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Testing the link between genome size and growth rate in maize

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Little is known about the factors driving within species Genome Size (GS) variation. GS may be shaped indirectly by natural selection on development and adaptative traits. Because GS variation is particularly pronounced in maize, we have sampled 83 maize inbred lines from three well described genetic groups adapted to contrasted climate conditions: inbreds of tropical origin, Flint inbreds grown in temperate climates, and Dent inbreds distributed in the Corn Belt. As a proxy for growth rate, we measured the Leaf Elongation Rate maximum during nighttime (LER\textsubscript{max}) as well as GS in all inbred lines. In addition we combined available and new nucleotide polymorphism data at 29,090 sites to characterize the genetic structure of our panel. We found significant variation for both LER\textsubscript{max} and GS among groups defined by our genetic structuring. Tropicals displayed larger GS than Flints while Dents exhibited intermediate values. LER\textsubscript{max} followed the opposite trend with greater growth rate in Flints than in Tropicals. In other words, LER\textsubscript{max} and GS exhibited a significantly negative correlation (r=-0.27). However, this correlation was driven by among-group variation rather than within-group variation - it was no longer significant after controlling for structure and kinship among inbreds. Our results indicate that selection on GS may have accompanied ancient maize diffusion from its center of origin, with large DNA content excluded from temperate areas. Whether GS has been targeted by more intense selection during modern breeding within groups remains an open question. 

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Testing the link between genome size and growth rate in maize

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ABSTRACT

Little is known about the factors driving within species Genome Size (GS) variation. GS may be shaped indirectly by natural selection on development and adaptative traits. Because GS variation is particularly pronounced in maize, we have sampled 83 maize inbred lines from three well described genetic groups adapted to contrasted climate conditions: inbreds of tropical origin, Flint inbreds grown in temperate climates, and Dent inbreds distributed in the Corn Belt. As a proxy for growth rate, we measured the Leaf Elongation Rate maximum during nighttime ($\text{LER}_{\text{max}}$) as well as GS in all inbred lines. In addition we combined available and new nucleotide polymorphism data at 29,090 sites to characterize the genetic structure of our panel. We found significant variation for both $\text{LER}_{\text{max}}$ and GS among groups defined by our genetic structuring. Tropicals displayed larger GS than Flints while Dents exhibited intermediate values. LER$_{\text{max}}$ followed the opposite trend with greater growth rate in Flints than in Tropicals. In other words, LER$_{\text{max}}$ and GS exhibited a significantly negative correlation ($r = -0.27$). However, this correlation was driven by among-group variation rather than within-group variation – it was no longer significant after controlling for structure and kinship among inbreds. Our results indicate that selection on GS may have accompanied ancient maize diffusion from its center of origin, with large DNA content excluded from temperate areas. Whether GS has been targeted by more intense selection during modern breeding within groups remains an open question.

INTRODUCTION
It is well established that Genome Size (GS) varies greatly among species, and that much of this variation is caused by repeated sequences (Muñoz Diez et al. 2012, Grover and Wendel 2010). There is still however a surprising dearth of studies assessing within-species variation. Among plant populations, several investigations have reported GS stability (Ellul et al. 2002, Moscone et al. 2003) while there are a handful of well-documented examples of substantial GS variation (reviewed in Smarda and Bures (2010)). The extent of within-species GS variation as measured by the coefficient of variation ranges from less than 1% in *Hordeum lechleri* (Jakob et al. 2004), around 2% in *Arabidopsis thaliana* (Long et al. 2013), 3.4% in *Camellia sinensis* (Huang et al. 2013) and in *Festuca pallens* (Smarda et al. 2007), and up to 6% in maize (*Zea mays ssp. mays*) and its closest wild relatives (*ssp. parviglumis* and *mexicana*), the teosintes (Muñoz Diez et al. 2013).

The factors driving GS variation remain a largely controversial issue. Several competing models have been proposed to explain among-species variations in GS. Interestingly, at least two of these models involve population genetic processes that may drive GS variation within species among populations, and ultimately preside over among-species GS variation (Agren and Wright 2011, Petrov 2001). The “mutational hazard” hypothesis (Lynch et al. 2011) posits that selection to maintain a constant per-genome mutation rate indirectly impacts GS. Providing that selection overcomes drift, the per base-pair-per-generation mutation rate correlates negatively with GS (Sung et al. 2012). Under this model, one expects within-species GS variation to be driven by differences in effective population size that condition the efficiency of natural selection.
against genome expansion. An alternative hypothesis asserts that positive natural
selection may indirectly influence GS variation through developmental or adaptive
phenotypes (Knight and Beaulieu 2008). In plants, the latter hypothesis has been
sustained by a handful of empirical studies demonstrating that GS correlates negatively
with development traits such as seedling (Mowforth and Grime 1989), root meristem
growth rate (Gruner et al. 2010), and cell cycle length (Francis et al. 2008). Small
genomes indeed presumably facilitate faster cell division and therefore a higher growth
rate (Knight et al. 2005, Rayburn et al. 1994).

Improving our understanding of intra-species genome dynamics is essential for
elucidating the diversification of GS among related species. Maize is an attractive model
to test whether GS is fine-tuned by positive natural selection. Not only does it display
the largest within-species GS variation in plants and an exceptional genome fluidity
(Chia et al. 2012), but is also characterized by a large effective population size - with
estimates ranging from 33,000 (Vigouroux et al. 2002) to ~600,000 (Gossmann et al.
2010) and 993,000 individuals (Beissinger et al. 2016), and a worldwide distribution with
contrasted growing conditions. Actually, maize has a long-lasting history of research on
GS variation (for a review, see (Knight et al. 2005). The most recent and extensive
report on this question in maize landrace populations (Muñoz Diez et al. 2013) has
drawn several important conclusions: (1) GS varies primarily among landraces and
within-landrace variation is limited; (2) geographical coordinates (altitude, longitude,
latitude) are accurate predictors of GS; (3) GS correlates negatively with altitude. These
results corroborate significant GS difference between temperate and tropical inbred
lines in a sample of 17 improved inbred lines as reported by (Chia et al. 2012).
Altogether, these findings suggest that environmental-driven selection on life cycle length and growth rate could indirectly affect GS. To further validate this hypothesis, we measured GS and leaf elongation rate in 83 improved maize inbred lines of various origins in the purpose of establishing a link between GS and growth rate.

**MATERIALS AND METHODS**

We have sampled 83 maize inbred lines (inbreds) from the INRA Centre de Ressources Biologiques (Saint Martin de Hinx, France) and from the Maize gene bank at CIMMYT in Mexico (Table 1). In order to maximize GS and $\text{LER}_{\text{max}}$ variation, we sampled inbred lines from three of the genetic groups previously defined by Camus-Kulandaivelu et al. (2006): tropical inbreds (Tropicals) characterized by a long life-cycle from sowing to flowering, flint inbreds (Flints) grown in temperate climates with a short life-cycle, and Dent inbreds (Dents) distributed in the Corn Belt with an intermediate life-cycle. Our panel encompassed 33 Tropicals, 12 Flints and 13 Dents.

Genotyping of the 83 inbreds with the Illumina MaizeSNP50 array was either available (Bouchet et al. 2013) or generated for a subset of 11 inbred lines (Data S1). We analyzed 29,090 SNPs contributed by the Panzea project (Zhao et al. 2006) that were developed on a discovery panel of 14 maize and 16 teosinte inbreds. Genotypes of 83 lines on 29,090 SNPs are available in Data S1. We utilized FastStructure v1.0 (Raj et al. 2014) to evaluate the genetic structure of our sample using K=2 and K=3 as the number of genetic groups. We determined the memberships of each inbred to the
groups at K=2 and K=3 (Table 1). Kinship was computed from Astle and Balding (2009) using GenABEL (http://www.genabel.org, Aulchenko et al. 2010).

Plants from each inbred line were characterized for LER$_{\text{max}}$ in the phenotyping facility Phenodyn (http://bioweb.supagro.inra.fr/phenodyn/) in two experiments (Data S2). The first experiment included all 83 inbred lines with 3 replicated measurements per inbred. The second experiment was a biological replicate for 58 out of the 83 inbred lines, with 3 replicated measurements. Plants were grown in a Klaszmann substrate (30% clay, 70% peat) according to the protocol reported in Sadok et al. (2007b). Briefly, the LER$_{\text{max}}$ (in mm per hour) of the 6th leaf was measured every 15 min during nighttime from 12 to 4am, time at which LER is maximum. Measurements took place in the 4 to 7 days during which the leaf elongation rate of leaf 6 has no temporal trend over successive nights (Sadok et al. 2007a). A single measure is therefore an average of LER during 4 to 7 nights. Meristem and air temperature, light intensity and air relative humidity, were measured every 15 min. Plants were grown in the greenhouse with naturally fluctuating conditions (200 to 1100 μmol m$^{-2}$ s$^{-1}$ at noon time) under well-watered conditions. During the measurement period, meristem temperature was 18.5°C ± 0.2°C and 20.0 ± 0.8°C in Experiment 1 and 2, respectively. Both soil water potential (-0.11 and -0.15 MPa) and vapour pressure deficit (0.93kPa ± 0.14kPa and 0.98kPa ± 0.14kPa) were in the range most favorable for growth during measurements.

In parallel, we measured the GS of 3 to 5 individuals per inbred line - from the same seed lots used for the LER$_{\text{max}}$ measurements (Data S2). Inbreds were grown in a greenhouse in Gif-sur-Yvette (France) and transferred after 3 weeks to the Imagif facility.
in Gif-sur-Yvette. The total nuclear DNA amount was assessed by flow cytometry according to Mary and Brown (1993). *Pisum sativum* L. ‘Long Express’ (2C=8.37 pg) was used as an internal standard. Leaves of the internal standard and maize lines were chopped using a razor blade in a plastic Petri dish with 1 ml of Gif nuclei-isolation buffer (45 mM MgCl$_2$, 30 mM sodium citrate, 60 mM MOPS, 1% (w/v) polyvinylpyrrolidone 10,000, pH 7.2) containing 0.1% (w/v) Triton X–100, supplemented with 5 mM sodium metabisulphite and RNAse (2.5 U/ml). The suspension was filtered through 50 μm nylon mesh. The nuclei were stained with 50 μg/ml propidium iodide and kept 5 min at 4°C. DNA content of 5,000–10,000 stained nuclei was determined for each sample using a flow cytometer (CyFlow SL3, Partec-Sysmex. Excitation 532 nm, 30 mW; emission through a 630/30 nm band-pass filter). The total 2C DNA value was calculated using the linear relationship between the fluorescent signals from stained nuclei of the maize and the internal standard. We performed 3 technical replicates per plant. In addition, we employed the inbred line B73 (maize reference genome) to verify the flow cytometer calibration at regular time intervals.

The LER$_{\text{max}}$ and GS values were averaged among technical replicates (Data S2). LER$_{\text{max}}$ of 58 inbred lines replicated over the two experiments were compared using the Bland and Altman’s method (1986). The replicates were highly concordant with differences between replicates that did not differ from 0 ($t = -1.3$, $df = 28$, $P = 0.20$), and no correlation between differences between replicates and inbred line mean values ($t = -1.6$; $df = 27$, $P = 0.13$). GS measurement was replicated on 3 to 5 plants per line, except for three that were replicated twice and B73 for which we had 14 replicates. Given the high and variable replicates number, the Bland and Altman’s method could
not be applied. Instead, we performed a one-way ANOVA and showed that GS variation was mainly owed to inbred line differences ($R^2 = 89.7\%$), with only 10.3% variation across biological replicates. Means and standard deviations for $\text{LER}_{\text{max}}$ and GS across biological replicates for each inbred line are reported in Table 1, and mean values were used for further statistical analyses. All statistical analyses were performed using the R software.

The effect of genetic groups on $\text{LER}_{\text{max}}$ and GS was first tested using linear regression on quantitative memberships obtained from FastStructure. We also employed a one-way ANOVA with a qualitative classification of inbreds as Flints, Dents or Tropicals. In this case, inbreds were assigned to a group based on its highest membership coefficient as determined by FastStructure at $K=3$. We computed pairwise differences between groups using Tukey-Kramer contrasts. We tested the correlation between $\text{LER}_{\text{max}}$ and GS first by simple regression; second we corrected for genetic structure by adding qualitative or quantitative memberships obtained from FastStructure as covariates in the linear model; third, we used a mixed model declaring FastStructure quantitative membership as a fixed effect and kinship as a random effect (Yu et al. 2006).
RESULTS

We assembled a panel of 83 maize inbred lines to test the link between genome size (GS) and the leaf elongation rate (LER_{max}). We extracted genotyping data from 29,090 SNPs and assess genetic structuring of the panel. Our results revealed a clear separation between Tropicals and Flints, while Dents were found as admixed individuals when K=2. With K=3, the Dent inbreds form a distinct genetic group (Figure 1).

GS varied between 4.96 pg and 5.89 pg (Table 1) with a coefficient of variation of 3.6 %. LER_{max} ranged from 3.80 to 6.94 mm h^{-1} (Table 1) with a coefficient of variation of 13.7%. Figure 2 illustrates GS and LER_{max} variation within and among the 3 genetic groups, each inbred being assigned to the genetic groups of greater membership. For both traits, mean values significantly differed among groups (one-way ANOVA, GS : \(F_{(2;80)} = 52.7, P = 2.5 \times 10^{-15}\); LER : \(F_{(2;80)} = 4.47, P = 0.014\)). Confirming previous observations, Tropicals displayed a larger genome size than Flints (Chia et al. 2012) while Dents exhibited intermediate GS although non-significantly different from the Flints (Figure 2A). LER_{max} followed the opposite trend with Flints exhibiting higher values than Tropicals (Figure 2B). Consistently we found a significant effect of the degree of “Flintness” – membership to the Flint group for K=2 – on GS (Figure 2C) and LER_{max} (Figure 2D). The Pearson correlation coefficients were highly significant (\(r = -0.77, P = 2.1 \times 10^{-17}\) and \(r = 0.40, P = 2.0 \times 10^{-4}\) respectively for GS and LER_{max}).

To validate further this pattern, we investigated the correlation between LER_{max} and GS and found a significantly negative correlation (\(r = -0.29, F_{(1;81)} = 7.28, P = 0.008\), Figure 3). However, GS may correlate with relatedness among inbreds because measures of closely related inbreds, i.e. those that form a genetic group, are not
independent observations. In order to control this effect, we re-analysed the correlation between GS and LER\textsubscript{max} controlling for qualitatively (group assignation from the highest membership coefficient) or quantitatively (group membership coefficient) defined groups. We found that the group effect was significant ($F_{(2;77)} = 4.68, P = 0.012$).

However the correlation was no longer significant when controlling for either qualitative group origin ($F_{(1;77)} = 1.07, P = 0.31$, Figure 3) or quantitative group membership ($F_{(1;77)} = 0.003, P = 0.95$). As expected when kinship was added to the model, the effect of GS on LER\textsubscript{max} remained not significant ($P = 0.95$). The regression slope between GS and LER did not differ among groups as indicated by the non-significant group X GS interaction on the LER measurements ($F_{(2;77)} = 2.84, P = 0.065$).

Finally, we performed within-group analyses. Sample size was too limited (11 inbreds) to evaluate correlation within Dents. We found no correlation within Flints (24 inbreds). Tropicals (50 inbreds) however exhibited a negative trend, with small genome inbreds displaying a tendency towards faster growth rate than larger genome inbreds ($r = -0.26, F_{(1;48)} = 3.35, P = 0.073$).
DISCUSSION

That plants with smaller genomes may undergo more rapid replication time of their genome, which translates into faster growth rate than plants with larger genomes, is a prediction of the positive natural selection evolution model of genome size. This prediction is based on findings of positive correlation between GS and duration of the cell cycle in 110 angiosperm species (Francis et al. 2008). Maize originates from teosintes (Matsuoka et al. 2002) and are characterized by an important range of variation in DNA content (Muñoz Diez et al. 2013). Its genome is extremely fluid (Chia et al. 2012) and GS may evolve rapidly under selection (Rayburn et al. 1994). Realini et al. (2015) have recently reported a positive correlation between heterochromatin content and length of the vegetative cycle in 9 maize populations sampled from Northeastern Argentina. However a more direct effect of GS variation on growth rate has never been formally tested.

Here, we determined GS and leaf elongation rate (LER_{max}) in 83 improved maize inbred lines selected under contrasted climates. We measured LER_{max} in the developing 6th leaf during the linear phase of elongation, considered as a steady-state (Salah and Tardieu 1997). This state is commonly used for measuring cell division and/or tissue expansion (Tardieu et al. 2000). It therefore is a good proxy for growth rate in relation with the timing of cell cycle. Besides, the LER_{max} in maize is reproducible and independent of environmental conditions if corrected for temperature effect (Sadok et al. 2007b). It is also a highly heritable trait (Dignat et al. 2012).
Our sample contained inbred lines from three well-defined genetic groups, the Flints, the Dents and the Tropica... the recent history of admixture between Tropica... to form the Dent inbreds at the end of the 19th century (Labate et al. 2003).

Our sample corroborates previous observations from a restricted set of inbreds with temperate inbreds (Flints) exhibiting a significantly smaller GS than tropical (Tropicals) inbreds (Chia et al. 2012) (Figure 2A and 3A). Interestingly, $LER_{\text{max}}$ followed the opposite trend with Flints exhibiting higher values than Tropicals whether inbred group membership was considered as qualitative (Figure 2B) or a quantitative trait (Figure 3B). Note that Dents exhibit intermediate values bot for GS and $LER_{\text{max}}$ consistent with their admixed status.

At a first glimpse our results therefore support the hypothesis that smaller genomes exhibit a faster development rate. Because $LER_{\text{max}}$ is a good indicator of growth ability of other organs including reproductive organs (Dignat et al. 2013), it is tempting to speculate that selection for a faster-life cycle in early flowering Flint inbreds has indirectly impacted genome size.

However the negative correlation between GS and $LER_{\text{max}}$ was mainly driven by among-group variation (Figure 3), suggesting that the existing link between these variables at the origin of the groups was followed by uncorrelated changes during subsequent evolutionary history. Such a pattern has been reported among species, whereby accounting for the phylogenetic history of species altered the relationship between effective population size and GS (Whitney and Garland 2010). Noteworthy,
within Tropicals smaller genomes displayed a tendency towards faster growth rate than larger genomes. The coefficient of variation of GS was also greater in this group (26%) than in either Flints (22%) or Dents (19%). Tropicals are subjected to high variation in altitude that may exert selective pressure on GS. Additional sampling with limited structuring will be necessary to validate further this result.

Altogether, our results show that selection on GS may have accompanied ancient maize geographical diffusion from its center of origin, consistently with the idea that landraces/inbreds with large DNA content may be excluded from more extreme temperate climates.

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Figure 1 (on next page)

Group membership of 83 maize inbred lines inferred using FastStructure v1.0 (Raj et al. 2014) from 29,090 SNPs with ancestral group number K=2 (A) or K=3 (B).

The 83 inbred lines are ordered as in Table 1. Group names were a posteriori defined from the inbred lines with greatest membership with Flints (blue), Dents (red), and Tropicals (green).
Mean and standard errors across inbred lines for genome size (1-A) and LERmax (1-B) measures, for each genetic group as defined as Flints, Dents or Tropicals following their greatest membership using FastStructure at K=3 (Table 1).

For both traits, mean values significantly differ among groups (one-way ANOVA, GS : $F_{(2;80)}=52.7, P=2.5 \times 10^{-15}$; LER : $F_{(2;80)}=4.47, P=0.014$). Pairs of groups with similar letters exhibit non-significant difference in mean values.
**Figure 3** (on next page)

Relation between genome size (A) and LERmax (B) with Flintness as measured by the membership to the Flint group at K=2.

The Pearson correlation coefficients ($r=-0.77$ and $r=0.40$, respectively) are highly significant ($P=2.1 \ 10^{-17}$ and $P=2.0 \ 10^{-4}$, respectively).
Table 1 (on next page)

List of inbred lines with measures of Genome Size (GS), LERmax (LER) and membership at K=2 (Group 1, 2) and K=3 (Group 1, 2, 3).
| Inbred line | GS (pg) | LER (mm/h) | K2_G1 | K2_G2 | K3_G1 | K3_G2 | K3_G3 | K3_group |
|-------------|---------|------------|-------|-------|-------|-------|-------|----------|
| CH10        | 5.05    | 6.77       | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | Flint    |
| EP1         | 5.17    | 4.54 (0.149)| 0.928 | 0.072 | 0.940 | 0.000 | 0.060 | Flint    |
| F39         | 5.31    | 6.25 (0.610)| 0.879 | 0.121 | 0.894 | 0.000 | 0.106 | Flint    |
| F471        | 5.26    | 5.66 (0.113)| 0.867 | 0.133 | 0.905 | 0.000 | 0.095 | Flint    |
| FC16        | 5.27    | 6.83       | 0.670 | 0.330 | 0.675 | 0.000 | 0.325 | Flint    |
| FC209       | 5.05    | 6.21 (0.047)| 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | Flint    |
| FC24        | 5.41    | 5.99 (0.045)| 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | Flint    |
| FV2         | 5.20    | 5.40 (0.251)| 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | Flint    |
| FV65        | 5.21    | 6.65       | 0.868 | 0.132 | 0.876 | 0.000 | 0.124 | Flint    |
| FV7         | 5.24    | 6.30 (0.514)| 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | Flint    |
| FV71        | 5.10    | 5.14 (0.129)| 0.923 | 0.077 | 0.976 | 0.000 | 0.024 | Flint    |
| FV75        | 5.11    | 5.86 (0.575)| 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | Flint    |
| FV76        | 5.27    | 5.21       | 0.821 | 0.179 | 0.840 | 0.000 | 0.160 | Flint    |
| ND30        | 5.04    | 6.94       | 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | Flint    |
| NY302       | 4.96    | 5.23 (0.269)| 1.000 | 0.000 | 0.796 | 0.204 | 0.000 | Flint    |
| PB40R       | 5.28    | 5.04 (0.046)| 0.770 | 0.230 | 0.725 | 0.087 | 0.187 | Flint    |
| W85         | 5.19    | 5.24 (0.397)| 1.000 | 0.000 | 1.000 | 0.000 | 0.000 | Flint    |
| YUBR05      | 5.21    | 6.80       | 0.724 | 0.276 | 0.542 | 0.458 | 0.000 | Flint    |
| B73         | 5.21    | 5.42 (0.369)| 0.490 | 0.510 | 0.000 | 1.000 | 0.000 | Dent     |
| CI1872U     | 5.26    | 4.73 (1.046)| 0.305 | 0.695 | 0.000 | 0.729 | 0.271 | Dent     |
| EA1433      | 5.24    | 4.21 (0.519)| 0.416 | 0.584 | 0.206 | 0.420 | 0.373 | Dent     |
| FC1852      | 5.33    | 6.12 (0.249)| 0.494 | 0.506 | 0.000 | 1.000 | 0.000 | Dent     |
| FC252       | 5.23    | 4.80 (0.108)| 0.449 | 0.551 | 0.000 | 1.000 | 0.000 | Dent     |
| K64R        | 5.24    | 5.68 (0.249)| 0.313 | 0.687 | 0.052 | 0.538 | 0.410 | Dent     |
| KY21        | 5.20    | 5.56 (0.885)| 0.416 | 0.584 | 0.000 | 1.000 | 0.000 | Dent     |
| LAN496      | 5.17    | 5.97 (0.009)| 0.476 | 0.524 | 0.076 | 0.924 | 0.000 | Dent     |
| MBS847      | 5.17    | 4.45 (0.519)| 0.437 | 0.563 | 0.000 | 1.000 | 0.000 | Dent     |
| MO17        | 5.16    | 4.78 (0.107)| 0.448 | 0.552 | 0.000 | 1.000 | 0.000 | Dent     |
| N25         | 5.31    | 4.72 (0.377)| 0.466 | 0.534 | 0.000 | 1.000 | 0.000 | Dent     |
| N6          | 5.22    | 6.28       | 0.520 | 0.480 | 0.110 | 0.890 | 0.000 | Dent     |
| SC55        | 5.48    | 6.24 (0.244)| 0.271 | 0.729 | 0.045 | 0.493 | 0.462 | Dent     |
| SCMALAWI    | 5.45    | 6.44 (0.527)| 0.263 | 0.737 | 0.000 | 0.609 | 0.391 | Dent     |
| W117U       | 5.32    | 5.03       | 0.423 | 0.577 | 0.000 | 1.000 | 0.000 | Dent     |
| A6          | 5.87    | 4.46 (0.490)| 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | Tropical |
| L256        | 5.32    | 5.63 (0.323)| 0.460 | 0.540 | 0.465 | 0.000 | 0.535 | Tropical |
| BA90        | 5.41    | 5.54 (0.139)| 0.366 | 0.634 | 0.201 | 0.356 | 0.443 | Tropical |
| CLA17       | 5.80    | 5.67 (0.137)| 0.000 | 1.000 | 0.000 | 0.059 | 0.941 | Tropical |
| CML69       | 5.64    | 5.06 (0.416)| 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | Tropical |
| CML245      | 5.70    | 5.59 (1.009)| 0.330 | 0.670 | 0.201 | 0.273 | 0.526 | Tropical |
| CML247      | 5.64    | 5.12 (0.804)| 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | Tropical |
| CML254      | 5.50    | 5.71 (0.814)| 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | Tropical |
| CML287      | 5.48    | 5.89 (0.660)| 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | Tropical |
| CML312      | 5.31    | 4.06       | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | Tropical |
| Code   | Value  | Mean | SD  | Median | Min  | Max  | Location |
|--------|--------|------|-----|--------|------|------|----------|
| CML333 | 5.54   | 0.073| 4.88| (0.640)| 0.051| 0.949| 0.023    | 0.061 | 0.917 | Tropical |
| CML340 | 5.51   | 0.068| 5.27|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| CML341 | 5.50   | 0.046| 4.53|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| CML344 | 5.58   | 0.092| 3.80|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| CML440 | 5.60   | 0.028| 4.21|-       | 0.063| 0.937| 0.063    | 0.000 | 0.937 | Tropical |
| CML91  | 5.44   | 0.053| 4.60| (0.802)| 0.109| 0.891| 0.032    | 0.149 | 0.819 | Tropical |
| CMLP1  | 5.60   | 0.087| 4.83| (0.020)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| CMLP2  | 5.59   | 0.080| 5.21| (0.457)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| CZL04006 | 5.51  | 0.142| 6.33|-       | 0.090| 0.910| 0.000    | 0.260 | 0.740 | Tropical |
| CZL0617 | 5.55  | 0.097| 5.27|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| CZL071  | 5.30   | 0.054| 6.52|-       | 0.089| 0.911| 0.028    | 0.119 | 0.853 | Tropical |
| EA1197  | 5.55   | 0.124| 5.90| (0.268)| 0.234| 0.766| 0.246    | 0.000 | 0.754 | Tropical |
| EA1201  | 5.56   | 0.164| 5.74| (0.492)| 0.152| 0.848| 0.152    | 0.000 | 0.848 | Tropical |
| EA1866  | 5.44   | 0.078| 6.47| (0.536)| 0.234| 0.766| 0.237    | 0.000 | 0.763 | Tropical |
| EA1712  | 5.34   | 0.012| 6.25| (0.486)| 0.199| 0.801| 0.208    | 0.000 | 0.792 | Tropical |
| F2834T  | 5.44   | 0.060| 5.14| (0.431)| 0.245| 0.755| 0.136    | 0.224 | 0.640 | Tropical |
| G37     | 5.65   | 0.096| 4.70| (0.010)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| DTPWC9-F115 | 5.55 | 0.072| 5.35|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| DTPWC9-F104 | 5.52 | 0.030| 4.59|-       | 0.000| 1.000| 0.000    | 0.062 | 0.938 | Tropical |
| DTPWC9-F31 | 5.65 | 0.068| 4.09|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| DTPYC9-F74 | 5.46 | 0.092| 5.37|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| DTPYC9-F46 | 5.49 | 0.105| 5.89|-       | 0.000| 1.000| 0.000    | 0.018 | 0.982 | Tropical |
| LPSC7-F64  | 5.45  | 0.004| 4.84|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| LPSC7-F71  | 5.41  | 0.044| 5.49|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| LPSC7-F103 | 5.45  | 0.019| 4.14|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| LPSC7-F86  | 5.49  | 0.084| 4.45|-       | 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| H16      | 5.37   | 0.029| 4.36| (0.150)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| KUI44    | 5.26   | 0.101| 4.63| (0.823)| 0.050| 0.950| 0.041    | 0.016 | 0.942 | Tropical |
| KUI11    | 5.54   | 0.050| 5.58| (0.073)| 0.000| 1.000| 0.000    | 0.042 | 0.958 | Tropical |
| KUI3     | 5.64   | 0.052| 4.17| (0.265)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| LP1037   | 5.30   | 0.037| 6.21| (0.678)| 0.340| 0.660| 0.249    | 0.175 | 0.576 | Tropical |
| LP1233   | 5.39   | 0.054| 5.97| (0.458)| 0.240| 0.760| 0.243    | 0.000 | 0.757 | Tropical |
| LP35     | 5.40   | 0.158| 5.69| (0.168)| 0.243| 0.757| 0.242    | 0.008 | 0.750 | Tropical |
| MO22     | 5.45   | 0.097| 5.40| (0.117)| 0.069| 0.931| 0.065    | 0.000 | 0.935 | Tropical |
| NC298    | 5.75   | 0.107| 4.77| (0.815)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| NC304    | 5.48   | 0.026| 5.02| (0.124)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| NC320    | 5.40   | 0.099| 5.61| (0.750)| 0.210| 0.790| 0.000    | 0.465 | 0.535 | Tropical |
| NC338    | 5.78   | 0.107| 4.98| (0.145)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| TZI18    | 5.89   | 0.044| 5.39| (0.112)| 0.000| 1.000| 0.000    | 0.000 | 1.000 | Tropical |
| ZN6      | 5.42   | 0.036| 5.61| (0.527)| 0.249| 0.751| 0.252    | 0.000 | 0.748 | Tropical |