

A new candidate cultivar of *Brachiaria* grass ‘Br-203’ developed with apomixis marker assisted selection, through a collaborative breeding activity of Thailand and Japan

Ganda Nakamane¹, Masumi Ebina^{2*}, Katsuhisa Shimoda², Sarayut Thaikua³, Wattanawan Srisomprn¹, Chalermpon Patipan¹, Kazuhiro Suenaga⁴, Sada Ando⁴, Kaori Kouki⁵, Genki Ishigaki⁶, Ryo Akashi⁶ and Yasuhiro Kawamoto⁷

¹Department of Livestock Development (DLD), Pak Chong, Nakhon Ratchasima, Thailand

²NARO-ILGS, Nasuhiobara, Tochigi 329-2793, Japan

³The United Graduate School of Agricultural Sciences, Kagoshima Univers, Nishihara, Okinawa 903-0213, Japan

⁴Japan International Research Center for Agriculture Science (JIRCAS), Tsukuba, Ibaraki 305-8686, Japan

⁵Okinawa Prefectural Livestock and Grassland Research Center, Nakijin, Okinawa 905-0426, Japan

⁶University of Miyazaki, Miyazaki, Miyazaki 889-2192, Japan

⁷University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

*Corresponding author e-mail: triticum@affrc.go.jp

Keywords: Apomixis, *Brachiaria*, Breeding, MAS selection, Tropical Forage

Introduction

Brachiaria grass (*Brachiaria* spp.) is one of most important tropical forage grass which belongs to family *Poaceae*, subfamily *Panicoideae*, tribe *Paniceae*, genus *Brachiaria*. *Brachiaria* grass has attracted attention because of a leading ‘Green Revolution’ over tens of millions of hectares in the Central Brazilian Cerrado in the 1970s. Breeding programs for *Brachiaria* grass have been conducted mainly by the International Center for Tropical Agriculture (CIAT) and the Brazilian Agricultural Research Corporation (EMBRAPA). Careful and effective recurrent selection of tetraploid sexual lines for vigor, growth habit, leafiness, and spittlebug resistance have spawned several successful hybrid cultivars: ‘Mulato’, ‘Mulato II’, and ‘Caiman’ (Miles *et al.*, 2004). Research activities on tetraploid sexual lines of *Brachiaria* spp. (Ishigaki *et al.*, 2010) have been also continued in tropical and subtropical Japan and Southeast Asia. Our recent breeding program for *Brachiaria* spp. has also got underway in the tropical monsoon region, particularly areas of Indo-China.

Marker-assisted selection (MAS) for breeding population has been previously done with some important crops and forages. Apomixis is one of important trait for breeding, which can lead eternal asexual propagation through seed. Previous report (Ebina *et al.*, 2005) revealed that the apomixis co-segregated marker could assist breeding selection.

In this paper, we report a new candidate cultivar ‘Br-203’ *brachiaria* grass crossed with a sexual maternal tetraploid parent of ‘Miyaoikoku’ and an apomixis paternal tetraploid parent of ‘Mulato’. After pre-selection by MAS for apomixis trait in Japan, effective and compact breeding populations have been transferred to Department of Livestock Development (DLD) Thailand. Major agronomical traits selections have been successfully performed in Thailand. The result shows the combination of MAS and conventional breeding method is a powerful and effective way of breeding of a new apomixis *bracharia* grass cultivar.

Materials and Methods

Plant Materials: Single plant cross was performed in 2008 and 2009 in Japan International Research Center for Agriculture Science (JIRCAS), Tsukuba, Ibaraki Japan by using ‘Miyaoikoku’ as a sexual maternal tetraploid parent and ‘Mulato’ as an apomictic paternal tetraploid parent. ‘Mulato’ was kindly provided by genetic resources section in CIAT. In 2010, a breeding population consisting 250 potted progeny was established in NARO-Institute of Livestock and Grassland Science (NILGS), Nasu-shiobara, Tochigi Japan in a greenhouse condition. At least three spikes from each progeny were kept until use for embryo sac analysis, selecting the mature spikes at a few days before flowering. Genomic DNA were extracted from each of progeny leaves by using CTAB method, and kept in refrigerator until use.

AFLP Linkage analysis: AFLP analysis was performed using the original protocol, as described in the manufacturer manual (Life Technologies Japan, Tokyo, Japan). Linkage analysis was performed for the complete set of AFLP markers using the F₂BC₁ model of Map Maker/EXP3.0.

Embryo sac analysis: The mode of reproduction (apomixis or sexual) was identified for each progeny by means of embryo sac analysis using methyl salicylate clearing and Nomarski differential interference contrast microscopy. At least 20 embryo sacs were analyzed for each of the 84 individuals as a testing population from a total of 250 progeny.

Agronomical traits measurement: After checking of two apomixis co-segregated AFLP markers scores out of 12 apomixis co-segregated marker, and measuring rates of seed maturity trait in Nishi-nasuno NARO Japan in the greenhouse condition, a total of 54 apomictic progeny indicating sufficient seed maturity rates out of initial 250 progeny population were transferred to Pak Chong, DLD Thailand as a few amount of seeds in March 2011. In Thailand, tested plant and fields were 4-5 replicates transplanted and established with NPK 100kg/ha as basal fertilizer. Through the growing season, the established plants were cut at 5-10 cm height for maintenance in a 45 to 60 days interval with NPK 50kg/ha as additional top-dressing fertilizer. After one year established and evaluated, three candidates for release as a new cultivar were selected by the total score of plant vigor. Consequently, forage dry matter yields of the three candidates with ‘Mulato II’ and ‘Kennedy’ as a comparison were measured as regional adaptability test (simulated forage field) at Pak Chong Thailand and the other four test site (Petchaburi, Phrae, SaKaeo, Sakon Nakhon) in Thailand in 2013-2014, according to the regional adaptability test manual (DLD Thailand). The other tests for major agronomic trait were performed at both Pak Chong in Thailand and Okinawa in Japan in 2012-2014, according to guidelines for the conduct of tests, for distinctness, uniformity and stability (UROCHILOA) provided by UPOV.

Results and Discussion

AFLP analysis and MAS breeding: Using 64 AFLP primer combination and 84 testing population out of 250 breeding population, an AFLP linkage map was constructed, comprising 29 linkage group with 272 informative AFLP markers representing ‘Mulato’. Twelve co-segregated AFLP markers appeared in a linkage group of the linkage map (Figure 1). The linkage map had a total length of 1423.2cM with an average interval of 5.23cM. Apomixis traits appeared in the group, whose length is 82.6cM, with 12 co-segregated AFLP markers with an average interval of 3.67cM, comprising a total 22 AFLP markers in the group (Thaikua *et al.*, submitted). Two apomixis co-segregated AFLP markers out of 12 markers were selected by its repeatability and legibility, and expand application to select apomixis plant among the breeding population. A combination selection MAS both for apomixis and seed maturity rates in green house condition was successfully done, consequently 54 progeny indicating apomixis and good seed maturity were selected.

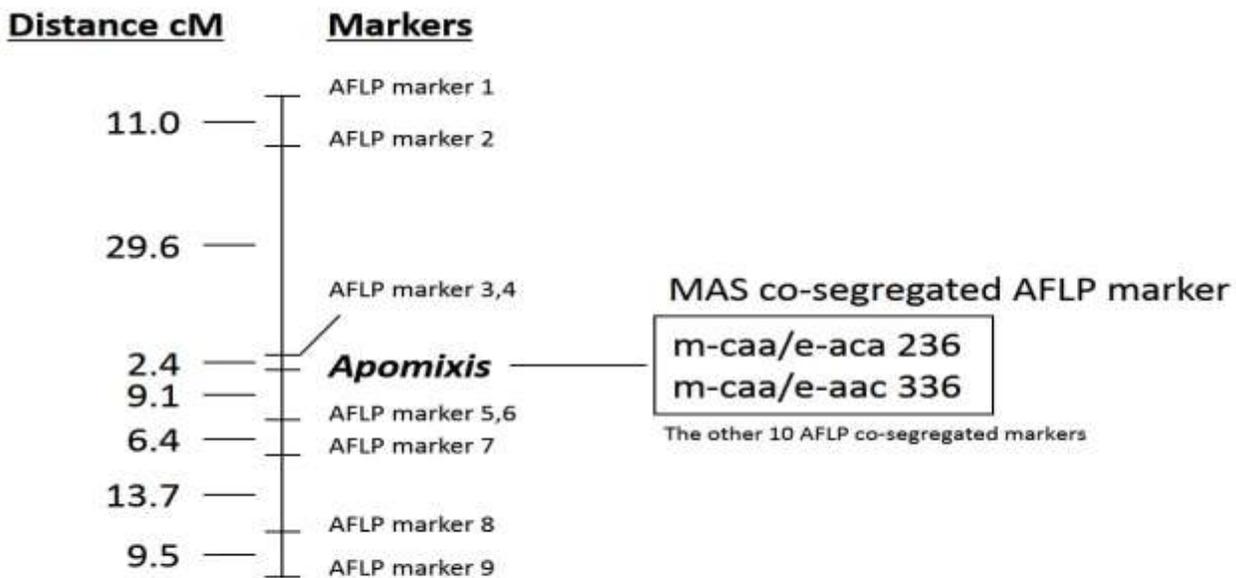


Figure 1 Apomixis linkage group of ‘Mulato’ Brachiariagrass, and apomixis AFLP markers for MAS

Breeding selection in Thailand: Breeding selections were mainly performed with plant vigour at initial growth in potted plants, and plant vigor in established field through one year growing season. At first, plant vigour at initial growth was checked, and 45 progeny were selected among the 54 progeny. Evaluations of plant vigour were continued for each of 45 progeny with 5 replications. After one year establishing, three candidates for release as a new cultivar were selected by the total score of plant vigour in May 2012. A preceding test for forage dry matter yield as a regional adaptability test (simulated forage field) in Pack Chong was started and reveled plant vigour facilitates the selection of high-yield lines, indicating a broad-sense heritability of 45.0% (Tsuruta *et al. in print*) with dry mater yield. In grass breeding, there is

usually little or no relationship between isolated plant vigor and grassland yield, preventing the selection of superior lines. However, this strong association indicated by its heritability between the performance of isolated brachiaria grass plants and grassland enables successful breeding selection based on isolated plant vigour. This method is used in the recurrent sexual line selection program at CIAT (Miles *et al.*, 2004).

Table 1: Dry matter yields and major agronomic traits

traits	Dry Matter Yield			CP	Seed maturity rate			Leaf		Flowering	
	two years average 5 sites average in Thailand	1st year 5 sites average in Thailand	2nd year 5 sites average in Thailand	In Pak Chong	Pak Chong no-cut before heading	Pak Chong cut before heading	In Okinawa	Width In Pak Chong	Length In Pak Chong	flowering column ratio In Pak Chong	Flowering time In Pak Chong
description	t/ha	t/ha	t/ha	%	%	%	%	cm	cm	1-9 rating	date
Br-203	19.28	21.27	17.28	17.2	10.7	28.6	7.1	1.95	20.2	4	27-Oct
Br-185	17.58	19.37	15.79	16.6	44.6	52.9	12.6	1.76	18.3	4	21-Oct
Br-226	16.96	19.35	14.58	17.0	26.7	33.4	23.6	1.79	19.2	4	24-Oct
Kennedy	16.98	19.96	14.35	14.3	-	-	55.6	1.15	10.0	6	17-Oct
Mulato II	16.50	18.31	14.69	16.1	-	-	42.5	1.09	10.4	1	5-Nov
LSD(5%)	*	*	*	*	-	-	-	-	-	-	-

Dry matter yields and major agronomic trait: Forage dry matter yields of the three candidates with ‘Mulato II’ and ‘Kennedy’ as a comparison were measured at least three times in a year as regional adaptability test (simulated forage field) performing two year at Pak Chong Thailand in 2012-2013, and the other four test site in Thailand in 2013-2014 (Table 1). Among the three candidates with ‘Mulato II’ and ‘Kennedy’ as a comparison, tentative named ‘Br-203’ exhibit the significant high yield indicating 19.2 t/ha/year (116% higher yield than ‘Mulato II’, 114% than ‘Kennedy’) in an average of two years test at five test sites in Thailand. The height of the plant of ‘Br-203’ at harvesting is also significantly high through two year, indicating good regrowth and extension. The high plant could be favourable to prevent weeds invading. Nutrient value for forage of crude protein (CP) of ‘Br-203’, in some good cases, indicates significant high of 17.2% in dry matter. This ratio of CP is almost same value as forage legume species. Seeds maturity rates varied from 7.1% to 28.6%, indicating less rates among the candidates, ‘Mulato II’ and ‘Kennedy’. However, the rate is almost similar to the old cultivar ‘Basilisk’, it have an appropriately responsible for commercial seeds propagation. ‘Br-203’ have wide and long leaf, moderate frequent flowering at moderate flowering time cultivar, indicating apomixis seed propagation.

Conclusion

We performed Brachiaria grass breeding using MAS for apomixis and selected breeding criteria. Quite effective breeding selections have successfully been carried out and a candidate for a new candidate cultivar ‘Br-203’ was selected. The new candidates for cultivar ‘Br-203’ exhibited apomixis, high yield, good forage quality with wide, long leaf, indicates comparatively low seed maturity, moderate frequent flowering at moderate flowering time.

References

- Ebina, M., H. Nakagawa, T. Yamamoto, H. Araya, S. Tsuruta, M. Takahara and K. Nakajima. 2005. Co-segregation of AFLP and RAPD markers to apospory in Guineagrass (*Panicum maximum* Jacq.). *Grassl. Sci.* 51: 71–78.
- Ishigaki, G., T. Gondo, K. Suenaga and R. Akashi. 2009. Induction of tetraploid ruzigrass (*Brachiaria ruziziensis*) plants by colchicine treatment of in vitro multiple-shoot clumps and seedlings. *Grassl. Sci.* 55: 164–170.
- Miles, J.W., C.B. do Valle, I.M. Rao and V.P.B. Euclides. 2004. Brachiariagrass. In: Moser L.E., B. L. Burson and L.E. Sollenberger (eds). *Warm-season (C4) grasses*, no. 45 *Agronomy*, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, USA. pp. 745–783.

Acknowledgement

Authors would like to gratefully thank to CIAT genetic resources section for kind providing ‘Mulato’ and genetic resources. This work was supported by JIRCAS project ‘The Establishment of the Sustainable and Independent Farm Household Economy in the Rural Areas of Indo-China’.