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1    **Evidence of a plant genetic basis for maize roots impacting soil organic matter mineralization**

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17

18    **Abstract**

19

20    Maize root traits associated with soil organic matter (SOM) mineralization were demonstrated to have a

21    heritable genetic basis. We show root length, root diameter and cumulative root-derived C mineralization

22    to be strong predictors of SOM-C mineralization and identify two candidate genes associated with

23    enhanced SOM-C mineralization rates. There is potential to target these genes to enhance release of

24    nutrients from SOM to support crop nutrition within sustainable maize cropping systems.

25

26    Keywords: maize breeding; soil organic matter mineralization; root traits; genome-wide association

27    study (GWAS)

28

29    The maize yield gap in sub-Saharan Africa (SSA) is approximately 60-80% (van Ittersum et al. 2016).

30    To achieve self-sufficiency by 2050, without expanding current maize area, the yield gap must be

31    reduced to 20% (van Ittersum et al. 2016). Drought stress regularly affects maize production in SSA

32    (Cairns and Prasanna, 2018). Low fertilizer use (<17 kg ha$^{-1}$) is another major factor contributing to this

33    yield gap (Thierfelder et al. 2018), particularly in female-managed plots (Farnworth et al. 2017) and is

34    exacerbated by low and variable economic returns on investment in the use of fertilizers (Jayne et al.

35    2018). Release of nutrients from positive priming of SOM (Kuzyakov et al. 2000) can supplement crop

36    nutrition and increase the return on investment in fertilizer use. Breeding for maize varieties that are

able to source and utilize nutrients from SOM for their nutrition will ensure more sustainable production where practices are applied that replenish SOM, such as from stover addition.

The importance of root trait diversity in shaping plant-associated microbiome composition and nutrient cycling, including SOM mineralization, is increasingly recognized (e.g. Mwafulirwa et al. 2016; Han et al. 2020; Zai et al. 2021). Genotypic information can be used to identify genomic regions associated with these traits and narrow the selection of candidates prior to phenotype-screening (Cooper et al. 2014). Identification of potential candidate genes for traits influencing rhizosphere processes paves the way for breeding to achieve more resilient and sustainable maize production. Here we investigate the genetic basis of root traits associated with differential SOM mineralization and identify potential candidate genes.

A set of 97 elite maize lines from a drought-tolerant maize for Africa (DTMA) panel and 8 commercial hybrids were selected for screening. Genotypes were grown (29 days) under controlled environment conditions in a granitic sandy soil from Domboshawa, Zimbabwe (-17.6091 Lat, 31.13374 Long). All the genotypes were replicated two times. In addition, some of the genotypes were randomly selected and replicated a few more times (Table S5). The experimental design was a sequential randomized block design, and included unplanted controls. Exposure to $^{13}$C-enriched $CO_2$ (20 atom% $^{13}$C) throughout the growth period enabled quantification of root- and SOM-derived $CO_2$-efflux rates at 16, 23 and 29 days after planting and cumulative C mineralization over the three weeks period of $CO_2$ measurement, following the approach of Mwafulirwa et al. (2016). The nine traits measured in this study were cumulative SOM-C mineralized (SOMC, µg C g$^{-1}$ soil), cumulative root-derived C mineralized (CRM, µg C g$^{-1}$ soil), average root diameter (ARD, mm), root length (RL, mm), root biomass (RBM, g), shoot biomass (SBM, g), total plant biomass (Stover, g), specific root length (SRL) and root-to-shoot biomass ratio (BM-RS). SBM and RBM were determined based on harvest dry weight (V3 growth stage) at 29 days after planting (shoots were cut at the soil surface and roots were carefully removed from the soil and washed in deionized water). Fresh roots were scanned, and root length and average root diameter measured using WINrhizo (Mwafulirwa et al. 2021). To explore the plant traits that could significantly explain the variation in SOM-C mineralization, correlation and stepwise multiple linear regression (SMLR) analyses were conducted for all genotypes (lines and hybrids) and for lines or hybrids separately. Further details of plant growth conditions, $^{13}$C-$CO_2$ labelling, measurements and phenotypic data are provided in Mwafulirwa et al. (2021) and supplementary materials. Details of genotyping of materials and methodology of genome-wide association study (GWAS) and genomic selection (GS) are provided in the supplementary materials.

72  We observed significant clustering of genotypes into six groups based on their phenotypic performance
73  (Fig. 1; Table S1), with the maize lines clustering distinctly from the hybrid varieties (Fig. 1). For inbred
74  lines, root diameter and root-derived soil $CO_2$ efflux (from root respiration and microbial mineralization
75  of rhizodeposits) were positively correlated (r = 0.39 and 0.24 respectively; $P < 0.05$) with SOM
76  mineralization rates (Fig. S1). SMLR indicated that root length, diameter, and root-derived $CO_2$ efflux
77  together explained 47% of variation in SOM mineralization ($P < 0.001$; Table S2) across inbred lines.
78  However, for hybrids, none of these traits were significantly correlated with SOM mineralization (Fig.
79  S1). This variation within tropical germplasm for maize root traits that correlate with SOM mineralization
80  rates suggests that exploration of the genetic basis of inbred lines can serve as a viable option for
81  targeted maize breeding for sustainable intensification.

82

83  Variation in SOM mineralization rates under plants is usually attributed to rhizosphere priming effects,
84  where the exudation of organic compounds and root turnover affects microbial activity and use of SOM
85  as substrate (Kuzyakov et al. 2000). While factors such as root physical impacts on soil structure,
86  nutrient and water availabilities may also affect microbial activity, our results are consistent with
87  differential priming impacts among inbred lines (Mwafulirwa et al. 2021). Increasing root diameter is
88  commonly associated with increased rates of exudation, resulting from greater photo assimilate supply
89  to thicker roots (Han et al. 2020; Zai et al. 2021). In turn, the magnitude of rhizosphere priming effects
90  has been found to be proportional to the substrate supply to rhizosphere microbial communities
91  (Paterson and Sim, 2013), suggesting the magnitude of rhizodeposition may be a key discriminating
92  factor between inbred lines. This interpretation is also supported by the strong improvement to the
93  variance explained when root-derived $CO_2$ efflux (partly from microbial use of rhizodeposits) is included
94  in the SMLR with SOM mineralization (Table S2).

95

96  Genotypic variances were significant for all traits that correlated with SOM mineralization, and broad-
97  sense heritability ranged from 0.18 to 0.65 (Table S3). Importantly, all traits showed normal distribution
98  of best linear unbiased predictions (BLUPs) which supports their quantitative inheritance (Fig. 2A). DNA
99  markers (199,755 markers) used for GWAS well represented the maize genome (Fig. S2A). The
100 principal components (Fig. S2B) and kinship heatmap (Fig. S3) indicated low levels of relatedness
101 among most pairs of inbred lines. GWAS for nine traits revealed 84 significant SNPs ($P < 5 \times 10^{-5}$; Fig.
102 2B; Table S4). Association analyses revealed 6, 25, 3, 7, 24,13, 8, 3 and 7 SNPs significantly associated
103 with SOMC, CRM, SBM, RBM, RL, ARD, SRL, BM-RS and stover, respectively (Fig. 2B; Table S4). The
104 distribution of significant SNPs across chromosomes varied between 4 in chromosome 8, to 15 in
105 chromosome 5 (Table S3). The allelic effect (difference in mean performance for trait value between
106 lines with the major or minor allele) for significant SNPs ranged from −1.27 to 1.43 for SOM
107 mineralization.

108

109 For all nine traits, a total of 23 potential candidate genes were identified (Table S4). Root-derived C
110 mineralization was negatively correlated with SRL and associated with candidate gene
111 *GRMZM2G141456* with functions for proteolysis, hydrolase activity and metallo-peptidase activity.
112 Genomic prediction correlations were -0.19 and -0.08 for SOMC and CRM, respectively (Fig. 2C).
113 Similar prediction correlations have been reported for less related populations or complex traits in maize
114 (Riedelsheimer et al. 2013), although not for SOM mineralization. Moderate prediction correlations for
115 other traits suggests genomic prediction can be used to improve these traits within a breeding program.
116

117 The genetic basis of variation in root exudation (amount and quality) is not well understood. In this
118 context, we have identified two candidate genes associated with enhanced SOM mineralization rates:
119 *GRMZM2G114362* with functions for proteolysis and peptidase activity, and *GRMZM2G170962* for
120 proton-transporting ATP synthase and ATP synthesis, which are associated with the regulation and
121 active release of root exudates into the rhizosphere. This suggests the potential for targeting these
122 genes to promote root exudate release and to stimulate the microbial community to release nutrients
123 from SOM for crop nutrition. This therefore holds great promise for enhancing sustainable production in
124 systems managed to enhance SOM, for example through return of stover to soil which is common in
125 conservation agriculture (Thierfelder et al. 2015), thereby promoting a more closed nutrient cycle, that
126 increases the return on investment in inputs. Root traits associated with SOM mineralization cannot
127 logistically be incorporated into maize breeding in SSA as this would require accurate phenotyping of
128 thousands of lines each year. However, our results suggest the potential of molecular breeding
129 approaches to enrich populations for markers linked with key traits associated with SOM mineralization
130 prior to field phenotyping.
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140 **References**

141

142 Cairns, J.E., Prasanna, B.M. 2018. Developing and deploying climate-resilient maize varieties in the
143 developing world. Current Opinions in Plant Biology 45, 226-230.

144

145 Cooper, M., Messina, C.D., Podlich, D., Radu Totir, L., Baumgarten, A., Hausmann, N.J., Wright, D.,

146 Graham, G. 2014. Predicting the future of plant breeding: complementing empirical evaluation with

147 genetic prediction. Crop and Pasture Science 65, 311-336.

148

149 Farnworth, C.R., Stirling, C., Sapkota, T.B., Jat, M.L., Misiko, M., Attwood, S., 2017. Gender and

150 inorganic nitrogen: what are the implications of moving towards a more balanced use of nitrogen fertilizer

151 in the tropics? International Journal of Agricultural Sustainability 15, 136-152.

152

153 Jayne, T.S., Mason, N.M., Burke, W.J., Ariga, J., 2018. Review: Taking stock of Africa's second

154 generation agricultural input subsidy programs. Food Policy 75, 1-14.

155

156 Han, M.G., Sun, L.J., Gan, D.Y., Fu, L.C., Zhu, B., 2020. Root functional traits are key determinants of

157 the rhizosphere effect on soil organic matter decomposition across 14 temperate hardwood species.

158 Soil Biology & Biochemistry 151, 108019.

159

160 Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects.

161 Soil Biology & Biochemistry 32, 1485-1498.

162

163 Mwafulirwa, L., Baggs, E.M., Russell, J., George, T., Morley, N., Sim, A., de la Fuente Cantó, C.,

164 Paterson, E., 2016. Barley genotype influences stabilization of rhizodeposition-derived C and soil

165 organic matter mineralization. Soil Biology & Biochemistry 95, 60-69.

166

167 Mwafulirwa, L., Paterson, E., Cairns, J.E., Daniell, T.J., Thierfelder, C., Baggs, E.M., 2021 Genotypic

168 variation in maize (*Zea mays*) influences rates of soil organic matter mineralisation and gross

169 nitrification. New Phytologist. https://doi.org/10.1111/nph.17537.

170

171 Paterson, E., Sim, A., 2013. Soil-specific response functions of organic matter mineralization to the

172 availability of labile carbon. Global Change Biology 19, 1562-1571.

173

174 Riedelsheimer, C., Endelman, J.B., Stange, M., Sorrells, M.E., Jannink, J-L., Melchinger A.E., 2013.

175 Genomic predictability of interconnected biparental maize populations. Genetics 194, 493-503.

176

177 Thierfelder, C., Rusinamhodzi, L., Ngwira, A.R., Mupangwa, W., Nyagumbo, I., Kassie, G.T., Cairns,

178 J.E., 2015. Conservation agriculture in Southern Africa: Advances in knowledge. Renewable Agriculture

179 and Food Systems 30, 328-348.

180

181 Thierfelder, C., Baudron, F., Setimela, P., Nyagumbo, I., Mupangwa, W., Mhlanga, B., Lee, N., Gérard,
182 B., 2018. Complementary practices supporting conservation agriculture in southern Africa. A review.
183 Agronomy for Sustainable Development 38, 16.

184

185 van Ittersum, M.K., van Bussel, L.G.J., Wolf, J., Grassini, P., van Wart, J., Guilpart, N., Claessens, L.,
186 de Groot, H., Wiebe, K., Mason-D'Croz, D., Yang, H., Boogaard, H., van Oort, P.A.J., van Loon, M.P.,
187 Saito, K., Adimo, O., Adjei-Nsiah, S., Agali, A., Bala, A., Chikowo, R., Kaizzi, K., Kouressy, M., Makoi,
188 J.H.J.R., Ouattara, K., Tesfaye, K., Cassman, K.G., 2016. Can sub-Saharan Africa feed itself? PNAS
189 113, 14964-14969.

190

191 Wen, W., Araus, J. L., Shah, T., Cairns, J., Mahuku, G., Bänziger, M., Torres, J. L., Sánchez, C., Yan,
192 J., 2011. Molecular characterization of a diverse maize inbred line collection and its potential utilization
193 for stress tolerance improvement. Crop Science 51, 2569–2581.

194

195 Zai, X.Y., Luo, W., Bai, W.Q., Li, Y.H., Xiao, X., Gao, X.E., Wang, E.T., Wei, G.H., Chen, W.M., 2021.
196 Effect of root diameter on the selection and network interactions of root-associated bacterial
197 microbiomes in *Robinia pseudoacacia* L. Microbial Ecology DOI: 10.1007/s00248-020-01678-4.

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**Figure captions**

**Figure 1.** Cluster plot showing six major groups (Ward linkage, squared Euclidean distance) of maize genotypes based on the measured traits: Cumulative SOM-C mineralized (SOMC), cumulative root-derived C mineralized (CRM), average root diameter (ARD), root length (RL), root biomass (RBM), shoot biomass (SBM), specific root length (SRL) and root-to-shoot biomass ratio (BM-RS) for 105 maize genotypes grown in granitic sandy soil (pH$_{H2O}$ 4.79; CEC 1.01 meq 100g$^{-1}$; total C 2.98 mg g$^{-1}$; total N 0.24 mg g$^{-1}$; sand 83.7%, silt + clay 16.3%) in controlled environment microcosms (22.5 x 5.5cm diam; 65% water holding capacity; soil bulk density 1.44g cm$^{-3}$) (Mwafulirwa et al. 2021). C mineralization was determined by continuous labelling with $^{13}$C-enriched $CO_2$ (20 atom% $^{13}$C) through a growth chamber (<10 µl L$^{-1}$ $CO_2$ outlet airflow; 12-hour daily photoperiod, 512µmol m$^{-2}$ s$^{-1}$ PAR). Soil $CO_2$ fluxes at 16, 23 and 29 days after planting were partitioned to SOM- and root-derived C based on their δ$^{13}$C signatures, following Mwafulirwa et al. (2016). Clustering formed six main groups, with lines mostly distributed in first five groups, whereas six hybrids (SC633, PGS61, Pristine 601, 30G19, PAN53 and ZAP55 in blue) are grouped together as group VI. Traits contributing high mean performance for clusters were specified on the figure. Pedigrees associated with the genotype codes can be found in Wen et al. (2011).

**Figure 2.** Phenotypic distribution of best linear unbiased predictions (BLUPs) for each trait (A), GWAS based Manhattan plots (B) and genomic prediction correlations (C) for nine traits from DTMA association panel. In the Manhattan plots each color represents different chromosome and the horizontal lines show the threshold p value at $P < 5 \times 10^{-5}$. SOM-C Cumulative soil organic matter C mineralized (µg C g soil$^{-1}$), CRM Cumulative root-derived C mineralized (µg C g soil$^{-1}$), SBM shoot biomass (g), RBM root biomass (g), RL root length (m), ARD average root diameter (mm), SRL specific root length, BM_RS root-to-shoot biomass ratio and Stover (total plant biomass, g).
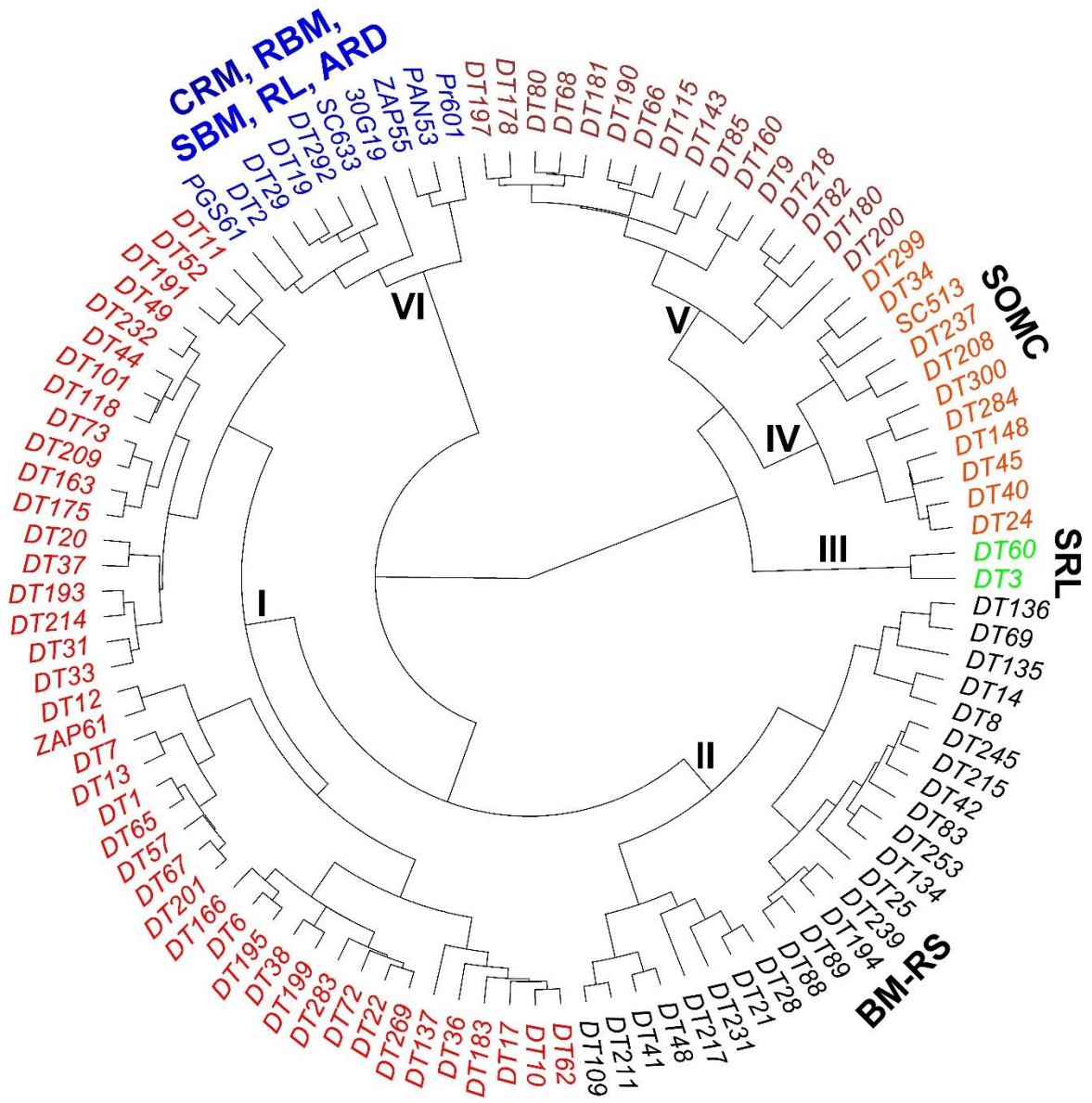
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**Figure 1**.

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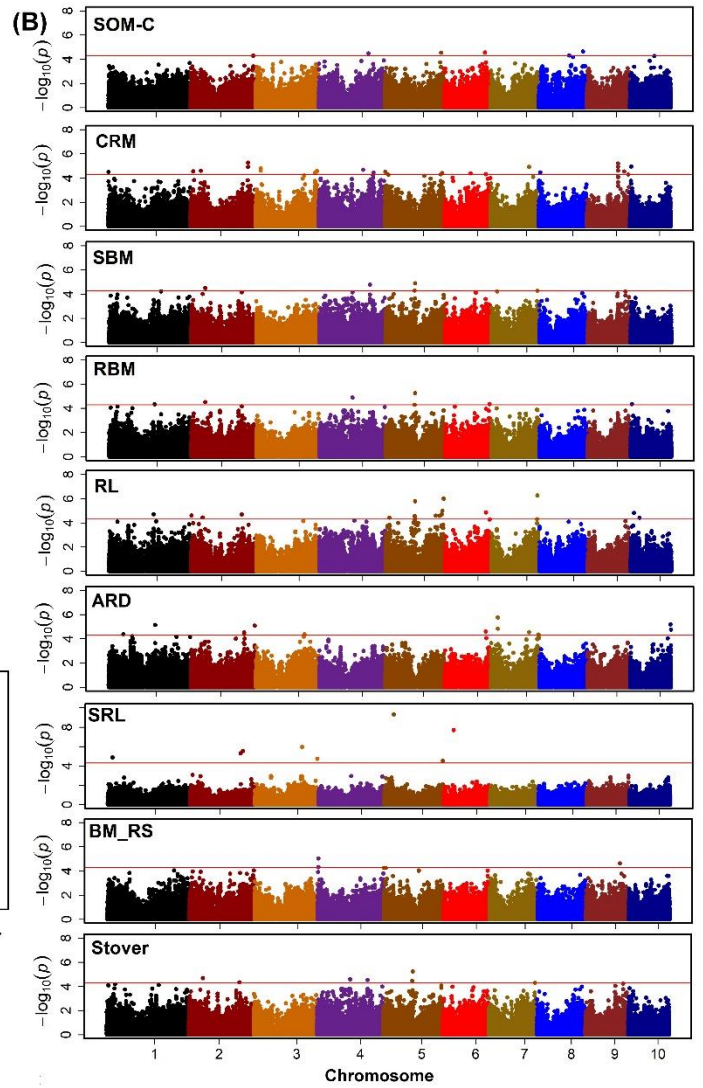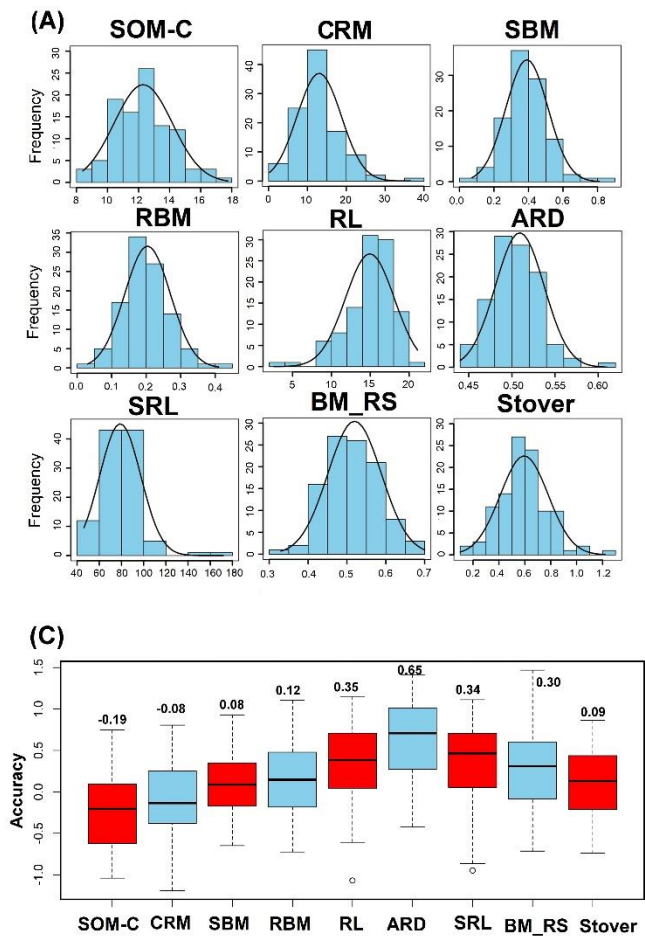236 **Figure 2**.
237