

1 **Utility of root cortical aerenchyma under water limited conditions in tropical maize (*Zea***
2 ***mays* L.)**

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15 **Running title: Root cortical aerenchyma in tropical maize (*Zea mays* L.)**

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20 **Abstract**

21 It has recently been shown that root cortical aerenchyma (RCA) formation substantially reduces
22 the metabolic costs of soil exploration, thereby improving rooting depth, water capture, and plant
23 growth under drought. The objectives of this study were to evaluate phenotypic variation of RCA
24 formation in Malawi maize germplasm and confirm the utility of RCA formation in different
25 agroecologies at field stations and on smallholder farms in Malawi. Forty maize accessions
26 collected across Malawi were grown under rainfed field conditions, and a subset contrasting for
27 RCA were grown under well-watered and water stressed conditions at two research stations and
28 under natural drought on farms across two agroecological zones in Malawi. We found substantial
29 variation for RCA in local germplasm ranging from 0 to 37% of root cross sectional area.
30 Suboptimal water availability increased RCA by 54% to 77% across environments. Under water
31 stress high RCA genotypes had 23% to 29% greater leaf relative water content, 67% to 96%
32 greater shoot biomass 70 days after planting, and 78% to 143% greater grain yield than low RCA
33 genotypes. Our results are consistent with the hypothesis that RCA improves plant growth under
34 drought by reducing the metabolic costs of soil exploration and improving water acquisition.
35 RCA has value as a selection criterion to improve the performance of maize and possibly other
36 cereal crops in water limited environments.

37 **Keywords:** *Zea mays* L., root cortical aerenchyma, drought

38 **Introduction**

39 Suboptimal water availability is a primary global constraint to crop productivity (Barker et al.,
40 2004; Campos et al., 2004), and is projected to worsen over the next several decades as the result
41 of global climate change (IPCC, 2014). Although developing crop cultivars with improved
42 adaptation to water stress is an avenue to mitigate drought impact, breeding for drought
43 adaptation using yield as a selection criterion is generally not efficient (Lynch, 2007; Wasson et
44 al., 2012; Watt et al., 2013). Yield is an integration of complex mechanisms at different levels of
45 organization affected by many elements of the phenotype and the environment interacting in
46 complex and often unknown ways. An alternative to yield selection is ideotype or trait-based
47 breeding that allows identification of useful sources of variation, informed trait integration, and
48 development of novel phenotypes (Araus et al., 2008; Donald, 1968; Lynch, 2007; Richards et
49 al., 2010; Wasson et al., 2012; York et al. 2013).

50 Root phenes ('phene' is to 'phenotype' as 'gene' is to 'genotype') that permit rapid exploitation
51 of deep soil domains can have significant impact on crop productivity under drought (Lynch,
52 2013). For this reason, root architectural phene states that enhance deep soil exploration such as
53 steep root growth angles can improve water acquisition (Manschadi et al., 2006; Lynch, 2013).
54 Root metabolic costs of soil exploration are quite substantial, and can exceed 50% of daily
55 photosynthesis (Lambers et al., 2002), therefore efficient utilization of metabolic resources for
56 soil exploration is a key aspect of plant adaptation to edaphic stresses (Lynch and Ho, 2005;
57 Lynch, 2007). This has been demonstrated in the case of limiting nitrogen, phosphorus,
58 potassium, and drought (Nielsen et al., 2001; Lynch and Ho, 2005; Zhu et al., 2010; Postma and
59 Lynch, 2010; Postma and Lynch, 2011; Jaramillo et al., 2013; Saengwilai et al., 2014a).

60 Anatomical phenotypes that reduce the maintenance requirements of root tissue can reduce the
61 metabolic cost of soil exploration and soil resource acquisition. Maize genotypes with less living
62 tissue in the root cortex had greater root growth, water acquisition, and plant growth under water
63 stress than genetically related lines with more living cortical tissue (Jaramillo et al., 2013). One
64 mechanism to reduce living cortical tissue is the formation of root cortical aerenchyma (RCA),
65 which converts living cortical cells into air space through programmed cell death (Evans, 2004).
66 RCA formation is widely known to be induced in response to hypoxia and functions to improve
67 oxygen transport to roots under hypoxic conditions (Jackson and Armstrong, 1999; Mano and
68 Omori, 2007; Mano and Omori, 2013). Increased RCA formation has been reported in response
69 to suboptimal availability of phosphorus (Drew et al., 1989; Fan et al., 2003), nitrogen (Drew et
70 al., 1989; Saengwilai et al., 2014a), sulfur (Bouranis et al., 2003; Bouranis et al., 2006), and
71 water (Zhu et al., 2010). RCA formation is associated with a disproportionate reduction of root
72 respiration in maize (Fan et al., 2003; Saengwilai et al., 2014; Zhu et al., 2010), reduction of
73 phosphorus (Fan et al., 2003) and nitrogen (Saengwilai et al., 2014) content in root tissue on a
74 volume basis, and improved growth in low P (Lynch, 2011) and low N (Saengwilai et al., 2014)
75 soil. Modelling studies using *SimRoot* suggest that RCA formation improves crop adaptation to
76 suboptimal nutrient availability by reducing the metabolic and nutrient cost of soil exploration
77 (Postma and Lynch, 2011a; Postma and Lynch, 2011b). Under water stress in the field maize
78 inbreds with more RCA had 5 times greater biomass and eight times greater yield than inbreds
79 with less RCA (Zhu et al., 2010). These results suggest that RCA has broad utility for improving
80 soil resource acquisition in stressful conditions.

81 Maize is the leading global crop and the dominant food crop in many developing nations
82 including Malawi, where it accounts for about 70% of the total caloric intake of rural people
83 (FAOSTAT, 2010). However, maize yields in Malawi remain low, averaging 1.4 t ha^{-1} , (i.e. one-
84 sixth the average yields in the US), which limits farm income and food security (Denning et al.,
85 2009; FAOSTAT, 2010). Food security and nutritional status of households in Malawi continue
86 to be threatened by recurring droughts which are further exacerbated by climate change (Burke et
87 al., 2009; Lobell et al., 2011; IPCC, 2014). The development of drought-tolerant cultivars is a
88 priority, as the use of drought tolerant cultivars may be the affordable option for improving food
89 security for small-scale farmers.

90 Our understanding of the utility of RCA for drought adaptation in maize is based on results from
91 simulations, and from analysis of North American inbred lines grown in controlled environments
92 and at agricultural research stations (Zhu et al., 2010; Postma and Lynch, 2011b; Postma and
93 Lynch, 2011c; Saengwilai et al., 2014a). The overall objective of this study was to evaluate the
94 utility of RCA in African maize germplasm in water-limited agroecosystems of southern Africa.
95 The specific objectives of this study were to i) assess the genetic variation of RCA formation in
96 Malawian maize landraces and regional breeding lines, ii) evaluate the agronomic performance
97 of maize genotypes contrasting for RCA formation under water limited conditions across
98 agroecological zones, and iii) to identify genotypes with high RCA for further use in a pilot
99 breeding program. Our results demonstrate that substantial variation for RCA formation exists in
100 local and regional maize germplasm and that RCA improves maize drought tolerance. In addition
101 to confirming the utility of high RCA under water stress, we also identified genotypes with high
102 RCA for use in the pilot breeding program.

103 **Materials and Methods**

104 *Maize agroecology in Malawi and experimental sites*

105 Malawi is a sub-tropical country in southeastern Africa with a sub-humid climate. The country in
106 divided into three main agroecologies based on climatic conditions and differences in altitude:
107 the mid-altitude or plateau areas (900-1200 meters above sea level), the lakeshore, and the shire
108 valley. Most of Malawi receives an average precipitation of 500 - 1000 mm per annum with
109 intermittent dry spells. Lakeshore areas are important agriculture areas and particularly
110 vulnerable to drought. Experiments were conducted in two agroecological zones (AEZs):
111 Lilongwe (mid-altitude plain) and Salima (Lakeshore). Combined, these two AEZs account for
112 more than 30% of all area planted to maize in Malawi per annum (Ministry of Agriculture and
113 Food Security 2014). In 2011 and 2012, field station trials were conducted at two locations:
114 Bunda Research farm of the Lilongwe University of Agriculture and Natural Resources in
115 Lilongwe, Malawi and the Chitala Agriculture Research Station in Salima, Malawi. On-farm
116 trials were conducted in 2013 at two locations: Mkwinda Extension Planning Area (EPA),
117 Lilongwe, Malawi and Chipoka EPA, Salima, Malawi. Salima AEZ was chosen because it is
118 located in the lake shore and more vulnerable to drought. Details of annual average rainfall
119 (Appendix 1) and soil type of these sites are shown in Table 1.

120 **Measurements common to all experiments**

121 *Root crown evaluation and root cortical aerenchyma determination*

122 Roots were evaluated 70 days after planting in all trials. To accomplish this, shoots of three
123 representative plants in each plot were cut just above the soil level. Root crowns were excavated
124 using the 'shovelomics' method (Trachsel et al., 2011). In brief, roots were excavated by
125 removing a soil cylinder of 30-40 cm diameter with the shoot at its center and a depth of 20-30
126 cm. Excavation was carried out using shovels and hand-held hoes. The excavated root crowns
127 were shaken briefly to remove a large fraction of the soil adhering to the root crown. The root
128 crowns were immersed in soapy water for 5-10 minutes in order to facilitate removal of the
129 remaining soil. The maize root system is characterized by four root types: the embryonic primary
130 root, seminal roots, shoot-borne crown roots, and shoot-borne brace roots (Hochholdinger,
131 2009). Three 8 cm root segments were collected 10-20 cm from the base of a representative
132 second whorl crown root of each plant for anatomical analysis as previously described (Burton et
133 al., 2012a). These segments were shipped to the Pennsylvania State University where they were
134 sectioned and analyzed for RCA. Root segments were sectioned using laser ablation tomography
135 (LAT) (Hall et al., unpublished) to obtain images for anatomical analysis. In brief, LAT uses a Q
136 switched pulsed UV laser (Avia 7000, 355 nm pulsed laser) to vaporize root tissue at the camera
137 focal plane ahead of an imaging stage. The sample is incremented, vaporized, and imaged
138 simultaneously. The cross-section images were taken using a Canon T3i camera (Canon Inc.
139 Tokyo, Japan) with 5X micro lens (MP-E 65 mm) focused on the laser-illuminated surface. Root
140 images were analyzed using *RootScan*, an image analysis tool developed for analyzing root
141 anatomy (Burton et al., 2012b). RCA was expressed as percentage of the root cortical area.

142 *Experiment 1: screening local maize germplasm for RCA formation*

143 Forty local maize landraces were screened under rainfed conditions for phenotypic variation of
144 RCA formation at Bunda College Research Farm in 2012 (Appendix 2). Local maize landraces
145 were obtained from Malawi Plant Resources Genetic Centre (MPRGC) at Chitedze Agriculture
146 Research Station, Lilongwe, Malawi and represented collections from all traditional maize
147 growing areas/ecotypes of Malawi (Appendix 1 & 2). The experiments were arranged as a
148 randomized complete block design (RCBD) with three replications. Each plot consisted of a
149 single 6 m long row with 0.75 m inter-row spacing and 0.25 m in-row spacing. Fertilizer was
150 applied to all plots at the rate of 60 kg N and 44 kg P ha⁻¹ at planting. An additional 40 kg N ha⁻¹
151 was top dressed 14 days after planting. The trial was kept free of weeds by manual cultivation.
152 Root crowns were excavated and RCA samples collected as described above. Genotypes
153 contrasting for RCA were selected from this data for subsequent experiment (Experiment 2 & 3).

154 *Experiment 2: field station evaluation of 20 local maize accessions contrasting for RCA across*
155 *two agroecologies*

156 A subset of 17 prescreened local maize accessions contrasting for RCA plus three open
157 pollinated varieties (OPVs: ZM523, SC403, and SC513) check lines were evaluated under well
158 watered and water stressed conditions at Bunda College research farm and Chitala agriculture
159 research station. These OPVs are currently used in the Farm Input Subsidy Program (Denning et
160 al., 2009; Holden and Mangisoni, 2013). This program helps resource-poor smallholder farmers
161 in Malawi to access fertilizer and improved maize varieties, which therefore are grown
162 throughout Malawi. The soils at both the sites are typical red soils classified as Oxic Rhodustalf
163 and are the most productive soils in Malawi (Table 1). The experiment was laid out as split-plot
164 design with two moisture regimes as the main plot factor and 20 genotypes as the subplot factor
165 replicated four times at both sites. The experiments were hand-planted on 3rd and 4th September
166 2012 at Bunda and Chitala respectively. Trials were planted in single row plots with 0.75 m
167 inter-row spacing and 0.25 m in-row spacing to give a plant population of 53000 plants ha⁻¹.
168 Drought stress was imposed by withdrawing irrigation water from 40 days after planting (DAP)
169 until maturity to simulate terminal drought. During the first four weeks of growth, the plots were
170 irrigated twice each week. For the rest of the experiment, the well-watered plants were irrigated
171 regularly to maintain soil water content near field capacity. Fertilizer was applied to all plots at
172 the rate of 60 kg N and 44 kg P ha⁻¹ at planting. An additional 40 kg N ha⁻¹ was top dressed 14
173 days after planting. The trial was kept weed free by hand weeding. Soil water content for both
174 well watered and water stressed treatments was monitored regularly during the experiment (Fig
175 1). Soil water content was monitored gravimetrically at two depths (25 and 50 cm). Midday leaf
176 relative water content (RWC) was used as a physiological indicator of plant water status. To
177 measure leaf RWC, fresh leaf discs (3 cm in diameter) were collected from the third fully
178 expanded leaf for three representative plants per plot at 60 days after planting and weighed
179 immediately to determine fresh weight (FW). Then the discs were immediately hydrated to full
180 turgidity (6 h) by soaking them distilled water, blotted dry and again weighed to determine turgid
181 weight (TW). Discs were then oven dried at 70°C for 72 h, and dry weight (DW) was
182 determined. Leaf RWC was calculated according to the equation: $RWC = 100[(FW - DW) /$
183 $(TW - DW)]$. Root crowns were excavated and RCA samples collected as described above.

184 *Experiment 3: on farm evaluation of 10 genotypes contrasting for RCA across two agroecologies*

185 Researcher-designed on farm trials were conducted in the 2013 growing season in the Mkwinda
186 Extension Planning Area and the Chipoka Extension Planning Area. A subset of 10 inbred lines
187 (AR716, M70-29-3, CML344, MANICA4, E21, M73-18, AR239, CML321, SW19, and
188 CML247) contrasting for RCA (Appendix 3) obtained from the Malawi Maize Breeding
189 Program at Chitedze Agriculture Research Station in Lilongwe, Malawi were planted. These
190 genotypes were selected based on previous screening in the field in Malawi (unpublished data).
191 The experiments were conducted in 14 farmers' fields within each EPA. A randomized complete
192 block design was used with each farmer as a replicate. Each replicate contained 10 genotypes
193 contrasting in RCA and plots consisted of three 4 m long rows with between-row spacing of 0.75
194 m. Distance between plants within the row was 0.25 m in all trials. The experiments were hand-
195 planted on 23rd and 24th January 2013 in Mkwinda EPA and Chipoka EPA respectively. Plants
196 were managed according to standard cultural practices used by farmers in the area, which is
197 completely un-mechanized, in order to assess the utility of RCA and plant overall performance
198 within the farmers' representative environment in these low input agroecosystems. Rainfall data
199 (in millimeters) was collected from each site using rain gauges mounted at each of the EPA
200 premises.

201 *Data analysis*

202 Data were analyzed using the R statistical package version 3.1.0 (R Development Core Team,
203 2014). The effect of soil moisture regime, genotype and their interaction on %RCA, relative
204 water content, shoot biomass, and grain yield was tested by analysis of variance using the linear
205 mixed effects model in *lme4* package in R (Bates et al., 2014). For on-farm data the effect of
206 'farmer' was considered as a random effect since the farmers were randomly selected from a
207 wider population of farmers in each EPA, while genotypes were considered to be fixed effects.
208 Comparison of genotypic means was carried out using the Tukey's HSD (honest significant
209 difference) test at 5% level of significance. Separate stability analyses were performed in order
210 to identify stable high-yielding accessions. Kang's (1993) selection index (YS_i) was calculated
211 from the grain yield data using the *agricolae* package in R (de Mendiburu, 2014). YS_i
212 incorporates both mean yield and stability in a single criterion. The stability function in
213 *agricolae* calculates YS_i by (i) determining the contribution of each genotype to GE interaction
214 by calculating variance (σ^2); (ii) then assigning ranks to genotypes from highest to lowest; (iii)
215 calculating the protected LSD for mean yield comparisons; (iv) adjusting yield rank according to
216 LSD; (v) determining significance of σ^2 , using an approximate F-test; (vi) assigning the stability
217 rating (S); (vii) summing adjusted yield rank and stability rating for each genotype to determine
218 the YS_i statistic; and (viii) calculating mean YS_i and identifying selected genotypes as having YS_i
219 $>$ mean YS_i (Kang, 1993). The YS_i statistic provides insight into a genotype's yield and yield
220 stability rating allowing for simultaneous selection for both criteria. Chi-square tests were
221 conducted to determine whether RCA phenotype grouping would affect the grain yield stability
222 for a genotype.

223 Results

224 *Substantial genotypic variation for RCA exists in Malawian maize (Experiment 1)*

225 Substantial variation was found for RCA formation among local maize accessions evaluated 70
226 days after planting in the field (Table 2). Accessions were grouped into three categories based on
227 percent of the root cross sectional area occupied by RCA: low RCA (%RCA < 2.4), intermediate
228 (2.4 < %RCA < 12.1), and high RCA (%RCA > 12.1) (Table 2). Based on this phenotype
229 classification, a subset of 17 accessions with contrasting %RCA but otherwise similar root
230 phenotypes were selected (high RCA lines: accessions 139, 145, 148, 1992, 203, 250, 260, 403,
231 736, 787, and low RCA lines: accessions 172, 1875, 2012, 243, 297, 303, 539) to evaluate the
232 agronomic performance of maize genotypes contrasting for RCA under water limited conditions
233 across agroecologies.

234 *High RCA was associated with greater leaf water status, greater shoot biomass and grain yield* 235 *under drought in field station trials across two environments (Experiment 2)*

236 Both genotype and soil moisture regime significantly ($p < 0.05$) affected RCA formation (Table
237 3). RCA was increased by suboptimal water availability in high RCA genotypes by an average of
238 54% (Bunda) and 77% (Chitala) while the increase in low RCA genotypes was not significant
239 (Fig. 2 A,B, Table 3). In addition, the interaction of genotype×soil moisture regime was highly
240 significant ($p < 0.05$) for RCA formation (Table 3). Genotypes responded differently to water
241 stress in terms of RCA formation. Water stress reduced leaf relative water content by 18%
242 (Bunda) and 25% (Chitala) (Fig. 2 C,D, Table 4), shoot biomass at anthesis by 43% (Bunda) and
243 59% (Chitala) (Fig. 3 A,B and Table 4), and grain yield by 48% (Bunda) and 56% (Chitala) (Fig.
244 3 C,D and Table 4) relative to well watered conditions. Under water stress high RCA genotypes
245 had 29% (Chitala) and 23% (Bunda) greater leaf relative water content (Fig. 2 C,D), 96%
246 (Bunda) and 67% (Chitala) greater shoot biomass 70 days after planting, and 78% (Bunda) and
247 143% (Chitala) greater yield (Fig. 3, A,B,C,D) than low RCA genotypes. RCA expression was
248 well correlated with grain yield under water stress (Fig. 4). RCA phenotype did not affect leaf
249 relative water content, shoot biomass, or grain yield under well-watered conditions (Figs. 2-3).

250 *Genotype x environment interaction and stability analysis for grain yield*

251 The combined ANOVA across environments indicated that grain yields were significantly
252 affected by environment, which explained 67% of the total (G + E + GxE) variation, while
253 genotype and GxE explained 9% and 12% of observed variation, respectively. Yield
254 performance data across environments is presented in Table 5. The variability of genotype
255 performance from one environment to the other shows the existence of possible crossover G x E
256 interaction. In order to understand the nature of highly significant genotype by environment
257 interaction, stability analysis was used to identify stable high-yielding genotypes (Table 6).
258 Based on yield stability index (Y_{si}) value of more than 3.6, 10 genotypes (139, 145, 148, 203,
259 260, 403, 736, SC403, SC513, and ZM523) were identified as stable and high yielding (Table 6).
260 Interestingly all 10 of these lines were classified as high RCA. The chi-square test ($\chi^2 = 12.8, p =$
261 0.0003) demonstrated that RCA phenotype had a highly significant effect on grain yield stability.

262 *High RCA lines profited most in drier agroecologies on farm*

263 On-farm trials were conducted under rainfed conditions. The distribution of rainfall during the
264 experimental period is presented in Figure 5. The effective rainfall started in November at all
265 sites and tapered off in March in Chipoka and April in Mkwinda, subjecting plants in Chipoka to
266 terminal drought. Under drought, high RCA genotypes had 70% (Mkwinda) and 88% (Chipoka)
267 greater shoot biomass at 70 days after planting, and 40% (Mkwinda) and 147% (Chipoka)
268 greater yield than low RCA genotypes (Fig. 6 and Table 7).

269 **Discussion**

270 Using inbred maize lines grown in greenhouse mesocosms and field rainout shelters in the USA,
271 Zhu et al. (2010) showed that RCA reduces the metabolic costs of soil exploration, thereby
272 substantially improving rooting depth, water acquisition, plant growth, and yield under drought.
273 The main objective of the present study was to validate and extend these observations to maize
274 lines used by smallholder farmers in naturally occurring drought environments in Malawi. Our
275 results support the hypothesis that RCA improves maize drought tolerance. We observed
276 substantial variation for RCA formation in maize germplasm currently in use in Malawi, and
277 found that high RCA lines had greater leaf relative water content, plant biomass, and yield across
278 drought environments in trials on field stations as well as on smallholder farms (Figs. 2-6).

279 This study employed landraces that were created through a combination of natural selection and
280 selection by farmers across Malawi (Table 2, Appendix 1). Landraces usually have broader
281 intraspecific genetic diversity than modern cultivars; therefore, they are a valuable resource for
282 crop improvement (Ceccarelli, 1996). The diversity of landraces is a result of selection under
283 different soil and climatic conditions and is also strongly influenced by local practices and
284 specific demands for product quality and other characters. Genetic variation for RCA formation
285 has been reported in maize and related *Zea* species (Mano et al., 2006; Zhu et al., 2010; Burton et
286 al., 2013). In the current study we observed substantial variation for RCA formation among local
287 maize landraces collected across Malawi (Table 2, Appendix 1). The range for RCA observed
288 among maize germplasm accessions in this study is in agreement with previous reports in maize
289 (Zhu et al., 2010; Burton et al., 2013). However, average annual rainfall distribution as defined
290 in this study is not an indicator for RCA phenotype. Although the genotypes were originally
291 collected from areas with different average annual rainfall (Appendix 1), most of the accessions
292 were grouped in different clusters irrespective of rainfall distribution (Table 2). On the other
293 hand, this could be attributed to seed systems as practiced in Malawi whereby seeds can be
294 exchanged among farmers from different areas, which blurs differences that could have been
295 attributed to local adaptation.

296 A number of environmental stresses induce RCA formation, including suboptimal availability of
297 oxygen, water, nitrogen, phosphorus, and sulfur (Deacon et al., 1986; Drew et al., 2000;
298 Bouranis et al., 2003; Fan et al., 2003; Evans, 2004; Mano et al., 2006; Zhu et al., 2010; Mano
299 and Omori, 2013). Our study confirms the observation by Zhu et al (2010) that water stress
300 increases aerenchyma formation (Fig 3), although the magnitude of increase varied among
301 genotypes. Although drought induced RCA formation, accessions maintained their phenotypic
302 grouping consistently across soil moisture regimes and environments. These results are
303 consistent with other studies under drought (Zhu et al., 2010) and low N (Saengwilai, 2013).

304 However, some accessions were consistent across soil moisture regimes, while others had a
305 strong interaction with water regimes (Tables 3 & 4). RCA plasticity may be valuable in
306 avoiding negative tradeoffs to RCA formation under less stressful conditions, while genotypes
307 with stable RCA phenotypes may have value in breeding programs for stressful environments
308 such as those in Malawi and other developing regions. Collectively, these findings suggest that
309 breeders could select for genotypes with consistently high, low or plastic (i.e. induced by stress)
310 RCA formation.

311 Genotypes capable of supporting greater root biomass are able to develop the extensive deep root
312 systems required to fully utilize the soil moisture available in deeper soil domains (Ludlow and
313 Muchow, 1990; Ho et al., 2005; Hammer et al., 2009). Although it was not possible to quantify
314 root depth in this study, the maintenance of improved leaf water status under drought for high
315 RCA genotypes across drought environments (Fig 2 C,D) could be indicative of deeper rooting
316 that allows high RCA lines to utilize water in deeper soil strata. Increased water capture due to
317 RCA formation could have an autocatalytic effect on subsequent root growth by improving shoot
318 C gain, which in turn would permit greater root growth, increased water capture, etc. Zhu et al
319 (2010) reported that RCA formation increased mid-day leaf relative water content under water
320 stress, and this was attributed to improved access to moisture located deeper in the soil profile.
321 Saengwilai, (2013) working with recombinant inbred lines of maize in the field in South Africa,
322 found that RCA formation increased rooting depth and acquisition of ¹⁵N-labeled nitrate from the
323 subsoil. Maize genotypes with more RCA had greater shoot biomass and 58% greater yield than
324 genotypes with less RCA under low N conditions (Saengwilai, 2013). The utility of RCA under
325 suboptimal N is highly relevant in the present context since nitrate leaching results in the
326 accumulation of N in deep soil strata over time. This is analogous to water capture under drought
327 in the sense that in both cases the limiting soil resource is primarily available in deeper soil strata
328 (Lynch 2013).

329 RCA formation has been associated with reduction of root respiration (Fan et al., 2003; Zhu et
330 al., 2010; Saengwilai et al., 2014a). In general respiration can be divided into three major
331 categories: growth respiration which is considered as a one-time cost, maintenance respiration
332 for existing tissue, and ion uptake and transport into the xylem (van der Werf et al., 1988;
333 Lambers et al., 2002; Lynch and Ho, 2005). Simulation studies with maize showed that root
334 maintenance respiration reduced plant growth under nutrient limiting conditions by up to 72% in
335 comparison with plants with no root maintenance respiration (Postma and Lynch, 2011a; Postma
336 and Lynch, 2011b). In addition to reducing the carbon cost of soil exploration, RCA formation
337 reduces N and P content of root tissue, permitting internal reallocation of nutrients to growing
338 root tissue, which is particularly beneficial under conditions of low N and P availability (Postma
339 and Lynch, 2011a). The utility of RCA was greater for N capture in high leaching environments
340 with coarse textured soil and high precipitation, since leaching results in the accumulation of N
341 in deep soil domains over time. This is important since in many drought environments the topsoil
342 dries before the subsoil, and as drought progresses, roots must exploit increasingly deeper soil
343 strata to acquire water. These simulation results are supported by empirical studies in which
344 RCA formation was closely correlated with the ability of maize genotypes to maintain root
345 growth under suboptimal N (Saengwilai, 2013) and P stress (Fan et al., 2003).

346 RCA is not the only anatomical phenonomenon to influence root metabolic costs. A recent study has
347 shown that living cortical area (LCA: total transversal root cortical area minus RCA area) is a

348 better predictor of root respiration than RCA alone (Jaramillo et al., 2013). In that study a 3.5-
349 fold reduction in LCA was associated with a 2.5-fold improvement in plant growth under
350 drought. LCA is determined by cortical cell file number (CCFN; number cell files from the
351 epidermis to endodermis) and cortical cell size. Lynch (2013) proposed that large cortical cells
352 and reduced CCFN may also substantially reduce root metabolic cost of soil exploration and
353 thereby influence water acquisition from drying soil. This hypothesis has been tested empirically
354 in greenhouse mesocosms, field rainout shelters, and in the field in Malawi. Reduced CCFN and
355 large cortical cell size are associated with substantial reduction of root respiration, greater
356 rooting depth, greater water acquisition from deeper soil layer, and greater grain yield of maize
357 under drought (Chimungu et al., 2014a & b). Collectively, these findings strengthen the
358 hypothesis that reducing root metabolic costs increase water capture from drying soil.

359 Several root architectural and anatomical phenes states have been identified as effective strategies
360 for improving crop adaptation under abiotic stress (Lynch, 2013; Lynch et al., 2014). Root
361 architectural phenes determine the deployment of roots in specific soil domains in time and space
362 for resource foraging (Lynch, 1995). Anatomical phenes such as RCA are important in affecting
363 the metabolic efficiency of soil exploration, which is a key aspect of plant fitness under abiotic
364 stress (Lynch and Ho, 2005; Lynch, 2013; Lynch et al., 2014). The utility of a phene may depend
365 on the expression of other phenes in the plant phenotype (York et al., 2013). Such interactions
366 may be synergistic, neutral, or antagonistic. For example, greater lateral root development in
367 deep soil layers could improve nitrogen and water acquisition. However, increased lateral
368 branching adds metabolic costs which could influence the growth of other root classes (Walk et
369 al., 2006). This tradeoff could be alleviated by reducing the metabolic burden of root tissue via
370 RCA formation. Postma and Lynch, (2011a) using the structural-functional plant model *SimRoot*
371 showed that there is synergism between RCA and lateral root branching density in maize under
372 low P conditions. Recently, York et al. (2013) reported synergistic effects of root crown root
373 number and RCA on plant growth. Under low N conditions, total root length was greatest for
374 plants with intermediate numbers of crown roots, and increased formation of root cortical
375 aerenchyma increased total root length in these plants. In addition, Saengwilai et al., (2014b)
376 reported that reduced crown root number enhances nitrogen acquisition under low N conditions.
377 These results suggest synergism between root architectural and anatomical phenes. The utility of
378 an integrated phenotype should be greatest when phenes that reduce the cost of soil exploration
379 (such as formation of RCA) co-occur with phenes that increase soil exploration, such as steep
380 root growth angle and few but long root branches, which may synergistically improve resource
381 acquisition under abiotic stress (Lynch, 2013; Lynch et al., 2014).

382 RCA formation improves crop productivity by improving root metabolic efficiency under abiotic
383 stress. However, it may have subtle effects on other root functions. For example it has been
384 reported that aerenchyma formation impedes the radial movement of water (Yang et al., 2012)
385 and nutrients (Hu et al., 2014). Another potential tradeoff as suggested by Lynch et al., (2014) is
386 that RCA may affect mycorrhiza colonization or susceptibility to root pathogens. Roots
387 commonly suffer mechanical stress during their lifespan (Bennie, 1996). Depending on soil type
388 and condition, such stresses can be a result of swelling-shrinkage due to repeated wetting-drying
389 cycles or mechanical impedance. In this study trials were conducted in sandy clay loam to sandy
390 loam soils with little capacity for swelling-shrinking due to fluctuations in soil moisture. Soil
391 shrinkage can cause roots to collapse, therefore hindering water and nutrient acquisition
392 (Bengough et al., 2006). However, in many maize agroecosystems, root systems could be

393 exposed to the combination of antagonistic stress factors such as drought and soil compaction. In
394 this context, RCA formation could reduce the mechanical strength of the roots, which helps them
395 resist root compression due subsequent soil shrinkage associated with drying as drought
396 progresses especially in clay soil. Although potential tradeoffs to RCA formation are poorly
397 understood, our finding that RCA is beneficial in multiple stressful field environments indicates
398 that such tradeoffs are not common or obvious.

399 In low-input systems drought and low soil fertility are primary constraints to crop production.
400 The development, deployment and cultivation of drought tolerant and nutrient efficient maize
401 varieties represents an important opportunity to improve food security in developing countries,
402 where abiotic stress is among the principal factors limiting crop productivity (Lynch, 2007).
403 Selection for root phenes that increase crop productivity under abiotic stress, such as greater
404 RCA formation, should be a priority of breeding programs in developing countries (Lynch,
405 2014). Deployment of higher RCA in crop improvement programs will result in the development
406 of crop cultivars with enhanced soil resource acquisition efficiency, benefiting resource-poor
407 farmers in developing countries. Trait-based breeding, targeting the improvement of specific
408 phenes known to improve stress tolerance, has proved useful in deploying root phenes such as
409 basal root angle, basal root whorl number, root hair length and density which confers P-
410 efficiency in bean breeding programs in Central America and Mozambique (Lynch, 2007). Given
411 the utility of RCA under several edaphic stresses, RCA could be deployed using the same
412 strategy.

413 In conclusion, high RCA genotypes had greater shoot biomass and yield both in field station and
414 on-farm trials. Under natural drought, the benefits of RCA were greater in the drier on-farm
415 location. Environments used in this study include variation in soil properties such as soil biota
416 which may interact with phenes such as RCA. The fact that our results are in agreement with
417 results of (Zhu et al., 2010) in controlled environments and in rainout shelters in the USA
418 validates the utility of RCA under water limited conditions. These results collectively support the
419 value of RCA as a breeding target for improved maize adaptation to nutrient and drought stress.

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Tables

Table 1. General agro-climatic characteristics of the experimental sites used to evaluate maize genotypes in Malawi

Agroecological zone	Site	Location		Altitude (m.a.s.l)	Annual rainfall (mm)	Soil type	
		Latitude	Longitude				
Lilongwe	Bunda	14°10'S	33°48'E	1080	1031	Oxic Rhodustalfs, Lilongwe series, medium-textured sandy loam	
Salima	Chitala	13°28'S	33°59'E	600	967	Oxic Rhodustalfs, Chitala series, Sandy clay loam	
Lilongwe	Mkwinda	14°24'S	33°76'E	1032	1031	Oxic Rhodustalfs, Lilongwe series, medium-textured sandy loam	
Salima	Chipoka	13°99'S	34°51'E	483	786	Calcustepts, Salima series, medium-textured sandy clay loam	

*m.a.s.l is meters above sea level

Table 2. Passport data of 40 Malawi maize landrace accessions used in this study, showing accession code, accession number, and geographic origin, percent root cortical aerenchyma (%RCA), and classification group for RCA: %RCA < 2.4 as low RCA, 2.4 < %RCA < 12.1 as intermediate (Int) RCA, and %RCA > 12.1 as high RCA.

Code	Accession	*Agroecology	Origin	Latitude	Longitude	% RCA	Group by phenotype
1	386	Shire valley	Nsanje	-17.1	35.1	21.2	High RCA
2	403		Nsanje	-16.3	35.2	2.0	Low RCA
3	699	Machinga	Zomba	-15.4	35.5	6.7	Int RCA
4	696		Zomba	-15.3	35.2	21.9	High RCA
5	3411		Zomba	-15.2	35.3	0.2	Low RCA
6	750		Machinga	-15.1	34.5	17.5	High RCA
7	741		Machinga	-15.1	34.8	0	Low RCA
8	752		Machinga	-15	35	0.7	Low RCA
9	736		Machinga	-14.6	35	23.7	High RCA
10	787		Machinga	-14.5	35.3	2.4	Low RCA
11	811		Mangochi	-14.4	35.3	3.9	Int RCA
12	1772	Lilongwe	Ntcheu	-15	34.7	0.7	Low RCA
13	1992		Dedza	-14.2	34.3	6.6	Int RCA
14	2012		Lilongwe	-14.1	33.6	14.6	High RCA
15	2027		Lilongwe	-14	34	19.0	High RCA
16	1786		Dedza	-14.2	34.1	7.7	Int RCA
17	1857	Kasungu	Dowa	-13.3	33.7	1.9	Low RCA
18	1915		Kasungu	-12.5	33.8	0	Low RCA
19	250	Mzuzu	Mzimba	-12.1	33.9	14.7	High RCA
20	249		Mzimba	-12.1	33.9	0.1	Low RAC
21	243		Mzimba	-12.1	33.7	22.2	High RCA
22	193		Mzimba	-11.5	33.4	15.6	High RCA
23	3244		Mzimba	-11.5	33.4	1.3	Low RCA
24	3243		Mzimba	-11.5	32.9	0.2	Low RCA
25	218		Mzimba	-11.5	33.9	14.9	High RCA
26	203		Mzimba	-11.5	33.3	10.3	Int RCA
27	163		Nkhata bay	-11.4	33.6	22.1	High RCA
28	172		Nkhata bay	-11.4	34	30.4	High RCA
29	164		Nkhata bay	-11.4	34.1	0.4	Low RCA
30	145		Mzimba	-11.3	33.5	3.2	Int RCA
31	148		Mzimba	-11.2	34.4	6.6	Int RCA
32	303		Rumphi	-10.5	33.7	2.1	Low RCA
33	260	Karonga	Chitipa	-10.2	33.8	12.2	Int RCA
34	2862		Karonga	-10.1	34	0.3	Low RCA
35	297		Karonga	-10	33.6	17.1	High RCA
36	139	Salima	Salima	-14	34.5	8.0	Int RCA
37	569	Blantyre	Chiradzulu	-15.6	35.2	1.0	Low RCA
38	637		Thyolo	-16.2	35.2	22.9	High RCA
39	539		Mulanje	-15.4	35.4	1.3	Low RCA
40	629		Thyolo	-15.1	35.32	18.4	High RCA

*The country is subdivided into agroecologies based on various ecological factors by Malawi Plant Genetic Resources Centre (MPGRC) of Department of Agriculture Research and Services (DARS) of the Ministry of Agriculture and Food Security, Lilongwe, Malawi.

Table 3. Mean percent root cortical aerenchyma for 20 selected maize accessions 70 days after planting in the field both in well-watered (WW) and water stressed (WS) conditions at Bunda and Chitala. Root segments were collected 10-20 cm from the base of the second whorl crown roots. Data shown are means of 4 replicates each genotype for each environment (soil moisture by location): BUWS (Bunda well watered), BUWW (Bunda water stress), CHWW (Chitala well watered), and CHWS (Chitala water stress).

Accession	Group by phenotype	BUWW	BUWS	CHWW	CHWS
139	High RCA	7.7 ±1.4	12.9 ±1.3	11.8 ±2.6	16.9 ±1.7
145	High RCA	7.3 ±2.5	13.9 ±1.6	7.4 ±1.2	15.5 ±1.3
148	High RCA	8.1 ±1.2	15.1 ±1.4	6.1 ±1.2	26.8 ±1.4
203	High RCA	11.4 ±2.0	17.3 ±1.9	9.1 ±2.2	21.4 ±1.8
250	High RCA	12.6 ±2.4	14.3 ±0.9	15.7 ±0.9	20.0 ±1.3
260	High RCA	12.0 ±1.6	14.5 ±0.6	11.5 ±3.7	23.3 ±2.4
403	High RCA	9.1 ±1.6	14.8 ±1.3	13.7 ±1.6	31.6 ±2.2
736	High RCA	23.6 ±1.3	27.3 ±0.8	12.7 ±6.1	20.1 ±2.7
SC403	High RCA	15.8 ±2.5	18.3 ±2.3	13.9 ±2.3	16.9 ±2.4
SC513	High RCA	13.9 ±2.1	16.7 ±1.9	14.9 ±1.8	16.2 ±1.5
ZM523	High RCA	9.2 ±0.7	22.0 ±0.9	14.8 ±1.0	22.2 ±3.6
172	Low RCA	3.1 ±0.9	3.8 ±1.4	3.6 ±1.5	6.2 ±1.4
243	Low RCA	0.3 ±0.2	3.6 ±1.5	1.2 ±0.6	4.5 ±1.1
297	Low RCA	4.9 ±2.9	0.4 ±0.3	2.7 ±1.8	4.5 ±1.1
303	Low RCA	3.3 ±2.1	2.5 ±0.8	6.5 ±1.9	5.8 ±0.9
539	Low RCA	3.4 ±1.9	7.5 ±1.1	5.8 ±2.0	7.5 ±2.1
787	Low RCA	2.3 ±2.1	2.9 ±1.2	8.2 ±1.5	6.7 ±1.5
1857	Low RCA	1.2 ±1.2	1.9 ±0.9	1.6 ±0.8	7.4 ±1.5
1992	Low RCA	0.7 ±0.5	5.6 ±2.2	8.9 ±0.4	16.4 ±1.9
2012	Low RCA	3.2 ±1.1	4.8 ±1.8	16.2 ±2.9	22.6 ±5.2
		<i>*mse</i>	<i>P-value</i>	<i>ms</i>	<i>P-value</i>
	Treatment	654.8	0.015	1308.3	0.020
	Genotype	303.5	0.020	254.9	0.001
	Treatment x Genotype	58.2	0.003	64.8	0.023

*mean square error

Table 4. Summary of linear models for irrigation, genotype, and genotype × irrigation interaction for leaf relative water content (RWC), shoot biomass and grain yield in twenty maize genotypes examined across two environments: Bunda and Chitala.

Effect	Bunda			Chitala				
	d.f	RWC F value	Biomass F value	Yield F value	d.f	RWC F value	Biomass F value	Yield F value
Irrigation	1	75.1***	123.8***	676.3***	1	455.1***	341.1***	1120.2***
Genotype	19	1.8*	3.7***	6.8***	19	5.3***	3.9**	15.5***
Genotype x Irrigation	19	2.6**	1.8**	4.7***	19	4.0***	3.3**	8.1***
Residuals	106				81			

P < 0 '***', *P* < 0.001 '**', *P* < 0.01 '*'

Table 5. Mean grain yield (g plant⁻¹) for 20 genotypes across four environments (soil moisture by location): BUWS (Bunda water stress), BUWW (Bunda well watered), CHWS (Chitala water stress), and CHWW (Chitala well watered). Values shown are means of four replicates \pm SE.

Genotype	Group by phenotype	BUWW	CHWW	BUWS	CHWS
139	High RCA	132.0 \pm 5.6	131.7 \pm 9.3	88.7 \pm 3.4	86.2 \pm 2.9
145	High RCA	106.1 \pm 1.8	117.2 \pm 5	88.2 \pm 2.4	86.6 \pm 4.1
148	High RCA	141.5 \pm 13.7	121.4 \pm 6.5	78.4 \pm 2.8	87.9 \pm 3.2
203	High RCA	125.7 \pm 9.3	150.0 \pm 9.7	80.2 \pm 2.4	74.4 \pm 4.6
250	High RCA	106.9 \pm 2.0	117.4 \pm 10.4	75.6 \pm 4.5	69.0 \pm 5.8
260	High RCA	135.0 \pm 7.3	113.2 \pm 8.1	85.9 \pm 3.8	66.5 \pm 1.4
403	High RCA		114.5 \pm 5.4		58.3 \pm 3.1
736	High RCA	130.6 \pm 30.3		72.1 \pm 2.2	
SC403	High RCA	127.3 \pm 9.1	110.9 \pm 6	79.1 \pm 4.0	61.1 \pm 2.1
SC513	High RCA	124.3 \pm 4.9	115.7 \pm 5.8	82.2 \pm 3.9	81.9 \pm 2.7
ZM523	High RCA	128.6 \pm 3.1	137.9 \pm 4.9	75.4 \pm 6.2	86.7 \pm 1.7
172	Low RCA	102.3 \pm 2.6	110.3 \pm 6.1	40.8 \pm 1.8	27.5 \pm 3.2
243	Low RCA	107.5 \pm 6.8		32.5 \pm 1.4	
297	Low RCA	130.0 \pm 7.1	115.5 \pm 6	41.4 \pm 3.0	31.5 \pm 3.1
303	Low RCA	119.4 \pm 10.2	114.9 \pm 3.8	42.8 \pm 0.8	32.3 \pm 2.8
539	Low RCA	115.7 \pm 6.7	112.8 \pm 3.2	48.5 \pm 2.4	34.1 \pm 4.4
787	Low RCA	134.6 \pm 10.8	140.8 \pm 4.7	40.2 \pm 3.6	30.3 \pm 3.7
1857	Low RCA	113.0 \pm 8.7	134.6 \pm 11.6	39.7 \pm 3.5	23.1 \pm 8.3
1992	Low RCA	111.4 \pm 11.4	120.7 \pm 4.1	77.4 \pm 9.7	57.6 \pm 0.1
2012	Low RCA	142.0 \pm 4.2	109.4 \pm 3.4	43.6 \pm 2.5	54.8 \pm 2.1
Mean high RCA		125.8 \pm 3.5	122.9 \pm 4.3	80.5 \pm 5.6	75.8 \pm 3.6
Mean low RCA		119.5 \pm 4.4	119.5 \pm 4.5	45.2 \pm 4.3	36.4 \pm 4.6

Table 6. Simultaneous selection of genotypes based on yield and stability (Kang, 1993). Genotypes with yield-stability index (*YSi*) greater than 3.6 (i.e. the mean of *YSi*) were selected as high yielding and stable across environments.

Accession	Group by phenotype	Yield (g plant ⁻¹)	Rank	<i>YSi</i>
250	High RCA	92.2	10	3
403	High RCA	94.6	11	6 +
145	High RCA	99.5	13	8 +
SC513	High RCA	99.6	14	17 +
260	High RCA	100.1	15	10 +
SC403	High RCA	101	16	11 +
148	High RCA	107.3	17	12 +
203	High RCA	107.5	18	13 +
139	High RCA	109.6	19	14 +
736	High RCA	99	12	15 +
ZM523	High RCA	114.7	20	15 +
243	Low RCA	67.4	1	-10
172	Low RCA	70.2	2	-9
1857	Low RCA	76.4	3	-8
303	Low RCA	77.3	4	-7
539	Low RCA	77.7	5	-6
297	Low RCA	79.6	6	-5
787	Low RCA	86.5	7	-4
2012	Low RCA	87.4	8	-3
1992	Low RCA	91.7	9	0

*+ selected genotypes

Table 7. Mean shoot biomass (g plant⁻¹) and grain yield (g plant⁻¹) for ten maize genotypes examined in two field environments: Mkwinda and Chipoka on farm contrasting for RCA and classification group for RCA: %RCA < 10 as low RCA, and %RCA > 20 as high RCA. Values shown are means of ten replicates ± SE.

Mkwinda EPA				
Genotype	Group by phenotype	% RCA	Shoot biomass	Grain yield
AR239	High RCA	27.3 ±2.6abc	151.8 ±8.8a	104.8 ±2.4a
AR716	High RCA	28.1 ±5.3ab	146.7 ±7.7ab	104.2 ±1.9a
CML344	High RCA	34.8 ±3.8a	147.5 ±11.4a	104.6 ±3.2a
E21	High RCA	28.0 ±3ab	137.5 ±13.4ab	96.5 ±4.4ab
CML247	Low RCA	8.1 ±1.6d	85.7 ±4.1d	60.2 ±4.6d
CML321	Low RCA	6.3 ±0.9d	95.4 ±6cd	73.5 ±5.4cd
M70-29-3	Low RCA	10.9 ±2.2d	106.9 ±8.2bcd	69.3 ±3.1cd
M73-18	Low RCA	6.8 ±2d	126.5 ±10.6abc	78.9 ±5.5bc
MANICA4	Low RCA	7.9 ±5bcd	130.6 ±14.2abc	95.3 ±3.8ab
SW19	Low RCA	4.1 ±2.6cd	77.1 ±3.8d	60.6 ±4.9d
Mean high RCA		27.1±1.8	132.7±5.4	101.1±1.5
Mean low RCA		7.5±0.9	9.2±4.1	77.7±2.3
Chipoka EPA				
Genotype	Group by phenotype	% RCA	Shoot biomass	Grain yield
AR239	High RCA	36.4 ±2.1a	141.4 ±8.2ab	91.5 ±3.2a
AR716	High RCA	30.6 ±2.1ab	157.4 ±10.9a	96.2 ±5.8a
CML344	High RCA	26.1 ±3b	141.2 ±9.7ab	88.4 ±4.2ab
E21	High RCA	27.6 ±1.9b	150.9 ±7.9a	91.3 ±4.7a
CML247	Low RCA	6.3 ±2.3c	61.3 ±7.3d	32.1 ±2.8c
CML321	Low RCA	3.2 ±0.9c	70.8 ±8.2d	33.9 ±3.5c
M70-29-3	Low RCA	3.6 ±1c	69.0 ±6.2d	33.1 ±2.5c
M73-18	Low RCA	6.2 ±1.4c	79.4 ±9.2cd	39.1 ±3.9c
MANICA4	Low RCA	10.1 ±2.7c	107.9 ±13.5bc	71.4 ±4.9b
SW19	Low RCA	5.8 ±1.1c	61.1 ±6.9d	38.6 ±5c
Mean high RCA		26.1±1.6	132.3±5.7	87.8±2.3
Mean low RCA		4.9±0.6	70.3±5.4	35.4±1.6

Means followed by the different letter for each trait (grain yield and shoot biomass) are significantly different as determined by Tukey HSD (P<0.05).

Figures

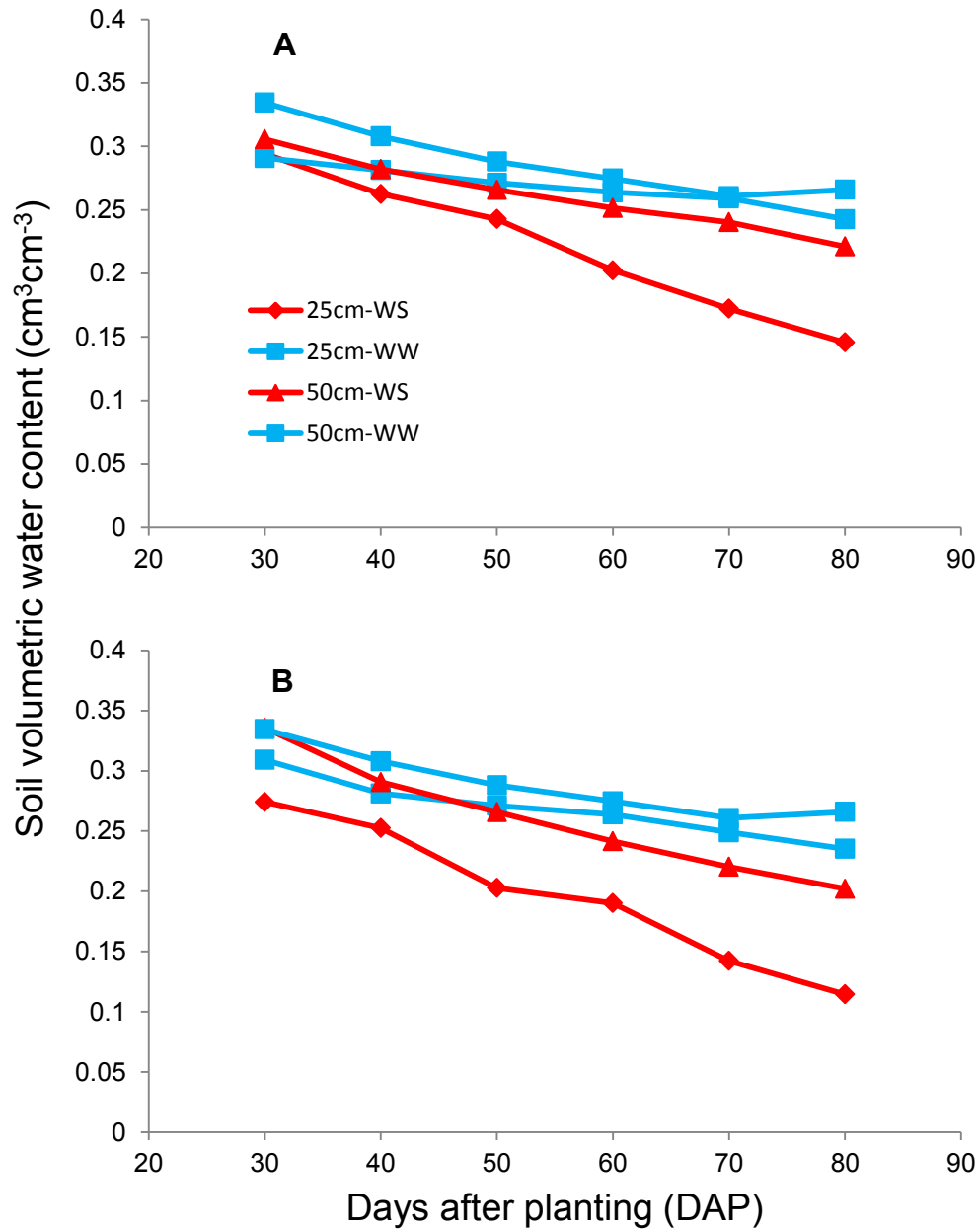


Figure 1. Soil water content under well watered (WW) and water stressed (WS) conditions in Bunda (A) and Chitala (B) at 25 and 50 cm.

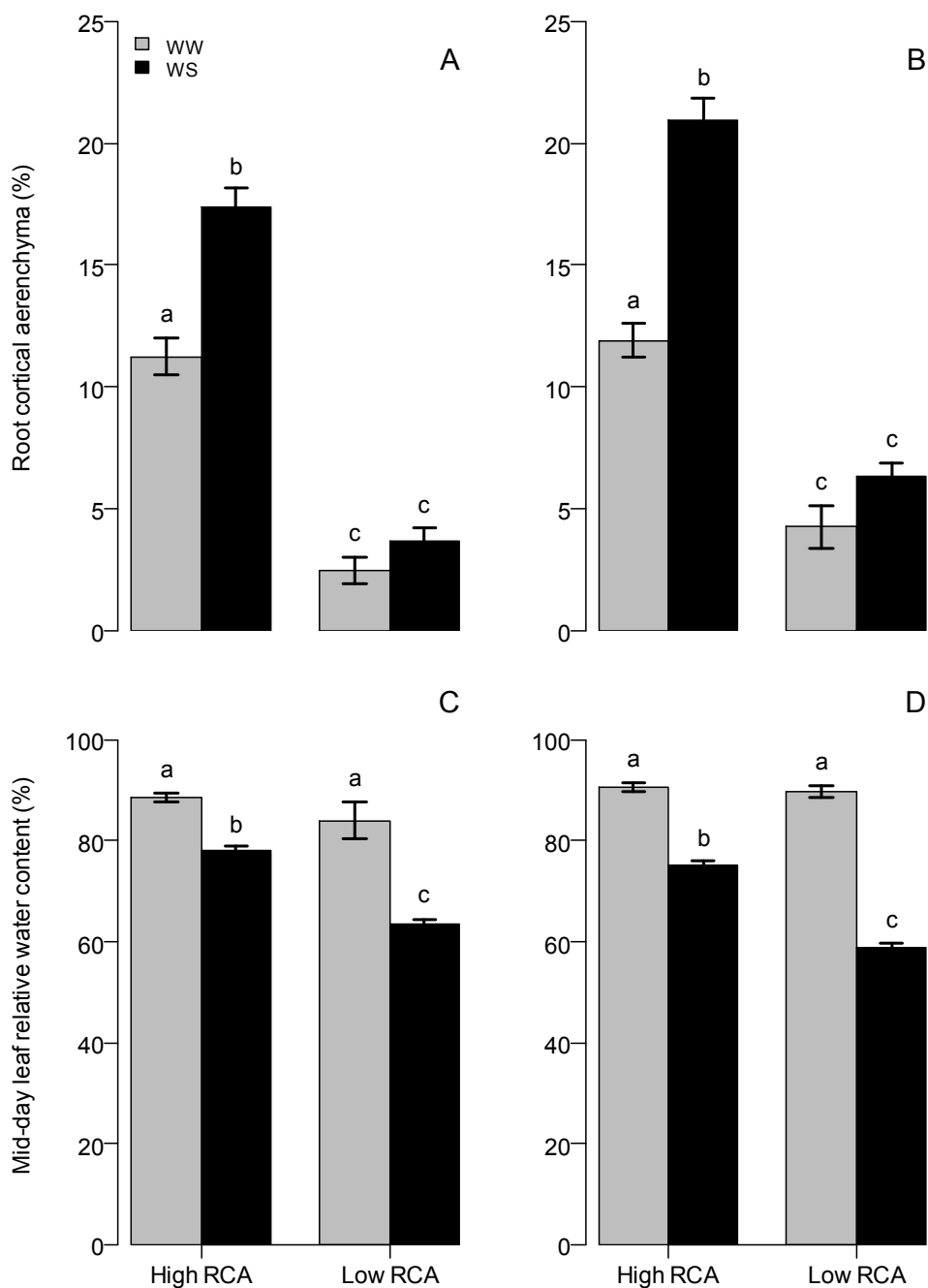


Figure 2. The proportion of root cortical aerenchyma and leaf relative water content for 10 high RCA and 10 low RCA maize genotypes under water stress (WS) and well watered (WW) conditions at 70 days after planting in two field environments: (A,C) Bunda and (B,D) Chitala. The data shown are means of 4 replicates \pm SE of the mean. Different letters represent significant differences ($p < 0.05$).

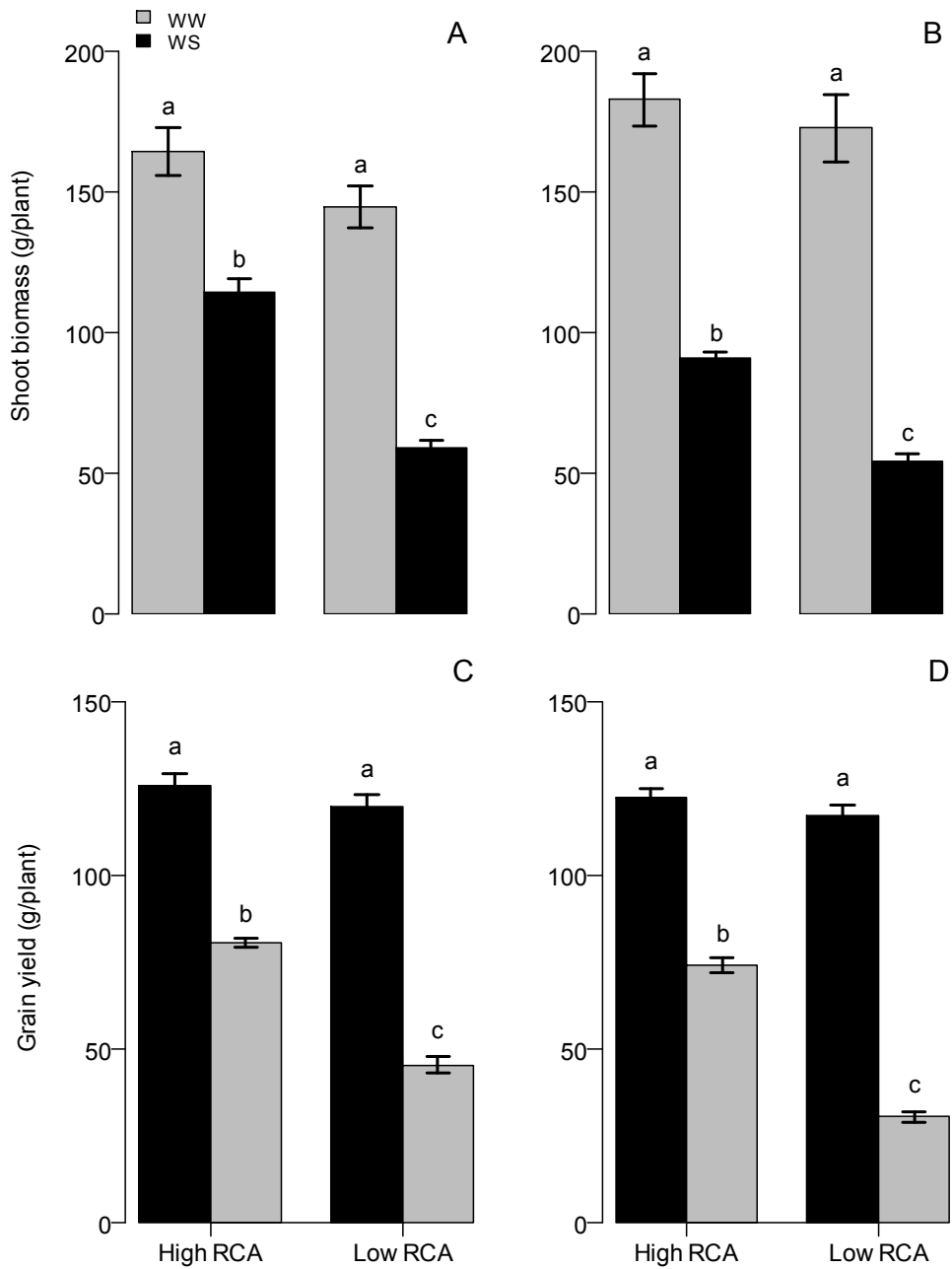


Figure 3. Shoot biomass and grain yield for 10 high RCA and 10 low RCA maize genotypes under water stress (WS) and well watered (WW) conditions at 70 days after planting in two field environments: (A,C) Bunda and (B,D) Chitala. The data shown are means of 4 replicates \pm SE of the mean. Different letters represent significant differences ($p < 0.05$).

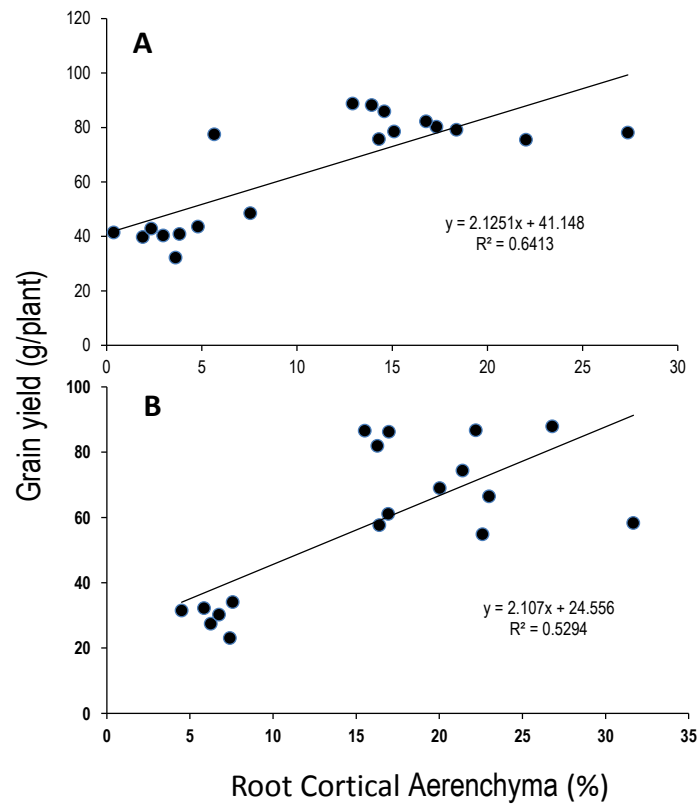


Figure 4. Correlation between yield and percentage of root cortical aerenchyma (% of cortical cross sectional area) under water stress condition in two field environments: **(A)** Bunda and **(B)** Chitala.

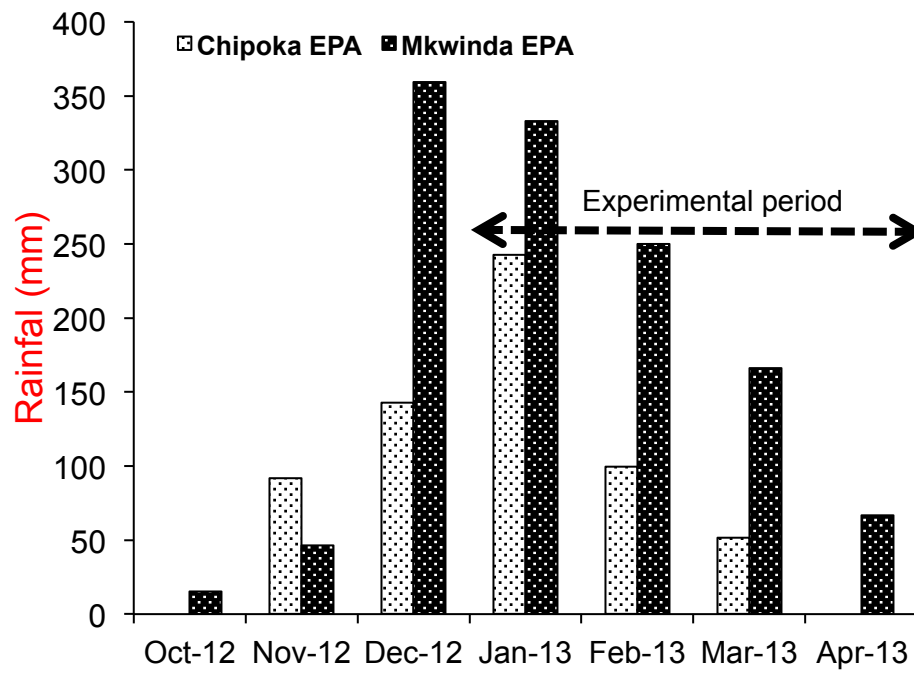


Figure 5. Rainfall distribution at on-farm experimental sites in Mkwinda EPA and Chipoka EPA during the 2012/13 growing season

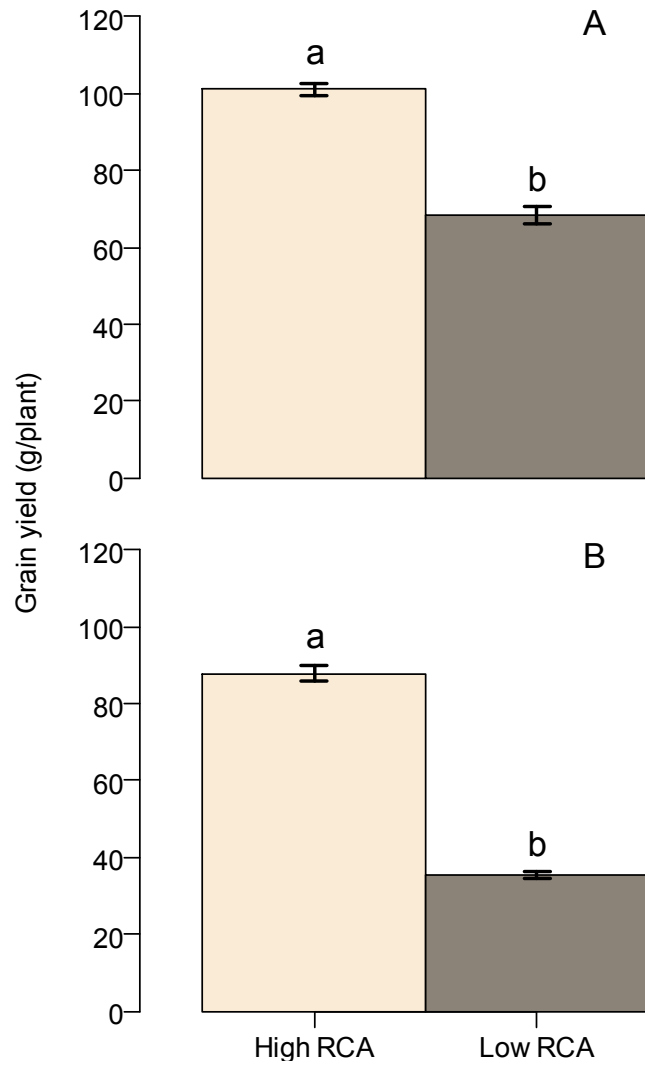
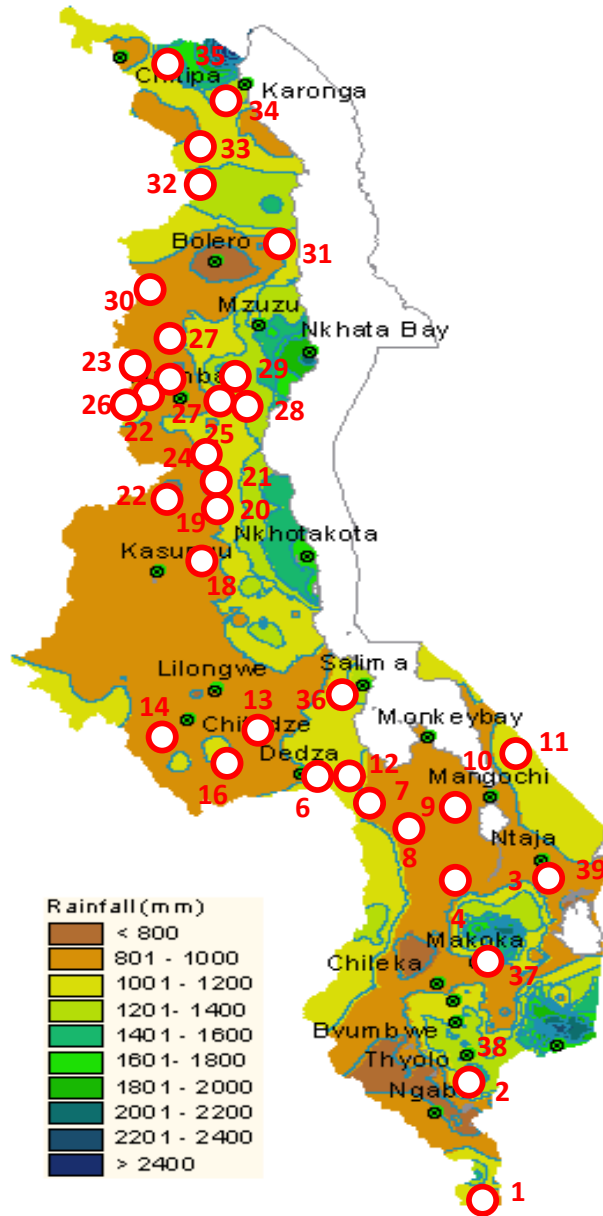


Figure 6. Grain yield for four high RCA and six low RCA maize genotypes on-farm across in two field environments: **(A)** Mkwinda and **(B)** Chipoka. The data shown are means of 10 replicates \pm SE of the mean. Different letters represent significant differences ($p < 0.05$).

Appendices

Appendix 1. Map of Malawi showing average annual rainfall distribution and geographical distribution of maize landraces evaluated in the study. Numbers correspond to accession codes in Table 2. (Source: www.metmalawi.com, accessed 10th September 2014).



Appendix 2: List of genotypes selected from Malawi maize breeding program populations and root anatomical phenes; RD, root diameter, TCA, total cortical area, CT, cortical thickness, SD,

stele diameter, CCFN, cortical cell file number, CCS, cortical cell size, and RGA, root growth angle. The data shown are means of 8 replicates \pm SE of the mean

Soil moisture	RIL	RD	TCA	CT	SD	CCFN	CCS	RGA
WS	139	1.9 \pm 0.1	2 \pm 0.1	0.5 \pm 0.1	1 \pm 0.1	12.7 \pm 0.4	264 \pm 4	46.9 \pm 5.8
WS	145	1.8 \pm 0.1	1.9 \pm 0.1	0.5 \pm 0.1	0.9 \pm 0.1	9 \pm 0.5	343 \pm 24	54.8 \pm 6.7
WS	148	1.7 \pm 0.1	1.6 \pm 0.2	0.5 \pm 0.1	0.8 \pm 0.1	12.9 \pm 1.4	352 \pm 24	51.9 \pm 9.4
WS	172	1.8 \pm 0.1	1.8 \pm 0.1	0.5 \pm 0.1	0.9 \pm 0.1	14.7 \pm 1.4	403 \pm 45	49.1 \pm 4.9
WS	203	1.5 \pm 0.1	1.5 \pm 0.2	0.5 \pm 0.1	0.6 \pm 0.1	10 \pm 0.7	348 \pm 47	43.9 \pm 5.1
WS	250	1.8 \pm 0.2	1.8 \pm 0.2	0.4 \pm 0.1	0.9 \pm 0.1	11.3 \pm 0.7	341 \pm 29	45.2 \pm 3.9
WS	260	1.7 \pm 0.1	1.7 \pm 0.2	0.5 \pm 0.1	0.8 \pm 0.1	11.9 \pm 0.9	304 \pm 46	50.9 \pm 4.8
WS	297	1.7 \pm 0.1	1.5 \pm 0.1	0.4 \pm 0.1	0.9 \pm 0.1	12.6 \pm 1.1	355 \pm 33	48.3 \pm 4.1
WS	303	1.5 \pm 0.1	1.4 \pm 0.2	0.4 \pm 0.1	0.7 \pm 0.1	13.9 \pm 2.1	298 \pm 37	46.5 \pm 5.3
WS	403	1.4 \pm 0.1	1.3 \pm 0.1	0.4 \pm 0.1	0.7 \pm 0.1	10 \pm 0	387 \pm 13	38.8 \pm 5.1
WS	539	1.6 \pm 0.1	1.6 \pm 0.1	0.4 \pm 0.1	0.8 \pm 0.1	14.4 \pm 0.5	291 \pm 8	39.9 \pm 4.5
WS	736	1.6 \pm 0.1	1.6 \pm 0.1	0.5 \pm 0	0.7 \pm 0.1	12.4 \pm 0.7	423 \pm 18	40.1 \pm 3.3
WS	787	1.7 \pm 0.1	1.8 \pm 0.1	0.5 \pm 0.1	0.9 \pm 0.1	14.9 \pm 0.6	351 \pm 19	41.3 \pm 6.9
WS	1857	1.5 \pm 0.1	1.3 \pm 0.1	0.4 \pm 0.1	0.7 \pm 0.1	12 \pm 0.5	267 \pm 10	42.4 \pm 7.2
WS	1992	1.8 \pm 0.1	2.1 \pm 0.3	0.5 \pm 0.1	0.9 \pm 0.1	15 \pm 1.1	284 \pm 15	45.1 \pm 7.0
WS	2012	1.4 \pm 0.1	1.2 \pm 0.2	0.4 \pm 0.1	0.8 \pm 0.1	10.8 \pm 0.9	302 \pm 19	42.4 \pm 6.2
WW	139	1.5 \pm 0.1	1.3 \pm 0.1	0.4 \pm 0.1	0.8 \pm 0.1	11.1 \pm 0.7	390 \pm 46	49.6 \pm 5.3
WW	145	1.8 \pm 0.1	1.9 \pm 0.1	0.5 \pm 0.1	0.9 \pm 0.1	12.4 \pm 0.9	391 \pm 25	50.5 \pm 4.2
WW	148	1.6 \pm 0.1	1.5 \pm 0.2	0.4 \pm 0.1	0.8 \pm 0.1	12.4 \pm 0.8	254 \pm 24	41.2 \pm 5.6
WW	172	1.6 \pm 0.2	1.6 \pm 0.2	0.4 \pm 0.1	0.8 \pm 0.1	12.4 \pm 1.2	362 \pm 45	46.8 \pm 4.1
WW	203	1.7 \pm 0.1	1.7 \pm 0.2	0.5 \pm 0.1	0.9 \pm 0.1	12.3 \pm 1.4	368 \pm 23	47.7 \pm 3.8
WW	250	1.6 \pm 0.1	1.6 \pm 0.1	0.5 \pm 0.1	0.8 \pm 0.1	9.4 \pm 0.4	531 \pm 10	43.8 \pm 2.9
WW	260	1.5 \pm 0.1	1.3 \pm 0.2	0.4 \pm 0.1	0.8 \pm 0.1	11 \pm 1	238 \pm 16	57.1 \pm 5.9
WW	297	1.7 \pm 0.1	1.7 \pm 0.1	0.5 \pm 0.1	0.8 \pm 0.1	12.8 \pm 0.8	320 \pm 38	51.8 \pm 4.3
WW	303	2 \pm 0.2	2.3 \pm 0.4	0.5 \pm 0.1	1 \pm 0.2	14.9 \pm 0.8	302 \pm 7	49.1 \pm 3.5
WW	403	1.7 \pm 0.1	1.7 \pm 0.1	0.4 \pm 0.1	0.9 \pm 0.1	13 \pm 0.5	248 \pm 22	48.3 \pm 5.2
WW	539	1.6 \pm 0.1	1.5 \pm 0.1	0.4 \pm 0.1	0.8 \pm 0.1	11 \pm 0.5	282 \pm 41	56.1 \pm 5.5
WW	736	1.4 \pm 0.1	1.1 \pm 0.1	0.4 \pm 0.1	0.7 \pm 0.1	10.3 \pm 1	277 \pm 14	54.2 \pm 5.0
WW	787	1.7 \pm 0.1	1.7 \pm 0.2	0.4 \pm 0.1	0.9 \pm 0.1	10.4 \pm 0.4	398 \pm 25	64.3 \pm 4.5
WW	1857	1.7 \pm 0.1	1.7 \pm 0.1	0.5 \pm 0.1	0.8 \pm 0.1	12.9 \pm 0.8	309 \pm 15	40.5 \pm 4.7
WW	1992	1.7 \pm 0.1	1.8 \pm 0.1	0.5 \pm 0.1	0.9 \pm 0.1	13.1 \pm 0.6	385 \pm 39	48.7 \pm 4.9
WW	2012	1.8 \pm 0.1	1.9 \pm 0.1	0.5 \pm 0.1	0.9 \pm 0.1	12.1 \pm 0.4	310 \pm 29	40.8 \pm 4.3

Appendix 3: List of genotypes selected from Malawi maize breeding program populations and root anatomical phenes; RD, root diameter, TCA, total cortical area, CT, cortical thickness, SD,

stele diameter, CCFN, cortical cell file number, CCS, cortical cell size and RGA, root growth angle. The data shown are means of 4 replicates \pm SE of the mean

Genotype	RD	TCA	CT	SD	CCFN	CCS	RGA
AR239	1.9 \pm 0.1	2.2 \pm 0.2	0.7 \pm 0.2	1.3 \pm 0.2	11.7 \pm 9.2	380.3 \pm 32.6	50.3 \pm 3.2
AR716	2.7 \pm 0.5	3.3 \pm 0.7	0.7 \pm 0.2	1.3 \pm 0.3	11.5 \pm 0.3	353.9 \pm 82.9	55.9 \pm 5.8
CML247	2.7 \pm 0.6	3.4 \pm 0.8	0.7 \pm 0.2	1.4 \pm 0.3	12 \pm 0.3	324.6 \pm 46.7	54.1 \pm 6.9
CML321	2.7 \pm 0.5	3.2 \pm 0.7	0.7 \pm 0.2	1.4 \pm 0.3	13.9 \pm 1.7	308.8 \pm 94.3	45.9 \pm 5.7
CML344	2.4 \pm 0.4	2.7 \pm 0.4	0.7 \pm 0.2	1.4 \pm 0.3	11.7 \pm 0.2	351.5 \pm 40.7	48.9 \pm 6.9
E21	2.6 \pm 0.5	2.5 \pm 0.3	0.7 \pm 0.2	1.3 \pm 0.3	12 \pm 2.1	339.9 \pm 98.2	41.0 \pm 4.8
M70-29-3	2.5 \pm 0.5	2.8 \pm 0.6	0.7 \pm 0.2	1.2 \pm 0.2	11.9 \pm 0.4	335.1 \pm 21.6	43.5 \pm 6.9
M73-18	2.6 \pm 0.5	3 \pm 0.6	0.6 \pm 0.2	1.4 \pm 0.3	12.1 \pm 0.3	385.4 \pm 88.3	45.9 \pm 4.4
MANICA4	2.6 \pm 0.5	3 \pm 0.7	0.7 \pm 0.2	1.3 \pm 0.3	11.7 \pm 0.3	330.1 \pm 45.4	56.7 \pm 5.6
SW19	2.7 \pm 0.5	3.3 \pm 0.7	0.7 \pm 0.2	1.4 \pm 0.3	12.1 \pm 0.3	357.1 \pm 56.7	45.8 \pm 4.1