soil samples were collected for laboratory tests (pH, organic matter, nitrogen, phosphorus, potassium, and texture) following the methods described by นพมาศ (2545).

The site was ploughed, disced, and harrowed before planting. Each plot size was 16 m^2 [4 m x 4 m] with 50 cm x 80 cm plant spacing.

Seedlings of the three grass lines were prepared in advance by planting four to five seeds of each line, on April 25-27, 2009, into black plastic bags. At one month of age (about 30 cm high), on May 26, 2009, seedlings were transplanted into the prepared trial plots (one position one bag). Fifty days after planting, a close cutting (5 cm above ground level) was done to adjust plot uniformity.

N was split and applied twice at half the rate of each treatment. The first fertilization just followed the closing cutting (Mulato II, BR02/1752, and BR02/1794 on August 10, July 27, and 13, 2009, respectively), and the second at six weeks after closing cutting (flowering started).

Due to the low fertility of Roi-et soil in the field, additional nutrients were applied according to past experiences. Dolomite, copper sulfate, and boron at the rates of 860, 20, and 10 kg/ha, respectively were applied at the time of land preparation before seedling transplanting. In addition, phosphorus (0-40-0) and potassium (0-0-60) at the rates of 20 and 50 kg/ha, respectively, were applied along with N in split applications.

Data of plant height (cm) and number of tillers were taken at 2, 4, 6, and 8 weeks after transplanting following the methods described by ไมเคิล และคณะ (2550).

At 50% flowering, inflorescences were tied together and covered with a nylon net-bag with an opening tied at the bottom. Six clumps of grass/plot were covered separately. When the mature seeds fell off within the bag, the opening was opened to drain out all the seeds for collection. Then the seeds were hand cleaned, air dried, and weighed for seed yield.

At 50% flowering, the number of inflorescences/clump, number of spikelets/raceme followed the methods described by Hare et al. (1999b). One raceme/inflorescence (three per clump) was randomly picked, and thousand seed weight and seed yield followed those described by ไมเคิล และคณะ (2550).

Meteorological data (rainfall, temperatures, and day length) were supplied from an agro-meteorological station next to the trial site and the Lower Northeast Meteorological Center, Ubon Ratchathani about 20 km to the north.

Laboratory tests (January - June, 2010) Design as above (1 Field trial) with four replications.

Grass seeds obtained from each treatment in the above field trial were tested following the rules of International Seed Testing Association (กองขยายพันธุ์พืช, 2543).

Cleaned seeds were stored in nylon net-bags at room temperature. At two months after harvesting, tests for seed quality began, which included seed purity, seed moisture content, seed germination, seed dormancy breaking, and accelerated seed ageing.

Seed dormancy breaking was done by soaking seeds (2, 4, and 6 months after harvesting in storage) in sulfuric acid (98%) for 0, 5, 10, and 15 minutes. Then the treated seeds were cleaned in running water and air dried before being tested for germination by using the top of paper method (in Petri dishes, one dish one replication).

Accelerated seed ageing was done by subjecting the seeds to 42°C and nearly 100% relative humidity, with the seeds spread out on a wire mesh, placed over water within a sealable glass jar and then placed in an oven for 0, 74, and 84 hours. Then the seeds were tested for germination by using the top of paper method as above.

Data obtained from all trials were analyzed by analysis of variance using IRRISTAT program, and Duncan's new multiple range tests were used for mean comparisons (Gomez and Gomez, 1983).

Results and Discussion

Weather Rainfall, day length, and maximum and minimum temperatures during this experiment, in general, conformed to the normal pattern (**Figure 1**). The grasses received 1,603.9 mm or 178.2 mm/month of rain, which was sufficient for growth. Haefele et al. (2006) suggested that brachiaria grasses needed only 83-125 mm of rain monthly for their growing cycle. It should be noted that during seed harvesting in November - December, rainfall had almost ceased, which improved seed quality at harvest.

Day length plays an important role in brachiaria grasses for growth and shorter days for flower induction (สายัณห์, 2548). Temperatures during this experiment were also favorable for grass growth. The average temperature was 27.3°C, which was within the optimum range of 25-35°C for brachiaria grass (สายัณห์, 2548).

Soil Pre-trial soil tests showed that the Roi-et series soil in the field was poor (pH 4.7, organic matter 0.76%, total N 0.03%, available P 32.45 ppm, and available K 26.15 ppm). This soil had up to 78.84% sand, and as described by เอิบ (2534) these soils are usually not suitable for cash crop production.

Growth Prior to the first N fertilization, growth of all three lines of brachiaria hybrid in the field was not uniform. However, following cutting and fertilization the plots became more uniform.

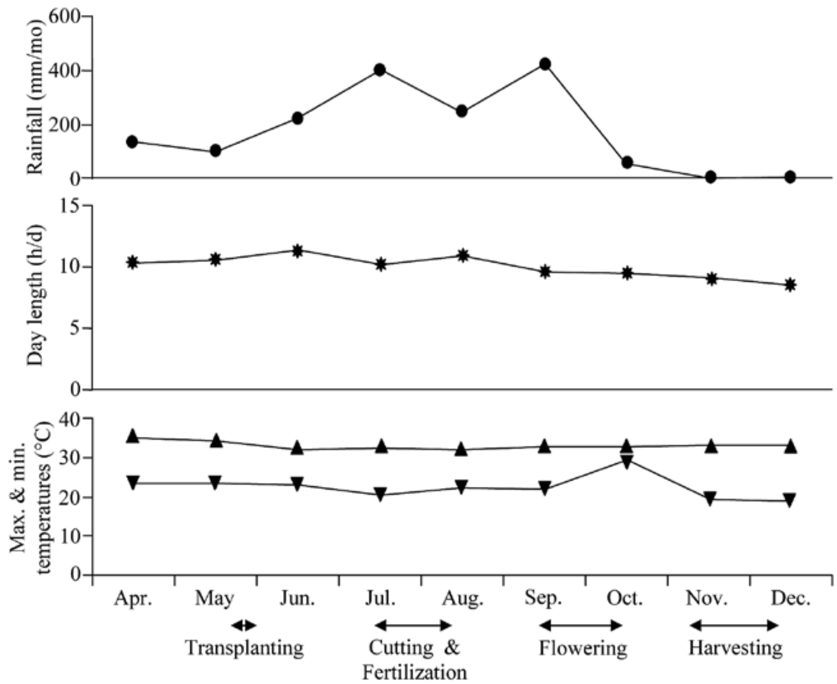

Figure 1 Monthly rainfall, average day length, and average maximun and minimum temperatures at Ubon Ratchathani University during the 2009 growing season.

All lines of grass were planted in plastic bags and transplanted into the field on the same day. However, close cutting, 50% flowering, and harvesting dates of all lines were different. BR02/1794, BR02/1752, and Mulato II flowered on September 12, September 28, and October 11, respectively; and were harvested on November 7, November 25, and December 10, respectively. This may be due to different genetic make-up between the lines (Miles and Hare, 2007). Consequently they responded differently to the environment, particularly day length. For seed production this might be good to have diverse lines for various conditions.

The interactions between N rates and brachiaria lines for height and tiller number were not significant (P>0.05; **Table 1**). Height of brachiaria hybrids were only affected by rates of N 2 weeks after transplanting, which was similar to the result reported by ศรัณยา และคณะ (2545) in *B. brizantha*. However, N significantly (P<0.05) affected tiller numbers of the grasses, with 40 kg/ha N producing nearly 50% more tillers than plants receiving no N. N at 80 kg/ha reduced tiller numbers. This reduction may have been due to an abundance of green leaves from high N reducing tillering, and was similar to the results reported by สมศักดิ์ และคณะ (2542), where high N application (300 kg/ha N) did not linearly increase tillers of Purple guinea grass. N at 40 kg/ha was the optimum at every growth stage. This suggests a stronger effect of genetic make-up on height and a stronger effect of N on tillering capacity.

The different lines of brachiaria hybrid, in general, performed similarly for both height and number of tillers, with only BR02/1794 having more tillers than Mulato II at 8 weeks after transplanting.

Table 1 Effect of nitrogen rates and hybrid brachiaria lines on plant height (cm) and number of tillers per plant.

Factor	Treatment	2 WAT[1/]		4 WAT		6 WAT		8 WAT	
		Height	Tillers	Height	Tillers	Height	Tillers	Height	Tillers
Nitrogen rate (kg/ha) (A)	0	44.8±1.00b[2/]	47.3±0.58b	66.3±1.28	62.0±1.00c	92.4±1.12	75.6±1.20c	120.5±1.02	83.3±1.28b
	20	48.7±1.23b	50.6±1.00a	68.8±1.05	73.3±1.04b	101.1±1.11	84.3±1.18b	126.5±1.09	94.3±1.25ab
	40	66.0±1.05a	57.3±0.99a	79.2±1.11	86.6±1.02a	112.2±1.05	98.6±1.05a	138.5±0.99	123.0±1.11a
	80	56.9±2.00a	47.3±0.89b	81.1±1.15	67.6±1.08b	106.1±1.02	80.3±1.09b	128.3±1.00	92.3±1.09b
	Average	54.1	50.6	73.9	72.4	103.0	84.7	128.4	98.3
Brachiaria line (B)	Mulato 2	55.1±1.05	50.7±1.11	72.5±1.12	74.5±1.10	107.2±1.08	88.7±1.08	123.7±1.09	95.5±1.28b
	BR02/1752	54.2±1.08	49.2±1.09	75.5±1.09	67.2±1.11	95.1±1.05	78.0±1.03	130.6±1.11	97.0±1.11ab
	BR02/1794	53.0±1.07	52.0±1.12	73.7±1.08	75.5±1.19	106.6±1.06	87.5±1.04	131.0±1.12	102.3±1.10a
	Average	54.1	50.6	73.9	72.4	103.0	84.7	128.4	98.3
	CV (%)	11.4	9.5	12.4	10	9.8	12.2	9.9	10.1
Level LSD ($p<0.05$)	A	10.0	6.8	ns[3/]	9.4	ns	6.3	ns	9.0
	B	ns	ns	ns	ns	ns	ns	ns	7.8
	A x B	ns	ns	ns	ns	ns	ns	ns	ns

[1/] weeks after transplanting;

[2/] Within a column for each effect, values followed by different letters are significantly different ($p<0.05$);

[3/] non significant

Yield components and seed yield The interactions between N rates and brachiaria lines for some major yield components and seed yield were not significant (P>0.05; **Table 2**). N at 40 kg/ha gave significantly (P<0.05) higher seed yields than other N rates. The highest seed yield at this N rate was a result of better yield components of numbers of inflorescences/plant (75.1), spikelets/raceme (45.0), and 1,000 seed weight (7.0 g). N has been shown to increase grass inflorescences (Humphrey and Riveros, 1986; and Loch et al., 1999) but not racemes (Hare et al., 1999b), and also has increased 1,000 seed weight in other brachiaria grasses (Gates and Slife, 1998; and Loch et al., 1999) and switch grass (Kassel et al., 1985). N rates at 0, 20, and 80 kg/ha N produced similar yields, which were 20% lower than yields from 40 kg/ha N. High rates of N at 80 kg/ha produced lower vegetative tiller numbers compared to N at 40 kg/ha (**Table 1**) and this then resulted in a lower number of inflorescences/plant (**Table 2**), which is the most important seed yield component (Hare, 2005).

The different brachiaria lines produced different seed yields, with BR02/1752 and BR02/1794 producing significantly (P<0.05) higher seed yields than Mulato II (**Table 2**). BR02/1752 produced 46% more seeds than Mulato II. The higher seed yields from BR02/1752 and BR02/1794 were due to the higher numbers of inflorescences/plant, racemes/inflorescence, and spikelets/raceme compared to Mulato II. BR02/1752 is now a promising brachiaria line as other forage parameters are comparable to Mulato II and it has been registered as cultivar Cayman by Grupo Papalotla (Eduardo Stern, personal communication) and has been registered for plant variety rights (Loch et al. 2011).

Seed purity and seed moisture content The interactions between N rates and brachiaria lines for seed purity and moisture content were not significant (P>0.05; **Table 3**). The purity of seeds harvested was not different and was high (90%), due to good management from the time of seed setting to harvesting to cleaning. These practices and high seed purity obtained are common for grass seed production in Thailand, for example, in Mulato (กานดา และคณะ, 2549), Ruzi (จุรีรัตน์ และคณะ, 2532), *Paspalum atratum* (สำราญ และคณะ, 2543), and Purple guinea (สมศักดิ์ และคณะ, 2542).

The moisture content of newly harvested seeds was not different (**Table 3**) due to good management during post harvesting. The seeds were air dried to 11-12% seed moisture content and this was safe for short term storage. After six months in storage, seed moisture content was not different and had further decreased to 8.0-9.2%. This was probably due to the decrease of relative humidity from the beginning to the middle of seed storage (relative humidity in December, 2009, January, February, March, and April 2010 was 91, 86, 89, 78, and 80%, respectively).

Seed dormancy Due to the high dormancy of new brachiaria seeds, two methods were employed to break seed dormancy, sulfuric acid soaking and accelerated ageing.

The interaction between N rates and brachiaria lines for seed germination after acid soaking was not significant (P>0.05; **Table 4**). Germination of brachiaria hybrid seeds, receiving different N rates and of different lines, and stored at three periods (2, 4, and 6 months) were similar. These results were similar to that reported in *P. atratum* (โอภาส และคณะ, 2544). However, length of time in storage and in sulfuric acid greatly increased seed germination. Hare et al. (2008) found similar results with Mulato hybrid brachiaria. Soaking in acid increased seed germination compared to control, with soaking up to 10 minutes giving optimum germination. Acid soaking for 15 minutes damaged many seeds and resulted in lower germination. For good pasture establishment, brachiaria hybrid seeds stored for at least six months and acid-treated should be used. All brachiaria hybrid seeds sold in Thailand are acid-treated (Hare et al., 2011).

Table 2 Effect of nitrogen rates and hybrid brachiaria lines on seed yield components and seed yield.

Factor	Treatment	Inflorescences /plant	Racemes/ inflorescence	Spikelets /raceme	1,000 seed weight (g)	Seed yield (g/m^2)
Nitrogen rate (kg/ha) (A)	0	62.5±1.28b[1/]	4.1±1.05	41.6±1.34b	6.6±1.99b	20.5±1.20b
	20	60.3±1.09b	4.2±1.11	42.3±1.25b	6.8±1.28b	21.2±1.12b
	40	75.1±1.19a	4.6±1.08	45.0±1.83a	7.0±1.34a	26.4±1.23a
	80	63.8±1.14b	4.4±1.99	43.5±1.05ab	6.8±1.30b	21.0±1.19b
	Average	65.4	4.3	43.1	6.8	22.3
Brachiaria line (B)	Mulato 2	47.7±1.34b	4.0±1.20b	29.3±1.18c	6.7±1.14	14.9±1.14b
	BR02/1752	75.6±1.23a	4.5±1.19a	51.3±1.15a	6.9±1.05	27.4±1.11a
	BR02/1794	73.0±1.13a	4.5±1.21a	48.7±1.10b	6.8±1.23	24.6±1.10a
	Average	65.4	4.3	43.1	6.8	22.3
	CV (%)	20.9	11.0	6.0	3.5	19.4
Level LSD (p<0.05)	A	11.3	ns[2/]	1.8	0.1	3.1
	B	9.8	0.3	2.1	ns	3.6
	A x B	ns	ns	3.7	ns	ns

[1/] Within a column for each effect, values followed by different letters are significantly different (p<0.05);
[2/] non significant

Table 3 Seed purity and seed moisture content of hybrid brachiaria grasses as affected by N rates and grass lines.

Factor	Treatment	Seed purity[1/] (%)	Seed moisture content (%) At harvesting	Seed moisture content (%) Six months after harvesting
Nitrogen rate (kg/ha) (A)	0	89.3±1.03	12.0±1.13	8.0±1.14
	20	90.3±1.11	13.0±1.11	9.0±1.13
	40	90.3±1.09	12.0±1.12	8.0±1.12
	80	90.3±0.99	11.0±1.13	9.0±1.15
	Average	90.0	12.0	8.5
Brachiaria line (B)	Mulato 2	90.0±1.13	12.0±1.10	8.0±1.08
	BR02/1752	90.0±1.13	12.0±0.99	8.3±1.07
	BR02/1794	90.0±1.12	13.0±1.10	9.2±1.08
	Average	90.0	12.0	8.5
	CV (%)	4	7	5
Level LSD (p<0.05)	A	ns[2/]	ns	ns
	B	ns	ns	ns
	A x B	ns	ns	ns

[1/] at harvesting; [2/] non significant

Table 4 Effect of H_2SO_4 soaking times on seed germination (%) of hybrid brachiaria grasses.

Factor	Treatment	Seed soaking time (mn) of 2 month storage				Seed soaking time (mn) of 4 month storage				Seed soaking time (mn) of 6 month storage			
		0	5	10	15	0	5	10	15	0	5	10	15
Nitrogen rate (kg/ha) (A)	0	0	5.0±1.00	25.0±1.00	12.0±1.00	3.0±1.00	20.0±1.05	45.0±1.11	40.0±1.04	33.0±0.99	50.0±1.12	73.0±1.11	60.0±0.85
	20	0	7.0±0.99	30.0±1.02	18.0±1.11	6.0±0.99	23.0±1.02	50.0±1.15	39.0±1.08	36.0±0.95	53.0±1.00	77.5±1.00	69.0±0.95
	40	0	7.0±0.85	49.0±1.11	25.0±1.14	7.0±0.98	23.0±1.05	69.0±1.09	45.0±1.09	37.0±1.00	53.0±1.15	80.0±1.09	75.0±1.00
	80	0	8.0±0.90	39.0±1.09	20.0±1.09	6.0±1.00	33.0±1.28	58.0±1.11	47.0±1.11	36.0±1.12	59.0±1.02	84.0±1.08	77.0±0.98
	Average	0	6.7	35.7	18.7	5.5	24.7	55.5	42.7	35.5	53.7	78.7	70.2
Brachiaria line (B)	Mulato 2	0	5.5±1.00	26.9±0.98	10.0±1.00	4.0±1.11	19.0±0.99	49.5±1.02	37.0±0.99	29.0±0.99	51.0±0.85	76.0±1.00	69.0±0.99
	BR02/1752	0	7.7±1.13	35.0±0.99	19.0±1.02	7.0±1.00	29.0±0.98	53.5±1.06	45.0±0.99	37.0±0.98	53.0±0.99	80.0±1.13	69.1±0.98
	BR02/1794	0	7.0±0.99	45.0±0.98	27.0±1.03	6.1±1.12	26.2±0.99	63.5±1.04	46.3±0.98	40.5±1.00	57.3±0.98	80.0±1.14	73.0±1.00
	Average	0	6.7	35.7	18.7	5.5	24.7	55.5	42.7	35.5	53.7	78.7	70.2
	CV (%)	-	5	10	7	0	5	10	7	4	7	9	7
Level LSD ($p<0.05$)	A	ns[1/]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	A x B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1/] non significant

Table 5 Effect of accelerated ageing times on seed germination (%) of hybrid brachiaria grasses, two months after harvesting.

Factor	Treatment	Accelerated ageing time (hr)			
		0	74	84	94
	0	0	50.7±1.00	27.4±1.13c[1/]	16.4±1.15
Nitrogen rate	20	0	54.0±1.13	29.6±1.00b	17.0±1.14
(kg/ha)	40	0	59.0±1.15	29.0±0.99b	18.0±1.15
(A)	80	0	54.0±1.00	30.3±0.98a	17.3±1.00
	Average	0	54.3	29.0	17.1
Brachiaria	Mulato 2	0	50.5±0.99c	27.9±0.97	15.6±1.09b
line	BR02/1752	0	54.0±1.00b	29.3±1.00	18.0±1.15a
(B)	BR02/1794	0	58.4±1.13a	30.0±1.15	18.0±1.18a
	Average	0	54.3	29.0	17.1
	CV (%)	0	9.5	8.5	14.7
Level	A	ns[2/]	ns	0.7	ns
LSD	B	ns	1.2	ns	1.8
(p<0.05)	A x B	ns	ns	ns	ns

[1/] Within a column for each effect, values followed by different letters are significantly different (p<0.05);

[2/] non significant

The interaction between N rates and brachiaria lines for seed germination after accelerated ageing was not significant (P>0.05; **Table 5**). Results of dormancy breaking by using accelerated ageing method did not differ among N treatments and brachiaria hybrid lines (**Table 5**). Gobius et al. (2001) reported that different N rates also did not affect seed germination of *B. decumbens* cv. Basilisk, Gamba grass, and Jarra digit. Accelerated ageing increased germination percentage compared to control (0%), with 74 minutes being optimum (54.3%). Longer times (84 and 94 minutes) decreased seed germination. Exposing seeds to very high relative humidity for long periods increases seed moisture content and causes detrimental effects to seeds (Delouche et al., 1973) and consequently lowers germination. The laboratory test used was a modified test used for rice seeds which have tough seed coverings, whereas the grass seed coverings are thinner, and consequently the results obtained in this study were not satisfactory. The periods may have been too long and shorter time periods may have given high germinations.

For both dormancy breaking methods, acid-treated and accelerated ageing, untreated two months old brachiaria seeds did not germinate, whereas treated seeds germinated much better (**Tables 4 and 5**). At two months after harvest, seeds passing through accelerated ageing technique germinated better compared to those soaked in acid. But at six months after harvest, acid-treated seeds for 10 minutes gave germinations of 73-84% (**Table 5**). However, there are some points to be considered. Acid soaking took a shorter time to do but great care must be taken to avoid serious accident to the seed handler and the seeds as well, while accelerated ageing took quite a long time but was safe to the seed handler.

Conclusions

N fertilizer at 40 kg/ha was an optimum rate for seed production and growth (tiller number) of brachiaria hybrids in Ubon Ratchathani.

Among three lines of brachiaria hybrid studied, BR02/1752 and BR02/1794 produced higher seed yields than Mulato II.

The quality of brachiaria seeds produced in terms of purity and moisture content was not affected by N fertilization. Seed purity was very high and seed moisture content was low and safe for short term storage.

Dormancy in new brachiaria seeds was common, but declined over time. Seed dormancy could be broken by acid soaking for 10 minutes or accelerated ageing technique for up to three days. For commercial seed of brachiaria hybrids in Thailand, seeds are acid-treated for 10 minutes. Seeds stored for 6 months produced higher seed germination than seeds stored for 2 and 4 months.

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