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Kaposvár University, Faculty of Agricultural and Environmental Sciences, Kaposvár

Application of BovineSNP50 genotyping array in variability assessment in Pinzgau bulls

R. Kasarda, N. Moravčíková, V. Šidlová, I. Pavlík, O. Kadlečík, A. Trakovická

Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources
94976 Nitra, Tr. A. Hlinku 2., Slovak Republic

ABSTRACT

The aim of this study was to evaluate the level of SNP polymorphisms and describe the basic characteristic of the analysed population genotyped using the BovineSNP50 genotyping array, which has lot of applications in cattle such as genome association studies, genetic prediction of breeding values, estimation of genetic diversity and population genetic parameters and investigation of genetic relationships among cattle breeds. In total 19 purebred Pinzgau bulls were successfully genotyped with Illumina BovineSNP50 BeadChip (98.96% of SNPs) with call rate 0.995. Genotyping results from 54,906 SNPs revealed that 43,120 SNPs (78.96%) were polymorphic with average minor allele frequency 0.273±0.133. Within 43,120 SNPs genotyped, 98.19% were autosomal, with 776 polymorphic SNP on chromosome X and only one on chromosome Y. The average values of observed and expected heterozygosity across polymorphic loci were 0.375±0.157 and 0.362±126, respectively. Sufficient proportion of heterozygotes indicated the value of F_{IS} (0.037±0.031). Genomic data obtained for purebred Pinzgau bulls from the BovineSNP50 chip can be in further applied for evaluation of genetic diversity in Pinzgau breed as endangered population of cattle in Slovak republic.
(Keywords: cattle, MAF, polymorphism, SNP50 chip)

INTRODUCTION

Genomics is currently being utilized for genetic evaluations, parentage verification and screening for lethal recessives, congenital disorders and other mutations with large effects on performance in cattle populations (*Mullen et al.*, 2013). In recent years the SNP array has been developed, based on the discovery of numerous SNPs through genome sequencing. This technology has been evaluated as a new high-throughput genotyping technology since it enables simultaneous detection of the loci of a large number of SNPs (*Suekawa et al.*, 2010). The availability of many thousands of single nucleotide polymorphism (SNP) markers distributed across the genome has led to a new approach for genetic studies and applications (*Van Tassel et al.*, 2008). SNPs are currently used in genome wide-association studies (*Bolormaa et al.*, 2011; *Schopen et al.*, 2011); in genetic prediction of breeding values (*Meuwissen and Goddard*, 2010) or for estimation of genetic diversity and population genetic parameters (*Engelsma et al.*, 2012). Among livestock species, this technology has been applied most successfully in cattle, because factors such as evolutionary history, genetic structure, economics, etc. make cattle particularly suitable for the application of genome assisted selection

(*Nicolazzi et al.*, 2014). Genotyping of cattle using SNP array has become common practice in dairy cattle breeding programs applying genomic selection (*Mulder et al.*, 2012).

A number of SNP chips from Illumina and Affymetrix are available for cattle. These include 3K, 7K, 15K, 25K, 50K and more recently 800K from Illumina, and 650K and 3 million SNP panels from Affymetrix. In addition next generation sequencing technologies for low-cost sequencing of whole genomes are now available (*Khatkar et al.*, 2012). The release of the Illumina BovineSNP50 BeadChip in late 2007 has drawn attention from cattle breeders around the world to develop breeding programs that leverage association of these single nucleotide polymorphism (SNP) with economically important quantitative trait loci (QTL) (*Lu*, 2012). Moreover the availability of large numbers of SNP markers has resulted in new opportunities to estimate genetic diversity in more detail, and to improve prioritization of animals for conservation of genetic diversity. Conservation of genetic diversity in livestock breeds is important since it is, both within and between breeds, under threat. Over the last decades, genetic diversity of livestock populations had been alternatively measured using pedigree information or microsatellite data when genealogy is not available. Currently, the availability of high-density SNP chips has opened up new opportunities to evaluate genetic diversity based on genetic markers. Up to now, conservation decisions for gene banks were often based on pedigree information, while the use of high-dense markers may give a more detailed picture of the diversity across the genome (*Engelsma et al.*, 2011).

The objective of this study was to evaluate the level of SNP polymorphisms and describe the basic characteristic of the population genotyped, using the BovineSNP50 BeadChip.

MATERIAL AND METHODS

Breed description

The Pinzgau cattle is traditional dual purpose type breed of mountainous areas of Slovakia, introduced in 18th century (*Kasarda et al.*, 2008). It was imported from region of the Austrian Alps and populations' breeding in Slovakia is from its constitution over connected (*Pšenica*, 1990). The first imports of Pinzgau purebred animals were organized long time ago before 1894 when system of cattle recording has started on territory of Slovakia. The size of breed was improving and in 1958 it was officially accepted as Slovak Pinzgau (P) breed (*Kadlečík et al.*, 2013). In 1970 re-building of purpose and breed type of cattle started. In this period several breeding bulls were imported, from which 24% were of Pinzgau origin (*Pšenica and Tretinova*, 1998). Highest number of cows bred was 162 127 in 1970 (*Pšenica*, 1998). From 1970 to 1992 in pedigrees of Pinzgau cattle were introduced seven breeds, almost 50% Pinzgau bulls imported from Austria and 34% from Slovakia. From 1994 Pinzgau cattle in Slovakia is registered by FAO as endangered. After 1990 considerable decrease of Pinzgau population size was observed in Slovakia (*Kadlečík et al.*, 2011).

Sample collection and DNA genotyping

For genomic evaluation 19 AI Pinzgau proven sires were selected. Selection criterions were as follows: sires present on AI stations with reliability of breeding value over 65% and born from 1998 to 2006 in Slovakia. Except one, all sires were born in Slovakia, only Nero from lineage Nusil bought as young sire in Austria. Five sires had father of Slovak origin (26%) and only two grandfather (10%). All sires represent 13 lineages of

dual purpose Pinzgau cattle. Most frequent lineage was Nobel represented by 4 sires, followed by lineage Nusil by 3 sires and Origin of foreign sires in pedigrees was Austrian. In long term scope Austrian sires were often used for mating of sire dams. Genomic DNA for each of the 19 bulls semen samples was genotyped at a commercial lab using an Illumina BovineSNP50 Genotyping BeadChip.

Statistical analyses

Quality controls and computation for SNP data was performed with PLINK (*Purcell et al.*, 2007). The following quality control criteria (filters) were used to remove from further analysis any SNPs with less than 95% call rate, and SNPs with less than 0.05 MAF. SNP were tested for HWE ($P<0.001$) to identify possible typing error. Samples with more than 10% missing genotypes were removed from the study. Heterozygosities and fixation index (F_{IS}) for analyzed population were estimated.

RESULTS AND DISCUSSION

In this study was evaluated application of the BovineSNP50 genotyping array in the Slovak Pinzgau cattle population. Nineteen purebred Pinzgau bulls were genotyped with Illumina BovineSNP50 BeadChip. All samples were successfully genotyped (98.96% of SNPs). Total call rate (99.45%) was comparable with average rate reported by *Cooper et al.* (2013) across 3 dairy cattle breeds and *Mullen et al.* (2013) across beef and dairy cattle originating in *Bos taurus*. Similarly high values of call rates (>98%) were reported also for cattle breeds originating from *Bos indicus* (*Qwabe et al.*, 2013; *Neves et al.*, 2014). These results validated that the BovineSNP50 BeadChip is important genomic tool for different bovine breeds, which can be subsequently used for multiple application in GWAS, genomic selection or evaluation of genetic relationship between breeds.

Table 1

Minor allele frequencies

Frequency	Autosomes		Sex chromosomes	
	# loci	%	# loci	%
0.0–0.1	4713	11.131	83	10.682
0.1–0.2	9273	21.900	137	17.632
0.2–0.3	9749	23.024	199	25.611
0.3–0.4	9832	23.220	185	23.810
0.4–0.5	8776	20.726	173	22.265
Total # of polymorphic loci	*42343*		*777*	

Genotyping results from 54,906 SNPs revealed that 43,120 SNPs (78.96%) were polymorphic with minor allele frequency greater than 0.05 (*Table 1*). The level of polymorphic SNPs in the present study was higher than in previously reported study for cattle (*Mai et al.*, 2010; *Hulsegge et al.*, 2013). Minor allele frequency (MAF) is widely used to describe the genetic variability of two-allele SNPs, and refers to frequency of the least common SNP allele (*Haynes et al.*, 2012). Within 43,120 SNPs genotyped, 98.19% were autosomal, with 776 polymorphic SNP on chromosome X and only one on chromosome Y. The average MAFs across loci on autosomes were 0.273±0.133 and sex

chromosomes 0.272±0.132 with minimum value 0.053. The average values of MAF depend of cattle breeds. Considerable variations in MAF between breeds reported *Matukamelli et al.* (2009), who observed higher proportions of polymorphic SNP in Holstein and Angus cattle in comparison with African N'Dama and Sheko breeds. The average values of observed and expected heterozygosity across polymorphic loci were 0.375±0.157 and 0.362±126, respectively. Sufficient proportion of heterozygotes indicated the value of F_{IS} (0.037±0.031).

CONCLUSIONS

Molecular genetic tools are in present preferably used in description of genetic composition of populations. Statistical analysis of molecularly based data gives deeper insight into genetic variability and helps in evaluation and monitoring of populations´ diversity. Use of the Illumina BovineSNP50k BeadChip is standard tool in studies such as genetic diversity, genomic breeding values estimation, evaluation of population structure and genetic distances. Further research will be oriented on evaluation of diversity issues of the Pinzgau population.

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REFERENCES

Bolormaa, S., Hayes, B.J., Savin, K., Hawken, R., Barendse, W., Arthur, P.F., Herd, R.M., Goddard, M.E. (2011). Genome-wide association studies for feedlot and growth traits in cattle. Journal of Animal Science, 89. 1684-1697.

Cooper, T.A., Wiggans, G.R., VanRaden, P.M. (2013). Short communication: relationship of call rate and accuracy of single nucleotide polymorphism genotypes in dairy cattle. Journal of Dairy Science, 96. 3336-3339.

Engelsma, K.A., Veerkamp, R.F., Calus, M.P., Bijma, P., Windig, J.J. (2011). Pedigree- and marker-based methods in the estimation of genetic diversity in small groups of Holstein cattle. Journal of Animal Breeding and Genetics, 129. 195-205.

Engelsma, K.A., Veerkamp, R.F., Calus, M.P., Bijma, P., Windig, J.J. (2012). Pedigree- and marker-based methods in the estimation of genetic diversity in small groups of Holstein cattle. Journal of Animal Breeding and Genetics, 129. 195-205.

Haynes, G. D., Latch, E. K. (2012). Identification of novel single nucleotide polymorphisms (SNPs) in deer (Odocoileus spp.) using the BovineSNP50 BeadChip. PLoS One, 7.

Hulsegge, B., Calus, M.P., Windig, J.J., Hoving-Bolink, A.H., Maurice-Van Eijndhoven, M.H., Hiemstra, S.J. (2013). Selection of SNP from 50K and 777K arrays to predict breed of origin in cattle. Journal of Animal Science, 91. 5128-5134.

Kadlečík, O., Hazuchová, E., Pavlík, I., Kasarda. R. (2013). Diversity of cattle breeds in Slovakia. Slovak Journal of Animal Science, 46. 145-150.

Kadlečík, O., Kasarda, R., Pavlík, I., Hazuchová. E. (2011). Pedigree Analysis of Slovak Pinzgau Breed. Agriculturae Conspectus Scientificus, 76. 165-168.

Kasarda, R., Kadlečík, O., Mészáros. G. (2008). Trends of endangered population of Pinzgau Cattle in Slovakia. Archiva Zootechnica, 11. 82-87.

Khatkar, M.S., Moser, G., Hayes, B.J., Raadsma, H.W. (2012). Strategies and utility of imputed SNP genotypes for genomic analysis in dairy cattle. BMC Genomics, 13. 538.

Lu, D. (2012). Applications of the Illumina BovineSNP50 BeadChip in Genetic Improvement of Beef Cattle. Guelph, Ontario, Canada, 1.

Mai, M.D., Sahana, G., Christiansen, F.B., Guldbrandtsen, B. (2010). A genome-wide association study for milk production traits in Danish Jersey cattle using a 50K single nucleotide polymorphism chip. Journal of Animal Science, 88. 3522-3528.

Matukumalli, L.K., Lawley, C.T., Schnabel, R.D., Taylor, J.F., Allan, M.F., Heaton, M.P., O'Connell, J., Moore, S.S., Smith, T.P., Sonstegard, T.S., Van Tassell, C.P. (2009). Development and characterization of a high density SNP genotyping assay for cattle. PLoS One, 4.

Meuwissen, T., Goddard, M. (2010). Accurate Prediction of Genetic Values for Complex Traits by Whole-Genome Resequencing. Genetics, 185. 623-631.

Mulder, H.A., Calus, M.P., Druet, T., Schrooten, C. (2012). Imputation of genotypes with low-density chips and its effect on reliability of direct genomic values in Dutch Holstein cattle. Journal of Dairy Science, 95. 876-89.

Mullen, M.P., McClure, M.C., Kearney, J.F., Waters, S.M., Weld, R., Flynn, P., Creevey, C.J., Cromie, A.R., Berry. D.P (2013). Development of a Custom SNP Chip for Dairy and Beef Cattle Breeding, Parentage and Research. Interbull Bulletin, 47.

Neves, H.H., Carvalheiro, R., O'Brien, A.M., Utsunomiya, Y.T., Do Carmo, A.S., Schenkel, F.S., Sölkner, J., McEwan, J.C., Van Tassell, C.P., Cole, J.B., Da Silva, M.V., Queiroz, S.A., Sonstegard, T.S., Garcia, J.F. (2014). Accuracy of genomic predictions in Bos indicus (Nellore) cattle. Genetic Selection Evolution, 46. 17.

Nicolazzi, E.L., Picciolini, M., Strozzi, F., Schnabel, R.D., Lawley, C., Pirani, A., Brew, F., Stella, A. (2014). SNPchiMp: a database to disentangle the SNPchip jungle in bovine livestock. BMC Genomics, 15. 123.

Pšenica J., Tretinova, D. (1998). Share of foreign breeds in pedigrees of bulls os Slovak pinzgau breed (in Slovak). Agriculture, 44. 775-784.

Pšenica, J. (1990). Pinzgau cattle in Slovakia (in Slovak). Bratislava, Príroda, 184.

Pšenica, J., Bulla, J., Huba, J. (1998). Slovak pinzgau cattle (in Slovak). Slovenský chov, 16.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I.W., Daly, M.J., Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics, 81. 559-575.

Qwabe, S.O., Van Marle-Köster, E., Maiwashe, A., Muchadeyi, F.C. (2013). Evaluation of the BovineSNP50 genotyping array in four South African cattle populations. South African Journal of Animal Science, 43. 64-67.

Schopen, G.C., Visker, M.H., Koks, P.D., Mullaart, E., Van Arendonk, J.A., Bovenhuis, H. (2011). Whole-genome association study for milk protein composition in dairy cattle. Journal of Dairy Science, 94. 3148-3158.

Suekawa, Y., Aihara, H., Araki, M., Hosokawa, D., Mannen, H., Sasazaki, S. (2010). Development of breed identification markers based on a bovine 50K SNP array. Meat Science, 85. 285-8.

Van Tassel, C.P., Smith, T.P.L., Matukumalli, L.K., Taylor, J.F., Schnabel, R.D., Lawley, C.T., Haudenschild, C.D., Moore, S.S., Warren, W.C., Sonstegard, T.S. (2008). SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. Nat Methods, 5. 247-252.

Corresponding author:

Radovan Kasarda
Slovak University of Agriculture in Nitra
Faculty of Agrobiology and Food Resources
94976 Nitra, Tr. A. Hlinku 2., Slovak Republic
Phone: +421 37 641 4292
E-mail: radovan.kasarda@uniag.sk