Animal (2018), **12:2**, pp 205–214 © The Animal Consortium 2017
doi:10.1017/S1751731117001562

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Analyses of reaction norms reveal new chromosome regions associated with tick resistance in cattle

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(Received 12 December 2016; Accepted 22 May 2017; First published online 13 July 2017)

*Despite single nucleotide polymorphism (SNP) availability and frequent cost reduction has allowed genome-wide association studies even in complex traits as tick resistance, the use of this information source in SNP by environment interaction context is unknown for many economically important traits in cattle. We aimed at identifying putative genomic regions explaining differences in tick resistance in Hereford and Braford cattle under SNP by environment point of view as well as to identify candidate genes derived from outliers/significant markers. The environment was defined as contemporary group means of tick counts, since they seemed to be the most appropriate entities to describe the environmental gradient in beef cattle. A total of 4363 animals having tick counts (*n *= 10 673) originated from 197 sires and 3966 dams were used. Genotypes were acquired on 3591 of these cattle. From top 1% SNPs (410) having the greatest effects in each environment, 75 were consistently relevant in all environments, which indicated SNP by environment interaction. The outliers/significant SNPs were mapped on chromosomes 1, 2, 5, 6, 7, 9, 11, 13, 14, 15, 16, 18, 21, 23, 24, 26 and 28, and potential candidate genes were detected across environments. The presence of SNP by environment interaction for tick resistance indicates that genetic expression of resistance depends upon tick burden. Markers with major portion of genetic variance explained across environments appeared to be close to genes with different direct or indirect functions related to immune system, inflammatory process and mechanisms of tissue destruction/repair, such as energy metabolism and cell differentiation.*

Keywords: beef cattle, candidate genes, environmental gradient, gene function, single-step

Implications

The knowledge on single nucleotide polymorphism (SNP) effects and/or candidate genes interactions of tick resistance with the environmental burden of this parasite under reaction norm approach can be used to increase selection accuracy across environments and to choose best sires for different tick burden in genomic selection evaluations of this Hereford/Braford population.

Introduction

The cattle tick is a parasite that adversely affects livestock performance in tropical areas. Different studies have shown that cattle breed has a major effect on the level of tick burden and it is stated that *Bos indicus* cattle are more resistant to ticks than *Bos taurus* (Prayaga and Henshall, 2005). Likewise, some reports indicated that crossbred cattle (*B. taurus v. B. indicus*) carried more than four times ticks than *B. indicus*, or still that pure zebu cattle (*B. indicus*) are less infested with ticks compared with zebu–taurine crosses (*B. taurus v. B. indicus*) under identical field conditions (Wambura *et al.*, 1998). Relationships among skin thickness, hair length, coat score and tick count (TC) have been evaluated in native and composite cattle breeds like Nguri and Bonsmara (Marufu *et al.*, 2011).

The presence of genotype by environment interaction (G × E) can be important for beef cattle breeders as relative genetic merit will be environment-dependent. Although Brazil has provided genetic evaluations for tick resistance (Machado *et al.*, 2010; Cardoso *et al.*, 2015), these evaluations have not typically considered G × E. Despite genetic variation in response to environmental changes has been reported for economically important production traits

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(Cardoso and Tempelman, 2012; Silva *et al*., 2014) and complex traits (Mota *et al*., 2016a and 2016b), this is still neglected.

In genome-wide selection (GWS), marker by environment interactions can be used to identify and utilize G × E (Silva *et al*., 2014) in order to improve the accuracy of genomic-estimated breeding values for important traits over environments. However, markers effects or genetic variance explained are commonly estimated as the same across environments. In a recent study, Mota *et al*. (2016a) reported that genomic reaction norms might be an important tool to verify marker by environments interactions for tick resistance of Hereford and Braford cattle under GWS. These authors observed negative correlations between extreme (high and low tick burden) environments suggesting substantial G × E.

Nevertheless, genomic reaction norms can also be exploited to genetically characterize complex phenotypes candidate genes identification in proximity or linkage disequilibrium (LD) with markers across environments via genome-wide association studies (GWAS). Investigations of candidate genes have been reported for tick resistance for crossbred dairy cattle (Holstein × Gyr), on chromosomes 2, 3, 5, 7, 10, 11, 14, 23 and 27 (Gasparin *et al*., 2007; Machado *et al*., 2010), and beef cattle populations, on BTA 1, 2, 3, 5, 6, 7, 8, 10, 11, 12, 14, 15, 17, 18, 19, 23 and 27 (Porto Neto *et al*., 2011a and 2012; Mapholi *et al*., 2016), with different biological functions such as immune response. Gasparin *et al*. (2007) and Machado *et al*. (2010) working on a quantitative trait loci (QTL) mapping study reported QTL genomic regions controlling tick resistance and dependent upon the season ticks were counted. However, the genetic mechanism for tick resistance is not well established and the identification of candidate genes, useful for genomic selection application in commercial herds, needs to be more conclusive.

To our knowledge, despite the SNP availability and several GWAS reports for tick resistance (Porto Neto *et al*., 2010, 2011a, 2011b and 2012), the use of this source of information in G × E context remains unexplored. So far, there are no reports of candidate gene mapping studies under a genomic reaction norms framework in beef cattle. Toward this orientation, we aimed at identifying putative chromosomal regions and candidate genes associated with tick burden in Hereford and Braford cattle via genomic reaction norms.

Material and methods

Experimental procedures were approved by the Committee for Ethics in Animal Experimentation from the Federal University of Pelotas (Pelotas – RS, Brazil; Process number 6849).

Phenotypic, genotypic and pedigree data

Phenotypic data included 10 673 TCs on 928 Hereford and 3435 Braford cattle. A subset of 3545 was genotyped with the Illumina BovineSNP50 BeadChip (50 K; Illumina, San Diego, CA, USA). Pedigree information was highly incomplete due to use of multiple-sire matings, that is, 65% of unknown paternity. When the true sire was not identified via Mendelian conflicts (Wiggans *et al*., 2009), pedigree was reconstructed by creating 'virtual' ancestor for each identified half-sib family within multiple-sire groups and based on genomic relationships for the genotyped animals (Fernández and Toro, 2006). A total of 12 754 animals remained after pedigree reconstruction and pruning. Detailed phenotypic, genotypic and pedigree information were reported in our previous pedigree-based work (Mota *et al*., 2016a).

Contemporary groups (CG) were defined as a group of animals belonging the same herd, birth year and season (April to July; August to November and December to March), of the same sex and from the same management group. Following Mota *et al*. (2016b), CG means were set as environmental parameters because they are the appropriate entities to describe the environmental gradient in terms of cattle tick burden.

Statistical modeling and Bayesian inference

We fitted our best one-step genomic reaction norm model for tick resistance from a previous study (Mota *et al*., 2016b). Markov chain Monte Carlo was implemented using INTERGEN software (https://www.embrapa.br/pecuaria-sul), and by saving every 10th cycle from a total of 1 000 000, after 100 000 of burn-in ($n = 90\,000$ cycles). Further details referring to statistical modeling and Bayesian inference applied in this study can be found in Mota *et al*. (2016a and 2016b).

Derivation of single nucleotide polymorphism effects from predicted breeding values

Suppose that we can write $\mathbf{a} = [\mathbf{a_n}' : \mathbf{a_g}']'$ where $\mathbf{a_g}$ pertains to breeding values for genotyped animals and $\mathbf{a_n}$ pertains to breeding values of non-genotyped animals following Wang *et al*. (2012). We can similarly identify $\mathbf{b}$ as $\mathbf{b} = [\mathbf{b_n}' : \mathbf{b_g}']'$ it could be readily demonstrated that the equation used to solve the SNP intercept effect estimates is $\hat{\mathbf{u}}_0 = \mathbf{Z}'[\mathbf{Z}\mathbf{Z}']^{-1}\hat{\mathbf{a}}_\mathbf{g}$, whereas the equation used to solve the SNP-specific slope or reaction norm effects can be demonstrated to be $\hat{\mathbf{u}}_1 = \mathbf{Z}'[\mathbf{Z}\mathbf{Z}']^{-1}\hat{\mathbf{b}}_\mathbf{g}$. This is the best predictor of SNP effects in animals being $\mathbf{Z}$ is the matrix containing marker information. Hence, it is possible to determine a vector of SNP effect estimates ($\hat{\mathbf{u}}_\mathbf{w}$) for each environment ($\mathbf{w}$) as follows:

$$\hat{\mathbf{u}}_{(\mathbf{w})} = \hat{\mathbf{u}}_0 + \mathbf{w}\hat{\mathbf{u}}_1 \quad (1)$$

To verify the SNP effects pattern across environments, we followed Silva *et al*. (2014) and Verardo *et al*. (2016) and also identified top 1% SNPs ($n = 410$) presenting greatest effects (both directions) across tick burden. The number of shared SNPs across environments was used to reflect the similarity of SNP associations.

However, these studies basically assumed SNPs with highest effects. This is a simple statistics useful to verify SNP environment interactions, but that might be avoided for

gene detection, and consequently their functionality. Utsunomiya *et al*. (2014) presented a more reliable statistics to detect what they called 'outliers SNPs.' This is based on interquartile range over the percentage of genetic variance explained.

We applied the following formula:

$$\%\,\text{var} = 12 \times \text{IQR} + Q_3 \quad (2)$$

here %var is the percentage of genetic variance explained by each SNP; IQR is the interquartile range and Q_3 the third quartile of the distribution. Note that, we use the value 12 instead of 5 as in Utsunomiya *et al*. (2014) due to asymmetric genetic variance explained distribution (Supplementary Figure S1). The phenotypic/genetic variance explained by a single marker or windows of adjacent SNPs have been widely used as a criterion to identify candidates genes in single-step genome-wide association studies (ssGWAS) (Lemos *et al*., 2016; Melo *et al*., 2016; Valente *et al*., 2016; Zhang *et al*., 2016). The main reason is because there are no robust tests for SNP significance in ssGWAS.

The 50 K SNP panel data from Hereford and Braford cattle were pooled into a single-reference population for performing SNP effects and genetic variance explained across environments. This was assumed due to the genetic similarity between breeds. Braford animals are composites with a contribution of 62.5% of the Hereford breed as reported in a previous work on the same population (Biegelmeyer *et al*., 2016). These authors observed that the phase correlation estimates indicated that markers in LD at distances lower than 50 Kb in Hereford show quite similar levels of LD in Braford. Therefore, high proportion of these SNPs shares the same linkage phase. Another reason to not perform SNP effects or genetic variance explained per breed is the larger number of Braford ($n = 3435$) compared with Hereford ($n = 928$), which may accommodate differences in LD since Hereford are expected to contribute with 5/8 (62.5%) of Braford genome.

Gene mapping and overrepresentation analysis (ORA)

In order to provide gene identity and function mapped information, outliers or significant SNPs across low (LTI), medium (MTI) and high (HTI) tick burden environments represented, respectively, by 10th, 50th and 90th percentiles, were analyzed. To identify putative genes associated with the list of significant SNPs the package MAP2NCBI (Hanna and Riley, 2014) was used to generate a list of genomic features from the *B. taurus* (BUILD.6.1) genome. Furthermore, a Bioconductor package, that accesses and retrieves Ensembl data (Entrez IDs, Ensembl gene ID, HGNC symbols and more), R/BiomaRt, was used to download all genes (background genes) from *B. taurus* genome (ORG.MESH.BTA.DB), and map features within ±200 kb from the significant SNPs location. With this, in order to help interpreting the underlying genetic basis of tick resistance, the complete list of selected genes was used for enrichment analysis based on Medical Subject Headings (*MeSH*) vocabulary in Bioconductor retrieving statistically overrepresented annotations (Nelson *et al*., 2004). By merging *MeSH* terms and Entrez Gene IDs from the background and selected list of genes, *P-values* (Morota *et al*., 2016) were generated (Supplementary Table S1).

Results and discussion

Single nucleotide polymorphism by environment interaction

A total of 75 SNPs were consistently present across environments (Supplementary Table S2). Single nucleotide polymorphism effects estimated for these 75 consistent markers were plotted along the environmental gradient (Figure 1a). This figure indicates the presence of SNP by environment interaction due to an increase in SNP effect size in proportion to tick burden and SNP reaction norm line crossing across environments, especially in low tick burden. From these 75 SNPs, we also plotted the top 10 (Figure 1b), for better illustration. Although these 75 SNPs displayed relatively large effects at all levels, there were still changes in magnitude across environments, reinforcing the existence of SNP by environment interactions. A clear pattern was observed here: SNP effects increased as the tick burden increased regardless their magnitude and/or direction.

The Figure 1 also demonstrated that differences in SNP effects decreased in low tick burden, that is, SNP effects variation were greater at high level of tick resistance. It further indicates difficulty to identify superior breedstock in favorable conditions (low tick burden). To be clearer regarding marker effects varying across environments, Figure 2 presented effects for all ($n = 75$; Figure 2a) and top 10 (Figure 2b)

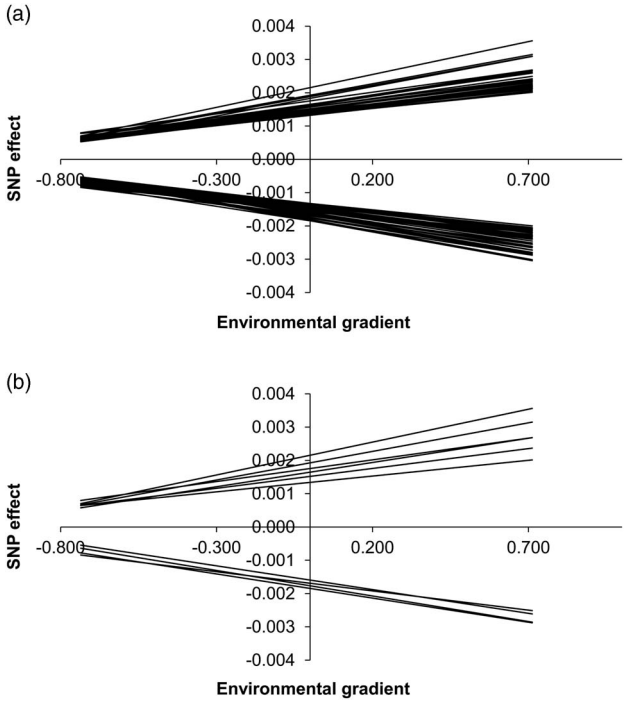

Figure 1 Single nucleotide polymorphism (SNP) effects for all ($n = 75$) (a) and top 10 (b) consistent SNPs across tick burden.

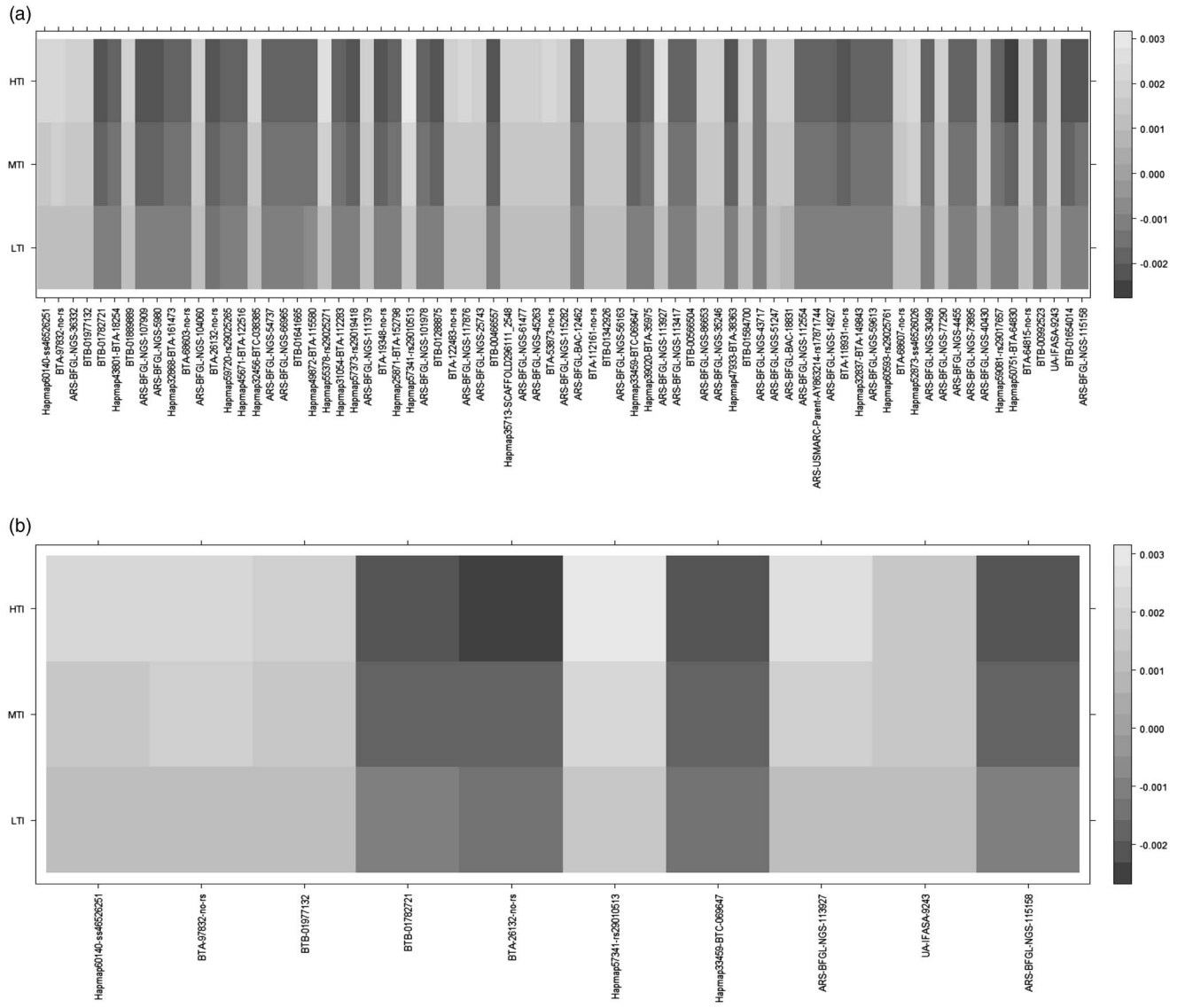

Figure 2 Single nucleotide polymorphism (SNP) effects for all ($n = 75$) (a) and top 10 (b) consistent SNPs at three different tick burden: low (LTI), medium (MTI) and high (HTI).

consistent SNPs over LTI, MTI and HTI. A trend that marker effects increase in magnitude as tick burden increases was noticeable. It implies that the identification of superior genetics for tick resistance requires exposure of animals to substantial environmental challenge (high tick burden).

Chromosome regions affecting tick resistance and overrepresentation analysis

The Manhattan plots showed SNP markers with larger portion of the genetic variance explained in three different environment levels associated with tick burden (Figure 3). The threshold of genetic variance explained were 0.15%, 0.08%, 0.29%, respectively for LTI, MTI and HTI. We declared 18, 16 and 18 SNPs as outliers or significant for low, medium and high levels, respectively. From those; 11 were coincident in all levels, 12 between low and medium, 11 between low and high and 15 between medium and high tick burden. On the other hand, we identified unique SNPs by levels: six (Hapmap32456-BTC-038385, Hapmap57373-rs29019418, Hapmap33459-BTC-069647, ARS-BFGL-NGS-113417, ARS-USMARC-Parent-AY863214-rs17871744 and ARS-BFGL-NGS-73895) in low and three (BTB-01475457, ARS-BFGL-NGS-52551, BTA-115451-no-rs) in high levels. Most of the SNPs explained the highest genetic variance proportion in medium and high levels ($n = 15$), whereas a subset of those ($n = 11$) were also significant in low tick burden. These results reinforce the presence of SNP by environment interactions in our study; as different patterns were observed according to the environmental condition in which the animals were raised.

The significant SNPs were detected in *B. taurus* chromosomes BTA 1, 2, 5, 6, 7, 9, 11, 13, 14, 15, 16, 18, 21, 23, 24, 26 and 28. From these SNPs, potential candidate genes were identified (Table 1) by using different sources for gene mapping (NCBI and Ensembl). Some of these SNPs had no gene identified; however, most of the potential candidate

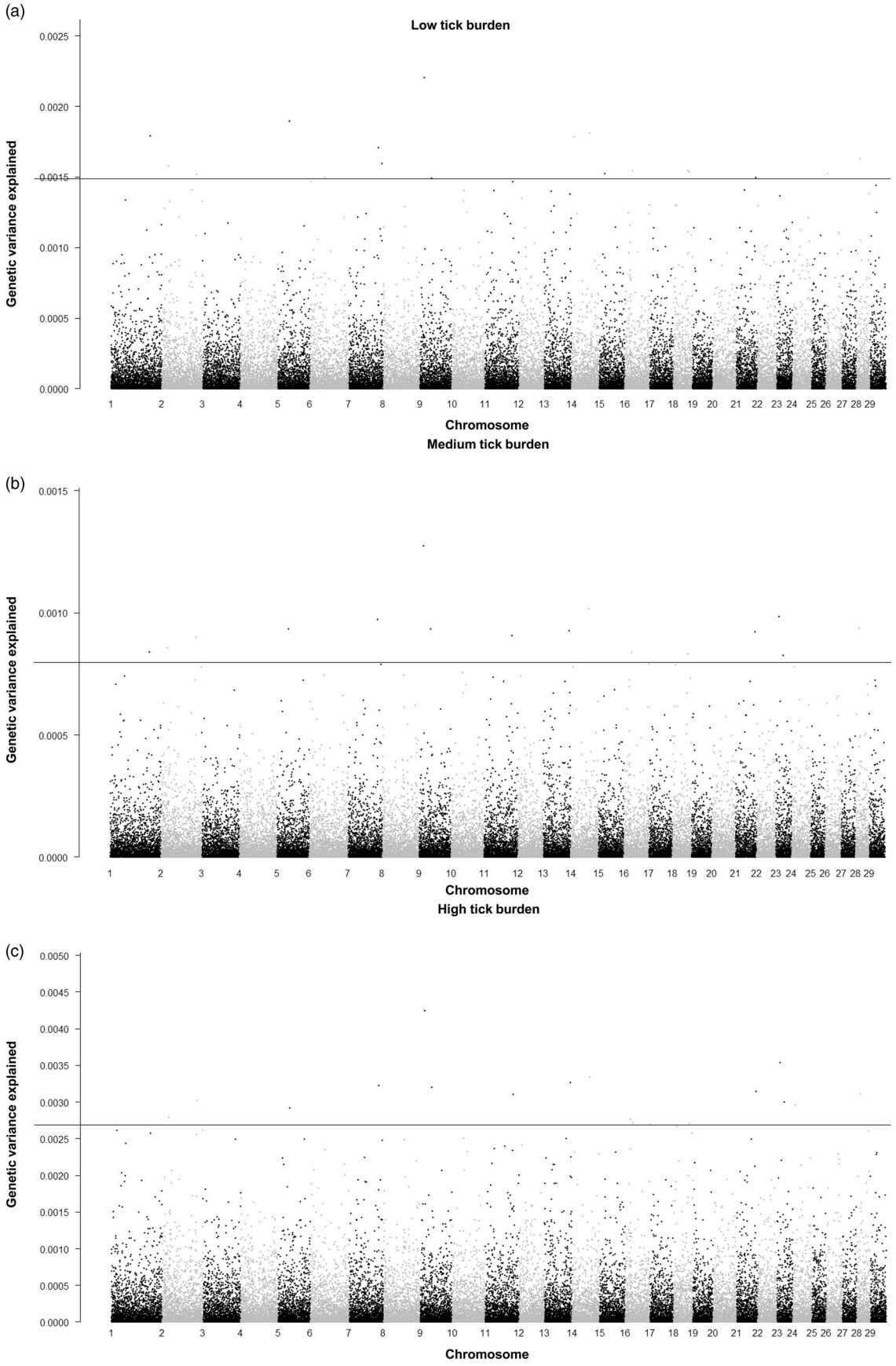

Figure 3 Manhattan plots at low (a), medium (b) and high (c) tick burden. Chromosomes 1 to 29 are shown separated by alternating colors. The corresponding horizontal line indicates the outliers/significant single nucleotide polymorphism markers based on genetic variance explained.

Table 1 *Single nucleotide polymorphism (SNP), chromosome (Chr), position (Pos), genetic variance explained (Var), candidate gene symbol mapped through Ensembl and/or NCBI database for outliers/significant SNPs at different tick burden (low, medium and high)*

SNP	Chr	Pos	Var	Gene via Ensembl	Gene via NCBI
			All tick burden		
BTB-01782721	2	21389327	1.89E-05	–	*HOXD1*
ARS-BFGL-NGS-107909	2	116934708	1.82E-05	*CCL20*	–
BTA-26132-no-rs	5	41799777	2.27E-05	–	–
Hapmap55378-rs29025271	7	96192503	2.05E-05	–	–
Hapmap57341-rs29010513	9	15767136	2.64E-05	*MYO6*	–
ARS-BFGL-NGS-63180	9	38575112	1.79E-05	–	–
ARS-BFGL-NGS-113927	14	51396430	2.17E-05	–	–
Hapmap47933-BTA-38363	16	29812601	1.85E-05	*H3F3B*, *H3F3A*, *TMEM63A*, *LIN9*, *SDE2*, *EPHX1*, *MIXL1*, *ACBD3*	*H3F3B*
BTA-118931-no-rs	18	52024379	1.84E-05	*BSP1*, *BSP3*, *BSP5*, *PLAUR*	–
Hapmap52873-ss46526026	21	66064177	1.79E-05	*SETD3*, *CCDC85C*, *BCL11B*	*SETD3*
Hapmap50751-BTA-64830	28	13270960	1.95E-05	*ZNF248*, *ZNF25*	–
			Low tick burden		
BTA-97832-no-rs	1	125283942	2.14E-05	–	–
Hapmap32456-BTC-038385	6	45216251	1.79E-05	–	*PPARGC1A*
Hapmap57373-rs29019418	7	108861104	1.91E-05	–	–
Hapmap33459-BTC-069647	14	6486431	2.14E-05	–	–
ARS-BFGL-NGS-113417	15	21732355	1.82E-05	–	–
ARS-USMARC-Parent-AY863214-rs17871744	18	46647177	1.85E-05	*PSENEN*, *UPK1A*, *TYROBP*, *PRODH2*, *HAUS5*, *APLP1*, *ETV2*, *NPHS1*, *LIN37*, *HSPB6*, *RBM42*, *NFKBID*, *IGFLR1*, *LRFN3*, *HCST*, *ZBTB32*, *KMT2B*, *ARHGAP33*	*LIN37*
ARS-BFGL-NGS-73895	26	6948695	1.83E-05	*PRKG1*, *DKK1*	*PRKG1*
			Medium tick burden		
BTA-97832-no-rs	1	125283942	1.38E-05	–	–
ARS-BFGL-NGS-116569	11	86751976	1.49E-05	*ROCK2*, *KCNF1*, *PQLC3*, *ATP6V1C2*, *C2orf50*	–
ARS-BFGL-NGS-36793	13	80239131	1.52E-05	*ATP9A*, *NFATC2*, *SALL4*	*ATP9A*
ARS-BFGL-NGS-111735	23	12997137	1.61E-05	*KCNK17*, *DNAH8*	*DNAH8*
ARS-BFGL-NGS-117031	23	25222914	1.35E-05	*BOLA-DQA2*, *BOLA-DQA5*, *BOLA-DQB*, *GCM1*, *ICK*, *FBXO9*	*ELOVL5*
			High tick burden		
ARS-BFGL-NGS-116569	11	86751976	8.17E-05	*ROCK2*, *KCNF1*, *PQLC3*, *ATP6V1C2*, *C2orf50*	–
ARS-BFGL-NGS-36793	13	80239131	8.60E-05	*ATP9A*, *NFATC2*, *SALL4*	*ATP9A*
BTB-01475457	16	21326517	7.29E-05	–	*ESRRG*
ARS-BFGL-NGS-52551	16	80859590	7.10E-05	*NR5A2*	*NR5A2*
ARS-BFGL-NGS-111735	23	12997137	9.33E-05	*KCNK17*, *DNAH8*	*DNAH8*
ARS-BFGL-NGS-117031	23	25222914	7.90E-05	*BOLA-DQA2*, *BOLA-DQA5*, *BOLA-DQB*, *GCM1*, *ICK*, *FBXO9*	*ELOVL5*
BTA-115451-no-rs	24	8540940	7.80E-05	–	–

genes pointed out were detected in all tick burden levels. Moreover, genes like *PPARGC1A*, *LIN37*, *PRKG1*, *UPK1A* and *DKK1* were found to be mapped surrounding SNPs exclusively identified in low, whereas *NR5A2* and *ESRRG*, only to high tick burden (Table 1).

Table 2 presented the significantly *MeSH terms* (*P-values* <0.01) returned by *MeSH* ORA that deserve particular attention in the area of diseases resistance/immunology. Each *MeSH term* was clustered into three categories: Diseases, Phenomena and Processes, and Chemical and Drugs.

The peroxisome proliferator-activated receptor γ coactivator 1 α (PPARγ/PGC-1α) protein, encoded by *PPARGC1A* gene, was related to a SNP located on BTA6. *PPARGC1A* is a metabolic switch, which transcriptionally activates a complex pathway of mitochondrial biogenesis and energy (lipid and glucose) metabolism. Despite *PPARGC1A (*or PGC-1α) being postulated as the most plausible gene underlying a QTL for fat yield (Streit *et al.*, 2013), the role of the PPARγ/PGC-1α pathway in the pathogenesis of liver cancer was investigated (Lee *et al.*, 2009) and PPARγ reported to mediate macrophage differentiation and inflammatory responses (Moore *et al.*, 2001).

Table 2 *-Statistically significant (*P*-value ⩽0.01) MeSH (Medical Subject Headings) terms (ID and name) associated to the selected genes for three categories (1 – Phenomena and Process, 2 – Diseases and 3 – Chemical and Drugs)*

Categories	MeSH term ID	MeSH term name	Gene	*P*-value
Phenomena and Process	D013499	Surface Properties	*BSP1*, *BSP3*, *BSP5*	0.00015
	D040681	Structural Homology (Protein)	*BSP1*, *BSP3*, *BSP5*	0.00015
	D059748	Proteolysis	*BSP1*, *BSP3*, *BSP5*	0.00023
	D017434	Protein Structure (Tertiary)	*PLAUR*, *PRKG1*, *ROCK2*, *BSP1*, *BSP3*, *BSP5*, *H3F3A*, *GLP1R*	0.0018
	D002199	Capillary Permeability	*PLAUR*, *ROCK2*	0.0035
	D002463	Cell Membrane Permeability	*PLAUR*, *BSP1*	0.004
	D006570	Heterochromatin	*H3F3A*	0.0053
	D009119	Muscle Contraction	*ROCK2*, *HSPB6*	0.0088
Diseases	D014552	Urinary Tract Infections	*UPK1A*	0.0053
Chemical and Drugs	D000949	Histocompatibility Antigens Class II	*BOLA-DQA5*, *BOLA-DQB*, *BOLA-DQA2*	0.00003
	D055655	NK Cell Lectin-Like Receptor Subfamily K	*TYROBP*, *HCST*	0.00027
	D006493	Heparin	*BSP1*, *BSP3*, *BSP5*	0.00047
	D005353	Fibronectins	*BSP1*, *BSP3*, *BSP5*	0.00075
	D051548	Histone Acetyltransferases	*ROCK2*, *H3F3A*	0.00075
	D055607	Receptors Natural Killer Cell	*TYROBP*, *HCST*	0.0012
	D051176	β Catenin	*PLAUR*, *DKK1*	0.0035
	D012701	Serotonin	*ROCK2*, *HSPB6*	0.004
	D006570	Heterochromatin	*H3F3A*	0.0053
	D036781	Fimbriae Proteins	*UPK1A*	0.0053
	D053766	Presenilin-2	*PSENEN*	0.0053
	D056245	Mi-2 Nucleosome Remodeling and Deacetylase Complex	*H3F3A*	0.0053
	D065636	Myotonin-Protein Kinase	*ROCK2*	0.0053
	D018080	Receptors GABA-B	*PRKG1*	0.01
	D024461	Myosin Type I	*MYO6*	0.01
	D050886	HSP20 Heat-Shock Proteins	*HSPB6*	0.01
	D051037	Large-Conductance Calcium-Activated Potassium Channel α Subunits	*PRKG1*	0.01
	D053499	Plasma Membrane Calcium-Transporting ATPases	*BSP1*	0.01
	D054481	Thioredoxin Reductase 1	*PRKG1*	0.01
	D059848	HLA-DQ α-Chains	*BOLA-DQA2*	0.01
	D060165	Uroplakins	*UPK1A*	0.01

PPARGC1A and steroid receptor coactivator-2 are important coactivators for the PPAR$_\beta$ regulatory functions, which in turn regulates the transcription of several genes implicated in metabolism, differentiation and immune functions (Neels and Grimaldi, 2014).

The *PRKG1* gene was mapped close to the SNP (ARS-BFGL-NGS-73895; BTA26) with significant effect only in low tick burden. This gene was previously reported to be involved in systemic lupus erythematosus, that is, an autoimmune disorder related to multiple organ systems including skin, musculoskeletal, renal and hematologic systems in humans (Kariuki *et al*., 2015). Despite *PRKG1* function was not as strongly supported in other immune cells such as T and B cells, it was in interferon-α-producing cells. In addition, *PRKG1* gene was also pointed out by *MeSH terms Large-Conductance Calcium-Activated Potassium Channel α Subunits*, *Receptors GABA-B* and *Thioredoxin Reductase* (Chemical and Drugs category) (Table 2). On the other hand, *BSP1*, *BSP3* and *BSP5* (mapped on BTA18) are genes associated to BTA-118931-no-rs marker across all (low, medium and high) tick burden levels, and related to some *MeSH terms*: Surface Properties; Structural Homology, Protein; Protein Structure, Tertiary; Cell Membrane Permeability; Heparin and Fibronectins and Plasma Membrane Calcium-Transporting ATPase.

The lin-37 DREAM MuvB core complex component, the protein product of *LIN37* gene mapped on BTA18 only in low tick burden level, is required for regulation of chondrocyte proliferation (Forristal *et al*., 2014). It is possible to infer that genes related to protein structure and cell proliferation may be involved with the inflammatory phase, adding to extracellular matrix remodeling, new blood vessels formation and epithelia (Theilgaard-Mönch *et al*., 2004). In fact, the *MYO6* gene, likewise related to cytoskeleton rebuilding (actin-myosin) in inflammatory conditions (Liao *et al*., 2013), is another significant gene associated within the *MeSH term* Myosin Type I. Thus, *MYO6* may play a critical role in epithelial barrier function.

The *DKK1* gene (Dickkopf-1) mapped on BTA26, as a major modulator of Wnt signaling could be involved in rickettsia infections pathogenesis, suggesting inflammatory effects

(release of proinflammatory cytokines interleukin (IL)-6, IL-8) if silenced (Astrup *et al*., 2012). This gene was associated to the β-catenin *MeSH term* (Table 2) jointly with *PLAUR* plasminogen activator, urokinase receptor gene. Plasminogen activator inhibitor-1 was mentioned to be involved with blood coagulation cascade. This is host defense first line that ticks have to defeat to successfully feed and subsequently transmit disease pathogens (Mulenga *et al*., 2001). It is known that uncontrolled inflammation may lead to tissue injury, and among many signaling pathways activated, the conserved pathway Wnt/β-catenin plays an important role in the expression of inflammatory molecules (Silva-García *et al*., 2014).

The candidate gene *NR5A2 was* mapped on BTA16, although associated to SNPs exclusively significant in high tick burden level. The nuclear receptor is a superfamily of eukaryotic transcription factors that are crucial for gene regulation and development. The *NR5A2* gene was reported to be enriched expressed in mammalian epithelial tissues and possibly related to mastitis immune response (Sharma and Jeong, 2013). In addition, Flandez *et al*. (2014) indicated that *NR5A2* is a novel pancreatic tumor suppressor, displaying histological abnormalities in the pancreas and showing impaired recovery from damage. This was followed especially by proinflammatory cytokine gene expression, hyper nuclear factor-*kb* activation and signal transducer. These findings led us to speculate about the relevance of this gene in terms of tick injuries in epithelial cells.

Another unique SNP (BTB-01475457) identified in high tick burden, was mapped to *ESRRG* gene, which is known to be strongly connected to T cell function via mitochondrial metabolism in autoimmune lupus disease (Perry *et al*., 2012; Choi *et al*., 2016). Perry *et al*. (2012) have reported mitochondrial functions decreased in $CD4^+$ T cells expressing the *NZM2410* allele of *ESRRG*. An old study in guinea pigs reported that significant resistance to tick burden can be transferred with viable T cells (Wikel and Allen, 1976). According to these authors, the tick resistance passage with T cells indicates a delayed hypersensitivity mechanism for tick resistance acquisition. These reports might explain the expression of this gene in high tick burden. Moreover, this gene was identified in a region from bottom 1% smoothed F_{ST} values, considered as potentially linked to purifying or balancing selection processes in domestic cattle (Porto-Neto *et al*., 2013).

On BTA23, the *DNAH8* and *ELOVL5* genes were identified in medium and high tick burden. *DNAH8* gene was recently highlighted as reproduction-related function by Fischer *et al*. (2015) in a Finnish Large White pig population. Newsworthy, the *ELOVL5* gene has already been reported as related to immune responses in Canadian Holstein cattle (Thompson-Crispi *et al*., 2014). These authors mapped *ELOVL5* as top 10 significant SNPs for antibody-mediated immune response (AMIR) in Holstein cows. Moreover, this genomic region is well known as a location of major genes associated with immune responses and resistance to diseases (Stear *et al*., 2001; Ellis and Codner, 2012; Thompson-Crispi *et al*., 2014). As reported by Thompson-Crispi *et al*. (2014), the expression of this gene showed superior immune responses for male and female Holsteins. As these authors had reported the significant association of *ELVOL5* with AMIRs, we might also infer that this gene is somehow related to tick immune responses. In addition, an important tool to identify less susceptible animals can be established since cattle with higher immune responses tend to have a lower occurrence of diseases.

The same authors above also reported candidate genes within the bovine major histocompatibility complex, such as *BOLA-DQA1* and *BOLA-DQB*. Our results from ORA through *MeSH* indicated *BOLA-DQA5*, *BOLA-DQB*, *BOLA-DQA2* (*MeSH term* D000949) as candidates to be related to the mechanisms of resistance to parasites in medium and high tick burden. These complex have already been reported by Martinez *et al*. (2006) showing interaction between the BOLA complex and innate and adaptive immunity traits in crossbred (Holstein $\times$ Gyr) cattle population.

The H3 histone, family 3B (H3.3B), a protein encoded by the *H3F3B* gene was detected in all tick burden levels and highlighted in some *MeSH terms* between Phenomena and Process and Chemical and Drugs categories (Protein Structure, Tertiary; Heterochromatin; Histone Acetyltransferases; Mi-2 Nucleosome Remodeling and Deacetylase Complex). Although histones are normally responsible for nucleosome structure of the chromosomal fiber, this gene has been also related to connective tissue disorders and inflammatory diseases (Gras *et al*., 2009).

In general, regarding the ORA *MeSH* results, it also worth to mention the *MeSH term* highlighted within those listed in Chemical and Drugs category, *Receptors Natural Killer Cell*, including genes like *TYROBP* and *HCST*. Natural killer cells are innate immune response effectors which function as regulatory cells in interactions with endothelial cells, dendritic cells, macrophages and T lymphocytes (Vivier *et al*., 2008).

Moreover, the significant *MeSH term Serotonin*, associated with Chemical and Drugs category, related *ROCK2* and *HSPB6* genes. Although these genes could play a role in the immune system, it is known serotonin is an inflammatory mediator that is rapidly released at the site of tissue injury by the vascular endothelium and attracted inflammatory cells. The serotonin is also related to the anti-inflammatory actions at feeding site exhibiting by tick salivary prostaglandins (Singh and Girschick, 2003).

Studies have been considering the signaling and Ca^{2+} binding protein genes in skin samples taken from cattle with known high (HR) and low (LR) resistance to ticks (Bagnall *et al*., 2009). Reported by those authors, an ATPase gene (*AT2A1* (*SERCA2*)- Sarco/endoplasmic reticulum calcium ATPase) was detected as differentially expressed between HR and LR cattle by cattle tick (*Rhipicephalus* (*Boophilus*) *microplus*) artificial challenge. This gene was suggested to be involved in tick attachment response. Similarly, we identified an ATPase gene (*ATP9A*), mapped on BTA13 in medium and high tick burden, and related to metabolic processes and phospholipid transport (Lee *et al*., 2005; Wei *et al*., 2010).

In terms of tick resistance these genes might be very important as tick bites cause activation of an inflammatory process influenced by cattle genetic composition and

previous exposure (Carvalho *et al.*, 2014). Most of the genes and biological/functional terms identified and discussed in Carvalho *et al.* (2014), are either part of the choreographed host defense responses (such as cutaneous interface) or the tick countermeasures in which the successful blood feeding and establishment of tick-borne infectious agents within the host occurs.

The presence of SNP by environment interactions was confirmed for tick resistance via one-step reaction norm models and SNPs that affect different tick burden levels could be identified. We reported outliers or significant SNPs associated with tick burden across environments and neighboring genes with different direct or indirectly molecular functions related to immune system, inflammatory process and mechanisms of tissue destruction/repair, such as energy metabolism and cell differentiation in Hereford/Braford cattle population.

The ORA through *MeSH* was able to enrich the gene biological knowledge and this tool has been applied successfully to mammals, including dairy cattle, swine and horse (Morota *et al.*, 2015). This information can be useful to further explore pathways and gene networks for validation and better dissection of candidate genes and their interaction with tick resistance/susceptibility mechanisms. By candidate genes validation, more reliable predictions are expected in genomic selection through SNP panels developed from the target population and taking into account individuals from different environments.

In addition, based on a previous persistence of LD phase in this population (Biegelmeyer *et al.*, 2016), which reflects their genetic relationships, knowledge about SNP effects or candidate genes by environmental interactions could be further used to increase accuracy of selection across environments and to choose best sires for different environments in genomic selection evaluations of this Hereford/Braford population.

Despite the main contribution of the present study in drawing interest toward SNP effects, genetic variance explained and candidate genes identified via linear genomic reaction norms, we are aware that a simple approach was used here for GWAS. Some future studies should applied higher-order of reaction norm models or even other specific GWAS models, such as Bayes-A, Bayes-B and Bayesian LASSO.

Acknowledgments

The authors thank Delta G Connection by providing the data; CAPES and CNPq by granting the scholarships. This project was supported by CNPq – National Council for Scientific and Technological Development Grant No. 478992/2012-2, Embrapa – Brazilian Agricultural Research Corporation Grants No. 02.13.10.002 and 01.11.07.002, and Agriculture and Food Research Initiative Competitive Grant No. 2011-67015-30338 from the USDA National Institute of Food and Agriculture.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731117001562

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