

Genomic approaches for dissecting complex traits related to quality production of range grasses

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ABSTRACT

Tropical grasses have been widely used as warm-season forage grasses in the warm temperate zone since the early 20th century because of their high yields; they have also been used as perennial forages in their native tropical areas. Increasing demand for animal production sparked by economic development in tropical countries is requiring breeders to improve native forage grasses in these countries. Considerable efforts have been made to breed accessions with improved characteristics and to develop new cultivars. However, cross-breeding is not common, owing to a lack of genetic information and to complexities related to polyploidy, high sterility rates, outcrossing, and apomixis. Nevertheless, several of the difficulties are being resolved by advanced research using molecular genetic tools, involving linkage analysis for the inheritance of genes for traits with major effects, such as apomixis. However, additional improvement is required for forage grasses with complex major traits that are controlled by multiple minor genes, such as forage yield, nutrient uptake, and sterility. In these cases, molecular tools can be combined with simple measurements of plant physical or morphological traits to support breeding. In addition, combining molecular tools with conventional breeding methods could lead to effective selection of promising breeding resources.

Key words: Apomixis, *Brachiaria*, Breeding, Complex traits, Digestibility, Heritability, Marker-assisted selection, *Panicum*, Tropical Forage, Yield.

Introduction

Many species of tropical grasses have attractive agronomic characteristics in their natural habitats. In any program for breeding of these grasses, the first decision that must be made is the choice of the most appropriate species based on the target area and proposed use. It is particularly important to determine the potential for expanding the range of a tropical grass species before developing new cultivars. The most important traits for tropical grasses include apomixis, forage yield, and nutrient value, and the challenge for breeders involves dissecting these complex traits. Molecular genetics provides powerful tools for dissecting traits with relatively simple inheritance, such as apomixis. Estimating the heritability of such traits provides important

information that can improve the power of selecting breeding materials based on characteristics such as forage yield. In some cases, such as nutrient value, simple regression analysis can be used when the trait is controlled by many genes. Here, I discuss how appropriate genomic tools can be combined with simpler measurements to improve the power of a breeding program for tropical grasses and so help breeders solve the complex problems that arise in tropical grass breeding.

Apomixis

Apomixis is an interesting and valuable trait (Ozias-Akins and van Dijk, 2007) because it enables the propagation of numerous individuals with genotypes identical to those of the maternal plant. This property improves

the speed and efficiency of breeding, so if a genotype for apomixis could be discovered, incorporating it in grass breeding would hold considerable promise. Accordingly, many research groups have worked to isolate genes related to apomixis and to develop the associated molecular markers. Guinea grass (*Panicum maximum*) is a model plant for apomixis in tropical grasses. Tsuruta *et al.* developed linkage analysis for apomixis in guinea grass, and revealed more than 250 molecular markers of apomixis loci in the progeny of 'Natsukaze'. The progeny of 'Paikaji' have also been tested using markers for sequence-tagged sites (STS) of amplified fragment-length polymorphisms (AFLP) from 'Natsukaze', and loci for at least eight markers that are strongly associated with apomixis have been revealed in the progeny of both 'Natsukaze' and 'Paikaji' (Tsuruta *et al.*, 2015).

The same techniques have been applied to genetic selection of *Brachiaria* grass. The breeding population derived from a tetraploid ruzigrass variety (*B. ruziziensis*, 'Miyaoikoku-1') (Ishigaki *et al.*, 2009) x a hybrid variety 'Mulato' in Thailand was analyzed using AFLP markers for apomixis (Tsuruta *et al.*, 2015). First, 84 randomly selected plants from a total of 250 progeny of this population were analyzed for apomixis with the AFLP markers. Subsequently, the mode of propagation (i.e., apomictic or sexual) was precisely determined by using the microscopic embryo sac method (Nakagawa, 1990). Using 64 AFLP primer combinations and the 84 promising plants, an AFLP linkage map was constructed, comprising 29 linkage groups with 272 informative AFLP markers representing the cultivar 'Mulato'. The linkage map had a total length of 1423.2 cM, with an average interval of 5.23 cM between markers. Twelve co-segregating AFLP apomixis markers appeared in one of the linkage groups. The apomixis

linkage group had a length of 82.6 cM, with an average interval of 3.67 cM between the 12 markers, comprising a total 22 AFLP markers in the group (Tsuruta *et al.*, 2015). Two of the 12 markers were selected on the basis of their repeatability, and were used to select apomictic plants among the breeding population. A combination of marker-assisted selection (MAS) for apomixis and seed maturity rates under greenhouse conditions in Japan was used to select 54 progeny that combined apomixis with good seed maturity. Breeding selection was subsequently performed to select for high plant vigor during initial growth in potted plants in Thailand, and 45 of the 54 progeny were selected (Nakamane *et al.*, 2015).

Forage yield

Forage yield is one of the most important traits for a forage grass. However, yield is a complex trait in many crops. With help from my research group, I attempted to develop a way to breed for improved forage yield by using the above mentioned breeding population that had been selected for apomixis, seed maturity rates, and initial growth. I focused on early vigor of isolated plants, with 5 replications (in Thailand). After 1 year, I selected three candidates for release as new cultivars based on the total score for vigor in May 2012. The forage dry matter yield of the three candidate plants was compared with those of two commonly used high yield cultivar of 'Mulato II' and 'Kennedy' in a regional adaptability test (in a simulated forage field) conducted for 2 years at Pak Chong, and four other test sites in Thailand. One candidate, Br-203, had a significantly higher forage yield than the others, at 19.2 t ha⁻¹ year⁻¹ (116% of the yield of 'Mulato II and 114% of 'Kennedy'), based on the average of 2 years of testing at the five sites. This candidate was selected on the basis of the vigor of the isolated plants, which

facilitates the selection of high-yielding lines, with a broad-sense heritability of 45.0% calculated according to Caradus and Woodfield (1990). In most grass breeding, there is little or no relationship between isolated plant vigor and forage yield, and this has prevented the selection of superior lines. However, in *Brachiaria* grass, the strong association between the performance of isolated plants and forage yield in a simulated forage field enables successful breeding selection. This method has also been used in a program of recurrent sexual line selection at the International Center for Tropical Agriculture (Miles *et al.*, 2004).

Nutrient value

In vitro dry matter digestibility (IVDMD) provides a good measure of plant nutrient quality, and is therefore an important trait for grass quality improvement. However, little work has been done on its inheritance. Thaikua *et al.* (2015a) evaluated the IVDMD of 17 *Brachiaria* germplasm to evaluate its suitability as breeding material, and defined selection criteria for high digestibility. They evaluated IVDMD, morphology, and water content, and calculated the correlations among the traits and their broad-sense heritability. Among the morphological traits, the leaf-stem index (the ratio of leaf width to stem diameter), the leaf-shape ratio (width divided by length), and leaf water content were most strongly correlated with whole-plant IVDMD ($r = 0.74^{***}$, 0.72^{**} and 0.79^{***} , respectively, $** : P < 0.01$ and $*** : P < 0.001$). The broad-sense heritability of IVDMD was high (0.75 to 0.93). Thaikua *et al.* (2015a) therefore showed that these three traits could be useful selection criteria for the breeding of *Brachiaria* with high digestibility, and that IVDMD could be improved in *Brachiaria* breeding because of its high heritability.

Furthermore, Thaikua *et al.* (2015b) developed a combined selection index for digestibility in a hybrid *Brachiaria* population, using leaf water content and leaf morphological traits. They examined the IVDMD of 49 individual hybrids, their leaf water content (LWC), leaf width (LW), leaf length (LL) and leaf shape ratio (LR; LW/LL), and constructed a selection index by means of linear regression:

$$\text{LWC-LW index} = 0.31 \text{ LWC} + 0.10 \text{ LW} \quad (R^2=0.35^{***})$$

$$\text{LWC-LR index} = 0.30 \text{ LWC} + 0.06 \text{ LR} \quad (R^2=0.35^{***})$$

$$\text{LWC-LW-LR index} = 0.30 \text{ LWC} + 0.07 \text{ LW} + 0.04 \text{ LR} \quad (R^2 = 0.39^{***})$$

($*** : P < 0.001$)

The combined index provided a better estimate of IVDMD than any of the individual factors ($R^2 = 0.27$ for LWC, 0.14 for LW, and 0.14 for LR, respectively). Using 45 additional plants as a validation population, they found above three potential selection indices: for LWC-LW index $R^2 = 0.35^{***}$, for LWC-LR index $R^2=0.20^{**}$ and for LWC-LW-LR index $R^2 = 0.31^{***}$ ($** : P < 0.01$ and $*** : P < 0.001$).

These results show the potential of using leaf water content and plant morphological traits to construct selection indexes that can be used to select candidate materials for improving digestibility in *Brachiaria* breeding.

Discussion and Conclusions

Apomixis, forage yield, and nutrient value are common targets for breeders of tropical grasses. The combination of molecular markers for apomixis with simple investigations of the vigor of isolated plants, supplemented by heritability analysis, provided a powerful tool for breeding *Brachiaria* with high forage yield. In addition, simple

measurements of leaf morphological traits, combined with regression and heritability analysis, provided a good estimate of forage quality. Providing a simple selection method for such relatively complex traits as yield and nutrient value will enable breeders to perform recurrent selection and possibly to select additional, more difficult and complex traits, such as late flowering time and seed yield.

However, we currently lack of information on the effects of multiple genes that have already been selected based on previous breeding targets and their effects on future target traits. If future target traits are controlled by the same major genes that were previously selected for, subsequent breeding efforts will be more difficult. In this case, correlation analysis to detect relationships among traits by using all available genetic resources will be an important tool. In addition, analysis of quantitative trait loci with complex ploidy and of high sterile material could identify the effects of new genes for important traits.

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References

- Caradus, J. R. and D. R. Woodfield. 1990. Estimates of heritability for, and relationships between, root and shoot characters of white clover I. Replicated clonal material *Euphytica* 46: 203-209.
- Ishigaki, G., T. Gondo, S. Suenaga and R. Akashi. 2009. Induction of tetraploid ruzigrass (*Brachiaria ruziziensis*) plants by colchicine treatment of in vitro multiple-shoot clumps and seedlings. *Grassland Science* 55: 164-170.
- Miles J.W., C.B. do Valle, I.M. Rao and V.P.B. Euclides. 2004. Brachiaria grass. In: L. E. Moser, 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, WI, USA. pp. 745-783.
- Nakagawa, H. 1990. Embryo sac analysis and crossing procedure for breeding apomictic guinea grass (*Panicum maximum* Jacq.). *Japan Agricultural Research Quarterly* 24: 163-168.
- Nakamane, G., M. Ebina, K. Shimoda, S. Thaikua, W. Srisomporn, C. Patipan, K. Suenaga, S. Ando, K. Kouki, G. Ishigaki, R. Akashi and Y. Kawamoto. 2015. A New Candidate Cultivar of Brachiariagrass 'Br-203' Developed with Apomixis Marker Assisted Selection, through a Collaborative Breeding Activity of Thailand and Japan. In: Proc. XXIII International Grassland Congress (Nov. 20-24, 2015), New Delhi.
- Ozias-Akins, P. and P. J. van Dijk. 2007. Mendelian genetics of apomixis in plant. *Annu. Rev. Genet.* 41: 509-537.
- Thaikua, S., M. Ebina, K. Kouki, M. Inafuku, H. Akamine, S. Shimoda, K. Suenaga and Y. Kawamoto. 2015a. Preliminary evaluation on digestibility and the relation to morphology and water content of *Brachiaria* spp. and their heritability. *Grassland Science* 61: 92-100.
- Thaikua, S., M. Ebina, K. Kouki, Y. Imai and Y. Kawamoto. 2015b. Development of selection indexes for dry matter digestibility in *Brachiaria* breeding. *Journal of Warm Regional Society of Animal Science, Japan* 58: in press.
- Tsuruta, S., K. Shimoda, K. Kouki and M. Ebina. 2015. The Present Status of C₄ Tropical Grasses Breeding and Molecular Approaches. *Japan Agricultural Research Quarterly* 49: 203-215.