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Seedling root system adaptation to water availability during maize domestication and global expansion

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Yu P., Li C., Li M., He X., Wang D., Li H., et al. (2024). Seedling root system adaptation to water availability during maize domestication and global expansion. *NATURE GENETICS*, 56(6), 1245-1256 [10.1038/s41588-024-01761-3].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/973534> since: 2024-07-05

*Published:*

DOI: <http://doi.org/10.1038/s41588-024-01761-3>

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# 1 Seedling root system adaptation to water availability during maize 2 domestication and global expansion

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54 **One sentence summary**

55 We demonstrated that fewer seminal roots in maize are beneficial under drought and identified genes  
56 controlling their environmental variation.  
57

58 **Abstract**

59 The maize root system has been reshaped by indirect selection during global adaptation to new  
60 agricultural environments. In this study, we characterized the root systems of >9,000 global maize  
61 accessions and its wild-relatives, defining the geographical signature and genomic basis of variation in  
62 seminal root number. We demonstrate that seminal root number has increased during maize  
63 domestication followed by a decrease to limited water availability in locally adapted varieties. By  
64 combining environmental and phenotypic association analyses with linkage mapping, we identified  
65 genes linking environmental variation and seminal root number. Functional characterization of the  
66 transcription factor *ZmHb77* and *in silico* root modelling provides evidence that reshaping root system  
67 architecture by reducing the number of seminal roots and promoting lateral root density is beneficial for  
68 the resilience of maize seedlings to drought .

## 69 Main Text

70 The spread of crops and expansion of cultivation from their ancestral habitats were accompanied by  
71 substantial phenotypic changes driven by a combination of direct farmer selection and environmental  
72 adaptation (Meyer and Purugganan 2013). Maize (*Zea mays* ssp. *mays*) was initially domesticated in  
73 southwest Mexico approx. 9,000 years ago from the wild lowland teosinte *Zea mays* ssp. *parviglumis*  
74 with subsequent admixture with the highland teosinte *Zea mays* ssp. *Mexicana* contributing substantially  
75 to modern populations (Hake and Ross-Ibarra 2015; Yang et al. 2023). Following domestication from  
76 *parviglumis*, maize spread to the highlands of Mexico and South America (Ross-Ibarra and Piperno  
77 2020; Fig. 1A). Subsequent adaptation to temperate climates allowed the expansion of maize from the  
78 tropics to diverse environments around the globe (Navarro et al. 2017; Swarts et al. 2017). Root system  
79 function is instrumental in colonizing new habitats (Ma et al. 2018) and acquiring resources, in particular  
80 water and nutrients in natural soils of different geographical origin (Eshel and Beeckman 2013). During  
81 domestication and diversification, the maize root system has become more complex by acquiring the  
82 capacity to form seminal roots, a feature largely absent in the maize progenitor teosinte (Hochholdinger  
83 et al. 2018; Lopez-Valdivia et al. 2022). In maize seedlings, the number of seminal roots determines the  
84 overall structure of the root system and thereby the depth and soil volume that roots can explore (Yu et  
85 al. 2016; Golan et al. 2018; Perkins and Lynch 2021). Seminal roots are formed endogenously in the  
86 embryo between 22–40 days after pollination (Hochholdinger et al. 2004). They are beneficial for  
87 nitrogen and phosphorus acquisition during maize seedling development (Perkins and Lynch 2021) and  
88 can persist and remain functional during the whole life cycle of the maize plant (Hochholdinger et al.  
89 2004). Nevertheless, the question of how the maize root system adapted its form and function during  
90 domestication and global expansion remains elusive. However, understanding the genetic basis,  
91 environmental drivers and the potential adaptive value of seminal root number variation to changing  
92 environments is essential to develop crops resilient to future climatic challenges.

## 93 Results

### 94 Variation of SRN follows maize domestication

95 We investigated the environmental and genetic factors driving diversity in seminal root number (SRN)  
96 in the genus *Zea*. We quantified SRN in a set of >9,000 *Zea* accessions representing the worldwide  
97 diversity across the major maize cultivating regions in the Americas, Europe, Asia and Africa. Our  
98 collection included 173 wild teosinte accessions, 4,868 traditional varieties and 4,049 modern inbred  
99 lines. Under controlled conditions, maize varieties produced up to 11 seminal roots in traditional varieties  
100 (table S1) and up to 14 seminal roots in modern inbred lines (table S2). Overall, maize varieties formed  
101 on average 3.3 seminal roots, while teosinte accessions (Fig.1B and C) did not produce any seminal  
102 roots in 23% of the accessions ( $n = 173$ ) (fig. S1A; table S3). Interestingly, although SRN was low across  
103 all teosinte accessions, highland teosinte (*Zea mays* ssp. *mexicana*) produced significantly more  
104 seminal roots than the lowland teosinte *parviglumis* (fig. S1B). These data are consistent with the  
105 previously advanced hypothesis that seminal root formation in *Zea* is a domestication trait (Burton et al.  
106 2013; Lopez-Valdivia et al. 2022).

107 Recently, it has been suggested that the increase of seed size during domestication was a prerequisite  
108 for seminal root formation (Perkins and Lynch 2021). In our study, SRN was only weakly correlated with  
109 seed size or the proportion of the embryo to the whole seed area across 2,429 modern inbred lines (fig.  
110 S2) and showed no relationship with embryo volume in a panel of diverse US traditional varieties (fig.  
111 S3). Thus, it is likely that the formation of seminal roots is independent from the process of seed selection  
112 during breeding. However, we cannot rule out the possibility that the other factors from the seed may  
113 have an effect on the SRN. To further investigate the relationship between seed traits and SRN, we  
114 evaluated an additional collection of 663 modern US inbred lines (table S4) and 975 globally distributed  
115 traditional varieties (table S5). We found that sweet corn, flour corn and popcorn varieties characterized  
116 by specific carbohydrate composition formed fewer seminal roots than other varieties in modern inbred  
117 lines (fig. S4A) or traditional varieties (fig. S4B). Analysis of near isogenic lines with mutants that alter  
118 the composition of the endosperm (*sugary1*, *shrunken2*) demonstrated that seminal root formation is  
119 independent of the amount of carbohydrates available during seed development (fig. S5). Thus, we  
120 hypothesize that the increase in SRN was part of domestication during the global expansion of maize,  
121 but was independent of seed traits in maize, which have been strongly modified by human selection and

122 breeding. It should be noted that sowing depth might have a potential effect on the variation of SRN,  
123 because modern varieties are usually planted closer to the soil surface than teosinte.

## 124 **Geographical and genomic signals of variation in seminal root number**

125 To determine whether and how SRN varies with environment, we applied machine learning to  
126 investigate the most important climatic and soil factors associated with SRN across 1,484 georeferenced  
127 traditional varieties sourced from diverse climatic and soil conditions (table S6). Traditional varieties  
128 which originated from arid regions had fewer seminal roots compared to those of other origins (fig. S6A).  
129 Using Random Forest modelling, we found that mean diurnal temperature range (MDR; Pearson's  $r = -$   
130  $0.36$ ,  $p < 0.001$ ), temperature seasonality (Pearson's  $r = -0.29$ ,  $p < 0.001$ ) and precipitation seasonality  
131 (Pearson's  $r = -0.07$ ,  $p = 0.010$ ) were the best environmental predictor of SRN followed by soil organic  
132 carbon (Pearson's  $r = 0.11$ ,  $p < 0.001$ ) and sand content (Pearson's  $r = -0.16$ ,  $p < 0.001$ ) (fig. S6B, C).  
133 High MDR and precipitation seasonality are important meteorological indicators associated with extreme  
134 climate such as deserts. Importantly, we further showed that paleoclimatic levels of precipitation in the  
135 mid-Holocene (ca. 6000 yrs ago) was a significant predictor of SRN (fig. S6D; Pearson's  $r = 0.30$ ,  $p <$   
136  $0.001$ ), highlighting the importance of rainfall level in the evolutionary patterns of maize. To better  
137 understand the relationship between SRN and environment, we combined selected environmental  
138 variables into a second predictive random forest model. Focusing specifically on Mexican maize, we  
139 identified a broad trend of decreasing SRN with increasing latitude (Fig. 2A). We used our trained model  
140 to predict SRN for an additional panel of 1,781 previously genotyped and georeferenced Mexican  
141 varieties (Fig. 2A; Navarro et al. 2017). Using the available genotypes and our predicted SRN values,  
142 we performed a genome wide association study (GWAS; Fig. 2B), identifying genomic loci linked to the  
143 combinations of environmental variables that themselves described SRN variation in our training set.

144 To phenotypically map SRN in Mexican maize, we generated and evaluated an eight-parent Multi-parent  
145 Advanced Generation InterCross (MAGIC) population generated from founders that spanned the  
146 previously observed latitudinal cline in SRN (Fig. 2C). Comparison of the results of predicted trait GWAS  
147 and MAGIC mapping identified several shared genomic regions, including a locus on chromosome 1  
148 linked to the previously described gene *rootless concerning crown and seminal roots* (*rtcs*; Fig. 2B;  
149 Taramino et al. 2007). The MAGIC population partially breaks down the population structure that can  
150 confound studies of local adaptation. On this basis, we used the MAGIC families to generate a genome  
151 wide predictive model for SRN and then applied this model to the eight founder haplotypes. Interestingly,  
152 our model recovered the latitudinal trend in SRN that we have observed in our broader sampling (Fig.  
153 2D). This result was robust to the removal of any single chromosome from the model, indicating that  
154 effects throughout the genome were contributing to the clinal trend, consistent with persistent directional  
155 selection and local adaptation. We examined more closely the region of the genome around *rtcs* by  
156 modelling separate allele effects for each of the eight founders, recovering evidence of an allelic series  
157 with effects ranging from positive to negative following the founder source from South to North (Fig. 2E).  
158 Thus, our ecological and genomic models suggest SRN variation is likely shaped by indirect selection  
159 for adaptation to new environments.

## 160 **Northern Flint drive seminal root differentiation**

161 Previous population genetic analyses have described the expansion of maize out of Northwestern  
162 Mexico and its subsequent adaptation to the dry environment of the Southwestern US (Arizona and New  
163 Mexico) (Merrill et al. 2009; da Fonseca et al. 2015). In our study, accessions sampled from the  
164 Southwestern US had remarkably low SRN (Fig. 3A). In fact, more than 57% (53/92) of Southwestern  
165 US accessions completely lacked seminal roots (Fig. 3B; table S2). Such seminal root defective  
166 phenotypes from the Southwestern US were more drastic than those of the investigated teosinte lines  
167 (fig. S1A; table S1). Interestingly, we observed such low SRN exclusively in the United States, Canada  
168 and some European countries (table S1), which associates with a higher share of Northern Flint, a group  
169 derived from the US Southwest (Doebley et al. 1986; Rebourg et al. 2003; Hu et al. 2021). Using a  
170 maximum-likelihood estimation, we evaluated the effect of Northern Flint germplasm on SRN across our  
171 sampling. We found that the proportion of alleles derived from Northern Flint germplasm negatively  
172 correlated with SRN in both the US (Fig. 3C) and modern European inbred lines (Fig. 3D). SRN was not  
173 significantly correlated with proportions of germplasm derived from Tropical highlands, Tropical lowlands  
174 or Southern dent (fig. S7). We next genotyped 778 geographically diverse US traditional varieties and  
175 confirmed that the proportion of introgressed Northern Flint germplasm correlated negatively with SRN  
176 (Fig. 3E). We also evaluated a collection of introgression lines carrying genomic regions of the typical

177 Northern Flint traditional variety Gaspé Flint (Salvi et al. 2011). The introgression lines with higher share  
178 of the Northern Flint genome formed fewer seminal roots than the other panels evaluated (fig. S8).  
179 Overall, these phenotypic and genetic analyses indicate that alleles derived from Northern Flint  
180 germplasm of Southwestern US origin are an important factor determining SRN during the local  
181 adaptation of maize to different environments.

## 182 **Seminal root variation contributes to root functional traits**

183 To determine the potential adaptive importance of SRN across different environments, we used *in silico*  
184 root models to determine the impact of SRN in the context of whole root system architecture using 218  
185 representative US maize traditional varieties (table S7). We first evaluated root architectural and  
186 morphological traits using a rhizobox system (Osthoff et al. 2019) to parameterize the structural-  
187 functional model CPlantBox (Zhou et al. 2020). The simulations illustrate that SRN negatively correlates  
188 with seedling primary root length and lateral root density along the primary root throughout the whole  
189 root system (Fig. 4A). We found that variation in SRN impacts seedling vigour by modulating the overall  
190 root system conductance, Krs (Fig. 4B). To explore whether changes in SRN will reshape root system  
191 architecture under realistic soil conditions, we used magnetic resonance imaging and positron emission  
192 tomography (MRI-PET) to compare the maize seminal rootless mutant *rtcs* to an isogenic wild type line  
193 that produced an average of three seminal roots (Fig. 4C). In the absence of seminal roots, the *rtcs*  
194 mutant produced an increased number of lateral roots. Water uptake in young maize has previously  
195 been shown to be dominated by lateral roots (Ahmed et al. 2016), suggesting that reducing in SRN to  
196 favour lateral root production may have an adaptive advantage for seed establishment in water limited  
197 conditions. We further characterized a specific Southwestern US traditional variety that we had identified  
198 to produce very few seminal roots but an enhanced number of lateral roots (Fig. 1C and S9). Thus,  
199 variation in SRN might drive the overall dimension and branching of whole root system, which will  
200 potentially determine the plant's capacity to capture water. We next used the CPlantBox realizations for  
201 each of the 218 traditional varieties to determine their standard uptake fraction (SUF) and demonstrated  
202 that the potentially relative contribution of lateral roots to total root water uptake decreases with  
203 increasing SRN (Fig. 4D). Based on these modelling results, variation in SRN might determine the  
204 overall absorptive surface by impacting lateral root formation.

205 We selected 66 representative traditional varieties (table S8) from the panel of 218 and experimentally  
206 measured transpiration rates in wet soil, finding no significant difference between groups (Fig. S10). We  
207 then used a soil-plant hydraulic model and determined that the stress onset limit (i.e., the point at which  
208 a small increase in transpiration provokes a large drop in leaf water potential at a given soil water  
209 potential), occurred at less negative leaf water potential in the traditional varieties with lower SRN (Fig.  
210 4E). Actually, maize traditional varieties with one seminal root require higher water flow rates per unit  
211 root length than traditional varieties with five seminal roots, which induces a local drop in soil water  
212 potential and exhibits an earlier stomatal closure (Abdalla et al. 2022; Cai et al. 2022). This allows  
213 sustaining similar transpiration rates. We propose that such adaptive stomatal behaviour leading to  
214 lower transpiration is beneficial for seedling maize subjected to water stress. In addition, salt-simulated  
215 drought conditions tend to increase the lignin accumulation along the tip of the primary root (fig. S11).  
216 Interestingly, traditional varieties with less seminal roots tend to respond more dramatically [than those](#)  
217 [with more seminal roots](#) especially under water stress condition (fig. S11), which facilitates root  
218 penetration through dry soil (Schneider et al. 2021). Thus, seminal root variation might contribute to the  
219 optimization of root architectural, hydraulic and physiological changes for improved plant tolerance to  
220 limited water availability.

## 221 **ZmHb77 regulates root system architecture and drought resilience**

222 To understand the genetic basis of variation in SRN in inbred maize, we performed GWAS using an  
223 association panel of 1,604 diverse modern inbred lines, which are mainly originating from the US, China  
224 and Europe and cover the maize heterotic groups used in the US and China (Li et al. 2022). We  
225 observed substantial variation in SRN, with values ranging from 0 to 12 with an average of 3 (table S3).  
226 We detected a total of 160 associated SNPs ( $p = 1.0e-05$ ), corresponding to 160 candidate genes  
227 underlying SRN (Fig. 5A, table S9). Among these candidate genes, we identified *rtcs*, which is known  
228 to regulate SRN in maize (Taramino et al. 2007). We next screened for novel mutants of these candidate  
229 genes in the *BonnMu* reverse genetics resource of maize (Marcon et al. 2020), and identified transposon

230 insertions in 5 distinct genes that resulted in reduced SRN (fig. S12; table S10). Among those five genes,  
231 one gene Zm00001d045398 on chromosome 9 was annotated as *Homeobox-transcription factor 77*  
232 (*ZmHb77*; Qiu et al. 2022). To further validate the function of *ZmHb77* in regulating root development,  
233 we generated two independent CRISPR/Cas9 knockout lines (KO#1 and KO#3) (Fig. 5B). Both mutant  
234 alleles KO#1 and KO#3 conditioned a significant reduction in SRN (Fig. 5C-D) coupled with an increase  
235 in lateral root density (Fig. 5E-F), suggesting that this gene plays a role in reshaping seedling root  
236 architecture by regulating SRN and lateral root density. Specifically, the haplotype analysis suggested  
237 that Hap 1 contributes to significant more seminal roots than the other haplotypes (Fig. 5G). We then  
238 carried out a soil cultivation box experiment with mutant and wild type plants under well-watered and  
239 drought conditions followed by re-watering. The mutants showed a significant advantage regarding  
240 growth and photosynthesis rate under both drought and re-watering conditions, although there were no  
241 visible differences under well-watered conditions (Fig. 5H-K). Interestingly, mutants with fewer seminal  
242 roots but more lateral roots were more tolerant to drought and have a higher survival rate than wild type  
243 plants after re-watering, while we observed no differences between mutants and wild type under well-  
244 watered conditions (Fig. 5H, L). These results support the notion that *ZmHb77* controls SRN and that  
245 SRN-dependent root architectural traits, in particular lateral root density improve drought tolerance as  
246 well as the recovery of drought stress.

#### 247 **Natural variation of the *ZmHb77* allele and function**

248 To explore the natural variation of *ZmHb77* in association with root architecture and drought tolerance,  
249 we first aligned our structural-functional model results to georeferenced locations across the US.  
250 Interestingly, root system hydraulic conductance showed a general gradient pattern from the Southwest  
251 dry area to the temperate region of the US (Fig. 6A), suggesting that root hydraulic conductance might  
252 have adapted with water availability. We then extended our drought analysis to the different traditional  
253 varieties and verified that Northern Flint varieties ( $n = 5$ ) with less seminal roots contribute to drought  
254 tolerance and resilience (fig. S13A) and showed a significantly higher biomass (fig. S13B) and stomatal  
255 conductance (fig. S13C) after re-watering. We next performed the haplotype analysis for *ZmHb77* allele  
256 in the traditional varieties and identified 41 high-confidence haplotypes (C allele) and the same number  
257 of A allele haplotypes (table S11). In particular, C allele haplotypes displayed significantly less seminal  
258 roots but significantly higher drought tolerance than the A allele haplotypes (Fig. 6B).

259 To further identify potential isogenic lines carrying the *ZmHb77* allele and drought tolerance based on  
260 Northern Flint sourced varieties, we evaluated the SRN, lateral root density and dry biomass under well-  
261 watered and drought conditions for the whole Gaspé Flint introgression library introgressed into B73  
262 (Salvi et al. 2011, 2021). We first demonstrated that GF111 (inbred line developed by repeated selfing  
263 and selected from Gaspé Flint) had a great advantage with respect to drought tolerance and resilience  
264 in comparison to the inbred B73 (Fig. 6C). Next, we explored the whole introgression population ( $n = 71$ )  
265 and identified that the lines with a higher share of the GF111 genome showed significantly ( $R^2 = 0.12$ ,  
266  $P = 0.0015$ ) less seminal roots, but significantly ( $R^2 = 0.38$ ,  $P = 7.3e-09$ ) higher lateral root density (Fig.  
267 6D). At the same time these genotypes provided drought tolerance as measured by the drought index  
268 of the dry biomass. Specifically, we identified four introgression lines (GF111<sup>ZmHb77</sup>) with *ZmHb77* alleles  
269 from GF111 and another four lines (B73<sup>ZmHb77</sup>) from B73, respectively. The GF111<sup>ZmHb77</sup> lines formed  
270 less seminal roots but a significantly higher lateral root density than the B73<sup>ZmHb77</sup> lines (Fig. 6D). We  
271 then performed an RNA sequencing experiment to explore the gene expression pattern in the embryo  
272 and root stele tissue. Interestingly, *ZmHb77* is in general lowly expressed in the embryo tissue but highly  
273 expressed in the root stele, where the lateral roots initiated (Fig. 6E), suggesting that the major function  
274 of *ZmHb77* is linked with lateral root formation. Based on the specific expression pattern of *ZmHb77*  
275 between the GF111<sup>ZmHb77</sup> and B73<sup>ZmHb77</sup> lines, *ZmHb77* might function in the promotion of seminal root  
276 formation but inhibition of lateral root density in maize seedlings (Fig. 6E). In particular, GF111<sup>ZmHb77</sup>  
277 lines displayed a strong drought tolerance as highlighted by a higher photosynthetic rate and stomal  
278 conductance (Fig. 6F, fig. S14). Indeed, less inhibition of *ZmHb77* on lateral root formation was  
279 demonstrated in the GF111<sup>ZmHb77</sup> lines under drought followed by re-watering (Fig. 6G). Interestingly,  
280 drought tolerance in maize driven by root architectural changes can be independently validated by the  
281 *rtcs* mutant and its wild type (fig. S15). Finally, we summarized our finding as a schematic model in  
282 which *ZmHb77* acts as a central modulator contributing to the promotion of seminal root formation but  
283 inhibition of lateral root density in maize seedlings. Such root architectural and functional plasticity  
284 provides maize seedlings a great potential to balance the external water constraints.

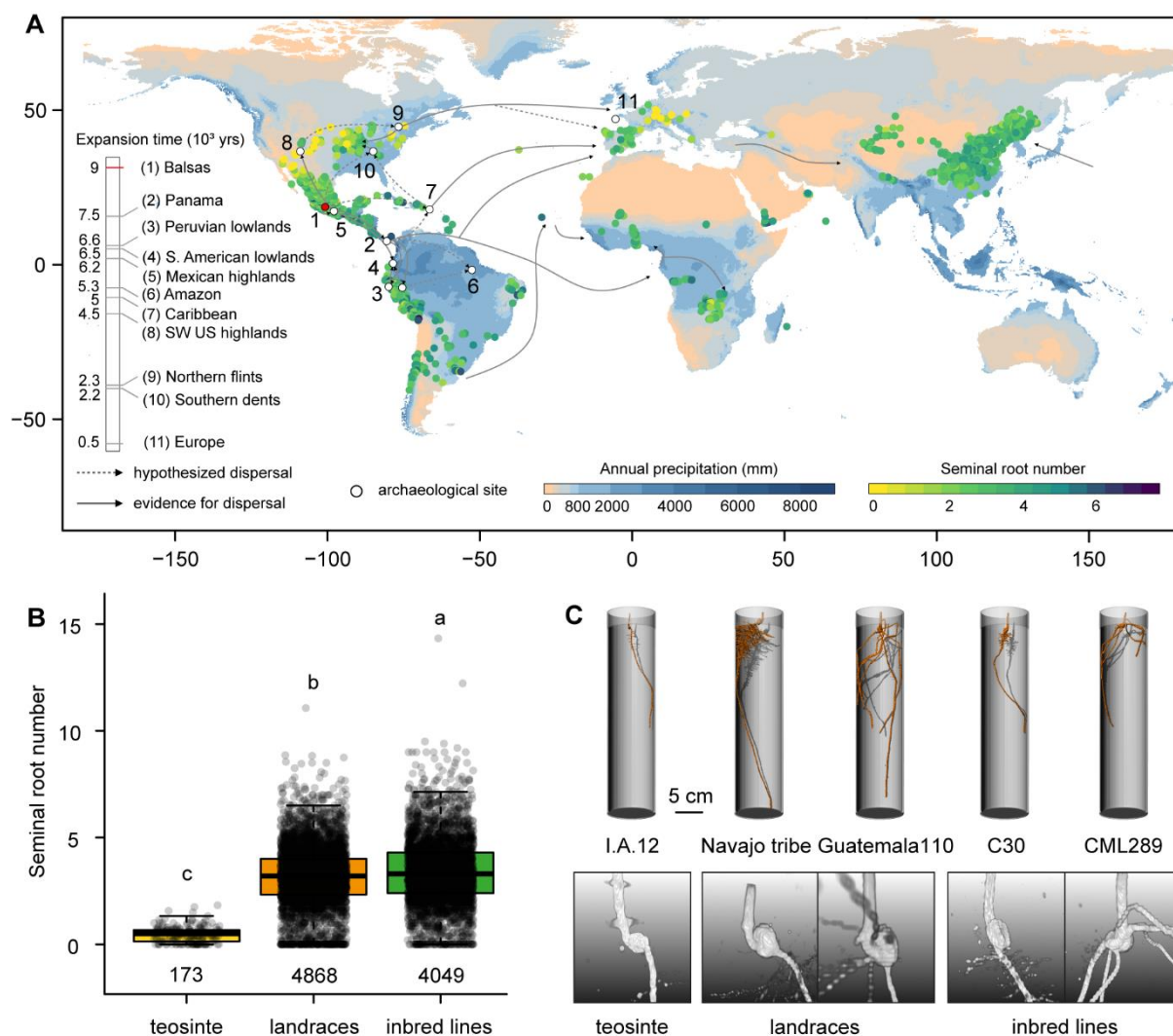
285

## 286 Discussion

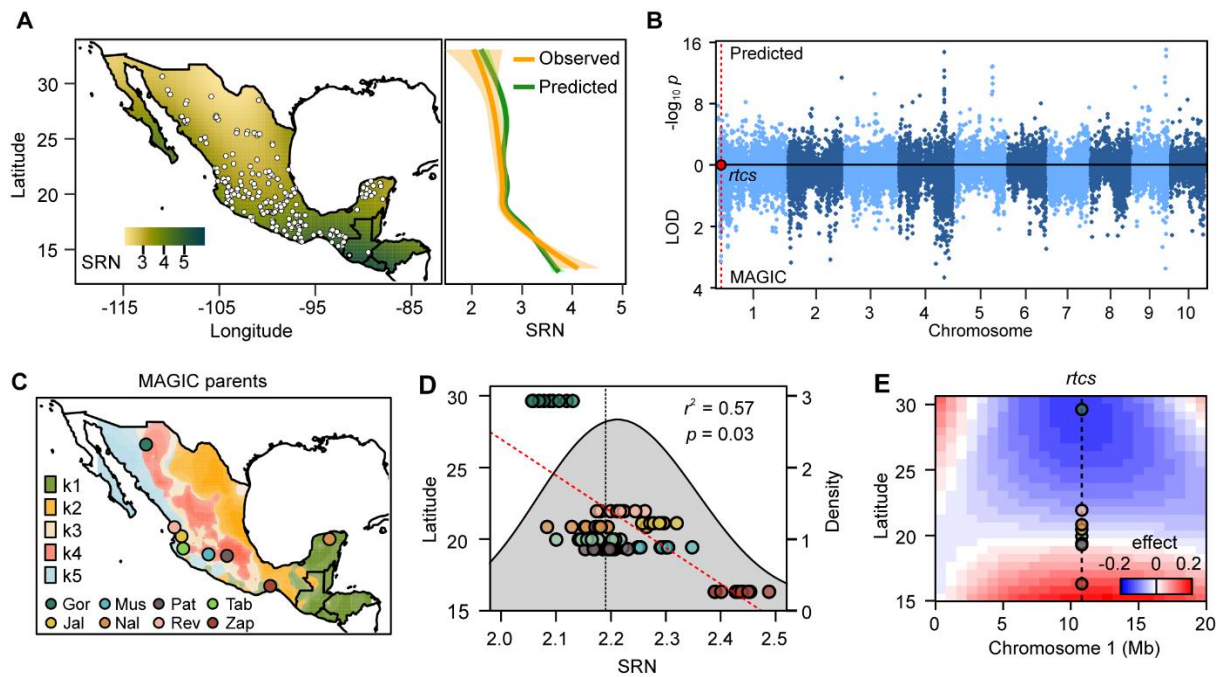
287 Plant root system architecture plays a critical role in the adaptation to environmental constraints (Giehl  
288 and von Wirén 2014; Ma et al. 2018). To date, little is known on how the formation and function of root  
289 systems evolved in space and time during domestication of agricultural crops. Nonetheless, it has  
290 remained unclear to what extent root trait adaptation was required to introduce maize to new  
291 environments and what role root traits played in maize domestication. Using the global diversity of the  
292 genus *Zea*, our study demonstrates that SRN varies between domesticated maize traditional varieties  
293 and modern inbred lines compared with their wild teosinte progenitors and suggests that variation of  
294 SRN might have played an overriding role during the process of maize domestication (Fig. 1; Lopez-  
295 Valdivia et al. 2022). In traditional maize varieties, the demographically distinct groups sweet corn, flour  
296 corn and popcorn sourced from Southwestern US have shown the fewest SRN (fig. S4). Independent  
297 lines of evidence indicate that adapted alleles derived from Northern Flint maize contribute to the  
298 variation of SRN in both modern inbred lines and traditional varieties (Fig. 3). Subsequent local  
299 adaptation of SRN is in line with the maize domestication history, in which Northern Flint originated from  
300 the Southwest US desert (Merrill et al. 2009; da Fonseca et al. 2015), and then expanded to the northern  
301 US and Europe (Tenailon and Charcosset 2011). We further applied ecological and genomic models  
302 and found a clinal trend in SRN across latitude and climatic factors (Fig. 2A; fig. S9). Recently, such an  
303 adaptive signature has been reported in the geographical adaptation of rice to local soil nitrogen  
304 availability (Liu et al. 2021). Here, we provide evidence for *rtcs*, a known determinant of SRN, to  
305 associate with variation in SRN along geographical gradients (Fig. 2E), emphasizing the importance of  
306 landscape and environmental factors in driving root trait differentiation.

307 In the near future, climate change will increase the incidence of drought, imposing a major threat to crop  
308 production (Jägermeyr et al. 2021). Improved adaptive capacity to flash drought is required for crops to  
309 mitigate such negative impacts in agricultural systems (Yuan et al. 2023). To tolerate stress and optimize  
310 the uptake of water even with a transient drought period, crops need to adapt root properties. We  
311 detected enhanced lateral root branching in both traditional varieties (Fig. 1C) and the *rtcs* mutants when  
312 seminal roots were absent (Fig. 4C), as well as recovering a similar result through *in silico* modelling  
313 (Fig. 4A). At the seedling stage, traditional varieties with fewer seminal roots can substantially reduce  
314 the carbon cost for the seed, and thus enable formation of highly dense and long lateral roots along the  
315 primary root (Fig. 4). Interestingly, we detected a significantly higher accumulation of lignin in the primary  
316 root tip of traditional varieties with few or no seminal roots under osmotic stress conditions (fig. S14).  
317 Such adaptive behaviour with enhanced lateral root branching in contact to water (Ahmed et al. 2016)  
318 and primary root lignification for better penetration of hard and dry soil (Schneider et al. 2021) improves  
319 plant tolerance to limited water availability, especially for the survival of seedlings after severe drought  
320 (Wang et al. 2016). In this context, we identified the transcription factor *ZmHb77* that affects overall root  
321 architecture by suppressing SRN but increasing lateral root density (Fig. 5C-F). Deletion of *ZmHb77*  
322 ultimately enhances survival of plants after recovery from drought (Fig. 5J, L). Indeed, domesticated  
323 wheat and barley have also been reported to form a larger number of seminal roots than their wild  
324 relatives (Grando and Ceccarelli, 1995; Golan et al. 2018). Based on the global warming scenario and  
325 an increasing incidence of drought, it is necessary to consider reducing the number of seminal roots in  
326 favour of lateral root branching for more efficient acquisition of soil water in the modern cultivars. It is  
327 important to note that such architectural plasticity will have its major impact during the seedling stage  
328 before crown roots become established (Hochholdinger et al. 2018) and sustain water uptake at later  
329 developmental stages. Our systemic analyses indicate that SRN is an important driver for the formation  
330 and pattern of lateral roots along the primary root (Fig. 6), thereby determining the overall absorptive  
331 surface and foraging capacity of crop roots. Variation in SRN alters hydraulic properties and may bear  
332 genetic potential to modify root plasticity and deepen our understanding of how plant roots sense and  
333 adapt to fluctuating water availability by hydropatterning (Orosa-Puente et al. 2018) or xerobranching  
334 (Mehra et al. 2022). Future studies need to address how SRN variation can optimize root development  
335 and hydraulic architecture for enhanced resilience in cereals (Maurel and Nacry 2015). Our results do  
336 not only reveal the past signature of domestication and adaptation of maize roots, but highlight the  
337 genetic potential to improve climate resilience in future crops.

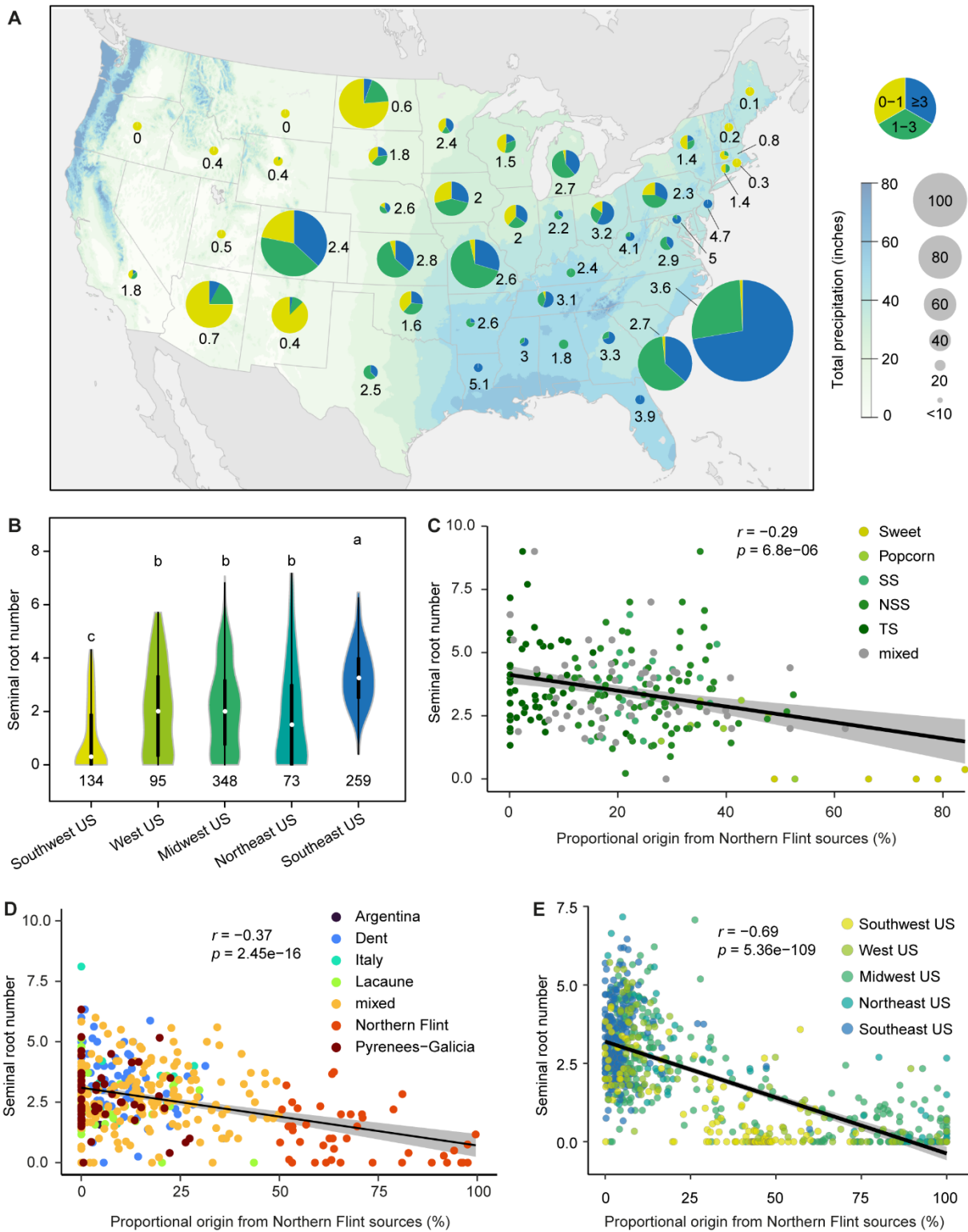




339  
 340 **Main figure 1. Maize evolutionary history resolves global organization of seminal root number**  
 341 **(SRN).** **A**, Geographical variability of SRN in maize traditional varieties ( $n = 2424$ ). SRN was determined  
 342 in globally collected traditional varieties of indicated geographical origin. Domestication and expansion  
 343 times for maize populations are indicated according to Ross-Ibarra and Piperno 2020. The global map  
 344 of average annual precipitation between 1991 and 2020 is derived from NOAA Climate.gov. Dot colours  
 345 from yellow to blue correspond to increasing SRNs. Solid arrows indicate the current evidence for global  
 346 maize dispersal. Dashed arrows indicate hypothesized dispersal. White dots indicate locations of  
 347 archaeological sites. **B**, Seminal root differentiation across the genus *Zea* including teosinte, traditional  
 348 varieties and modern inbred lines. Each dot indicates the average SRN of each investigated accession.  
 349 Number of analyzed seedlings per genotype: modern inbred lines  $n = 10$ ; traditional varieties  $n = 20$ .  
 350 Boxes span from the first to the third quartile, lines represent the median and whiskers include data  
 351 within the  $1.5\times$  interquartile range of the lower and upper quartiles. Data points outside of whiskers  
 352 represent outliers. Significant differences among groups are indicated by different letters (ANOVA,  
 353 Tukey's HSD,  $p = 0.001$ ). **C**, Reconstruction of root system architecture and initiation sites of seminal  
 354 roots by non-invasive magnetic resonance imaging (MRI) in natural soil. Teosinte: I.A.12 (Ames 21793);  
 355 Traditional varieties: Navajo tribe (PI 311229); Guatemala 110 (PI 490825); Modern inbred lines: C30  
 356 (Ames 26815); CML289 (Ames 32336).



357  
 358 **Main figure 2. Geographical and genomic signatures of SRN variation in Mexico.** **A**, SRN  
 359 decreases along a latitudinal gradient from South to North in Mexico. White dots indicate locations of  
 360 sampled native maize traditional varieties. Green saturation indicates increasing predicted SRN based  
 361 on a Random Forest model. The marginal plot depicts the calculated means of observed and predicted  
 362 SRN over latitude. **B**, Genomic loci of SRN variation. Miami plot shows GWA of SNPs predicted trait  
 363 values above the x-axis and with measured traits from a MAGIC population below the axis. Alternating  
 364 colours indicate the ten maize chromosomes. The *rtcs* gene was labelled accordingly. **C**, Source of the  
 365 eight founders of the MAGIC population. Shading on the map corresponds to an ancestry coefficient ( $K$   
 366 = 5) based on a broader genotyped panel. LOD, logarithm of odds. MAGIC founder traditional varieties  
 367 abbreviations: Gor, Gordo; Mus, Mushito; Pat, Palomero Toluqueno; Tab, Tabloniclo; Jal, Jala; Nal, Nal  
 368 Tel; Rev, Reventador; Zap, Zap Chico. **D**, Genome-wide effects in the MAGIC population support a  
 369 latitudinal dependency of SRN. Dots show predicted SRN for each of the eight founder parents based  
 370 on a genome wide model generated from the derived MAGIC families. Multiple points for each founder  
 371 indicate the result of dropping each chromosome in turn from the model. The trend line and correlation  
 372 are based on the complete model using all ten chromosomes. The grey curve shows the frequency  
 373 density of the whole population with the vertical line at the mean. **E**, MAGIC founder allele effects in a  
 374 20 Mb window around *rtcs*. Polynomial fit of marker effects against source latitude for the eight alleles  
 375 segregating in the MAGIC population. The vertical dashed line indicates the position of *rtcs*.



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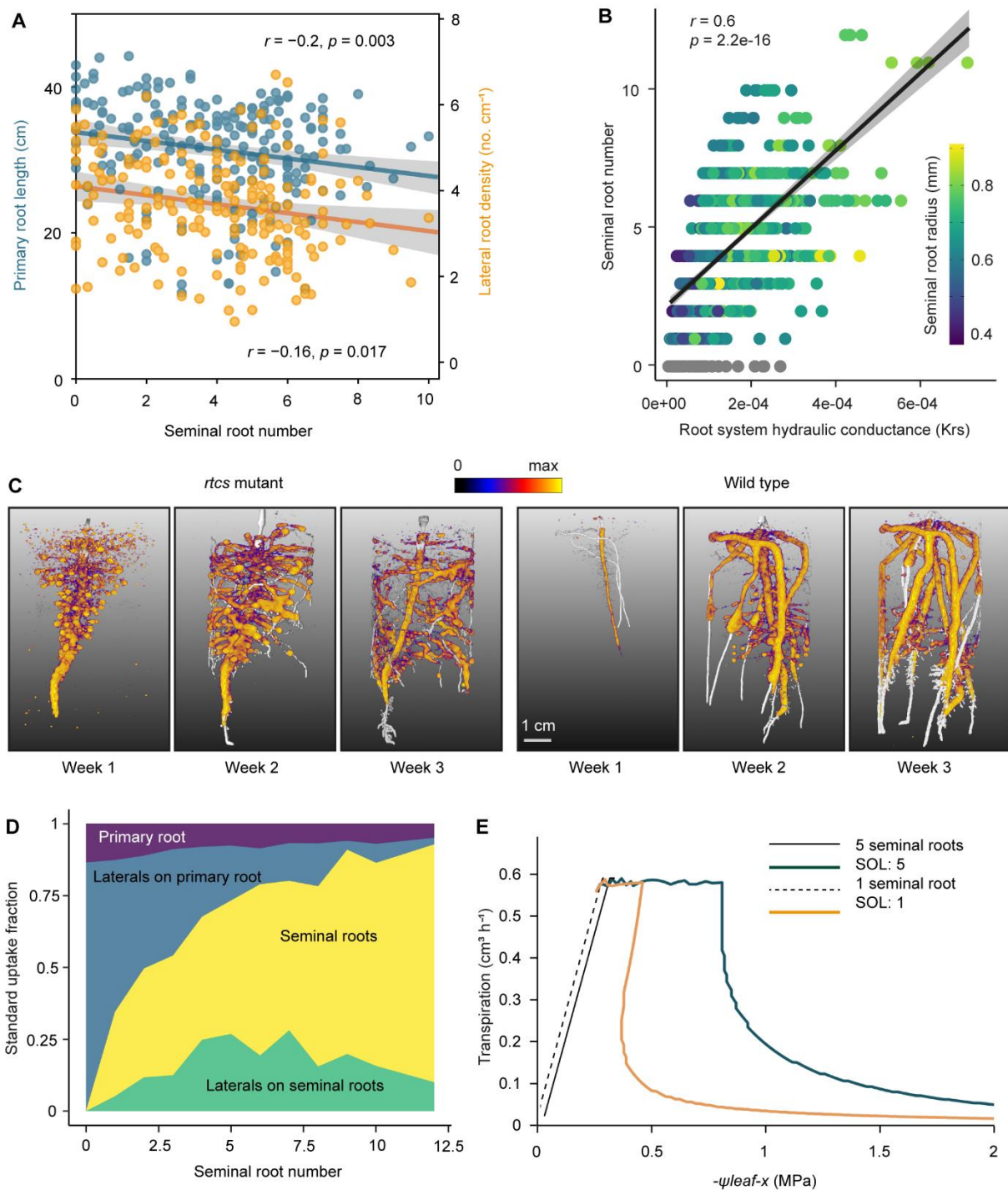
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**Main figure 3. Variation in seminal root number coincides with proportional origin from Northern Flint maize sources.** **A**, Patterns of water availability and seminal root differentiation across the US. The annual average precipitation (1991-2020) map is derived from NOAA Climate.gov. The size of the pie charts indicates the number of sampled traditional varieties accessions while coloured areas denote proportions of SRN classes. **B**, Violin plots show SRN variation in traditional varieties originating from different geographical regions of the US. The traditional varieties accessions were contributed by NCRPIS and CIMMYT. The geographical information of groups of traditional varieties derives from the narrative information of the US National Plant Germplasm System (<https://npgsweb.ars-grin.gov/gringlobal>). Sample sizes are highlighted with exact numbers. Different letters indicate significant differences among regional pools (ANOVA, Tukey's HSD,  $p = 0.001$ ). Boxes span from the

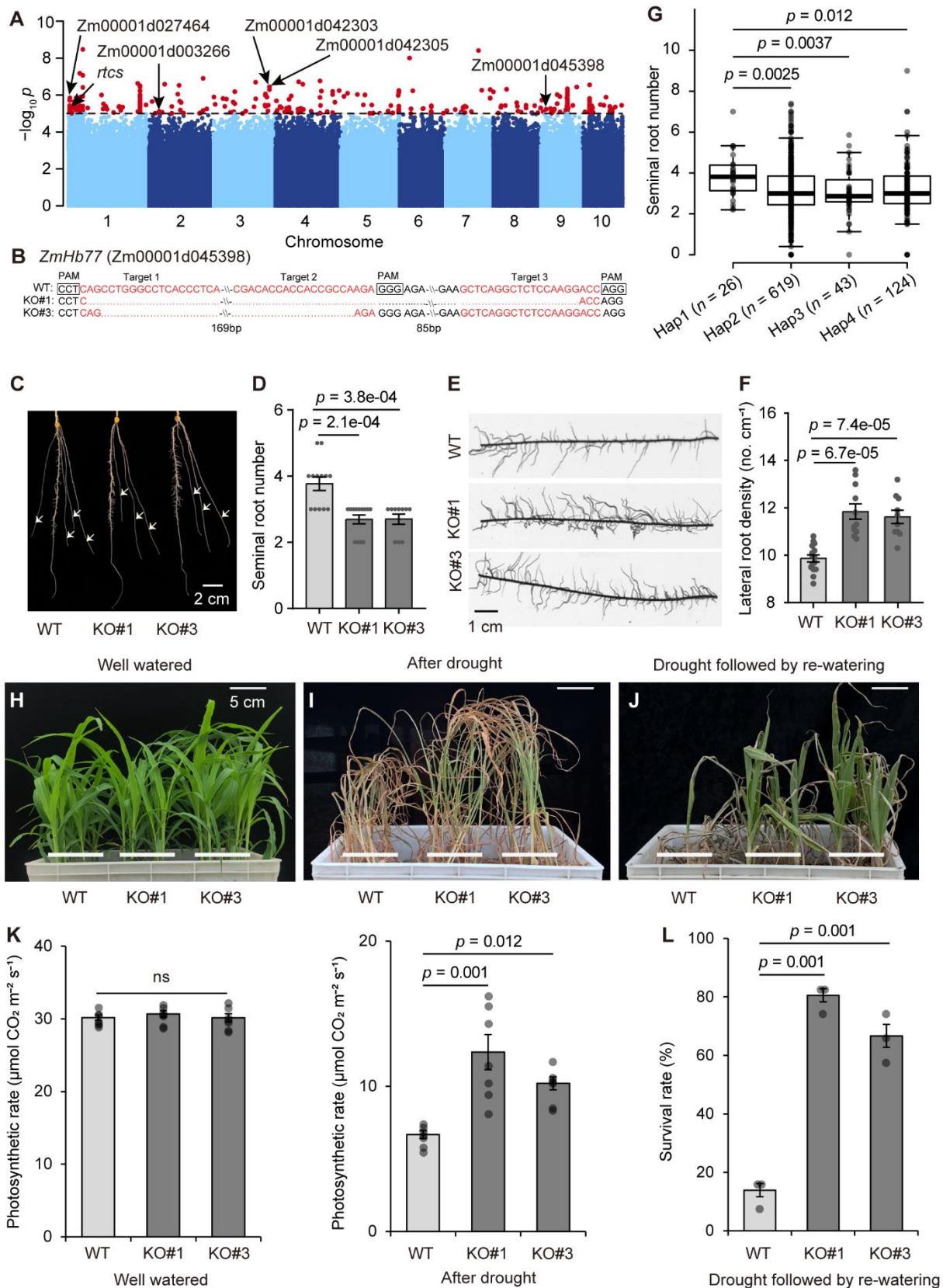
387 first to the third quartiles, centre lines represent median values and whiskers extend 1.5× the interquartile  
388 range of the lower and upper quartiles. Significant differences among different groups are indicated by  
389 different letters (ANOVA, Tukey's HSD,  $p = 0.001$ ). **C-D**, Correlation between SRN and the proportion  
390 of Northern Flint sources in the US Ames panel (**C**) and the European collection (**D**). Estimates of  
391 historical sources for individual Ames modern inbred lines and modern European inbred lines are  
392 extracted from Liu et al. (2003) and Gouesnard et al. (2017). Here, the proportion of Northern Flint  
393 sources was correlated with SRN across modern maize inbred lines. The  $p$  value denotes the probability  
394 at which the correlation coefficient is zero (null hypothesis). SS, stiff-stalk; NSS, non-stiff stalk; TS,  
395 tropical/sub-tropical; Mixed, mixture of these different germplasms. **E**, Correlation between SRN and the  
396 proportion of Northern Flint germplasm sources in US traditional varieties. The reference Northern Flint-  
397 sourced traditional varieties were defined according to Doebley et al. (1986). Scatter plots show  
398 combined SRN data of traditional varieties from different geographical origins with best fit (solid line)  
399 and 95% confidence interval (grey shading) for linear regression ( $p = 5.4e-109$ ,  $n = 778$ ). Different  
400 colours of dots correspond to different geographical origin of investigated traditional varieties.



401

402 **Main figure 4. Variation in SRN drives overall root architectural and hydraulic properties.** A, SRN  
 403 is negatively correlated with rooting depth of the primary root and lateral root density in different maize  
 404 traditional varieties accessions grown in a rhizobox system. Scatter plots show combined seminal root  
 405 data of traditional varieties grown in the rhizobox and linear regression with best fit (solid line) and 95%  
 406 confidence interval (shaded area) ( $n = 218$ ). B, Seminal root variation affects overall root hydraulic  
 407 properties. Root system conductance (Krs) is based on 2D images of root systems grown in the rhizobox  
 408 and simulated root architecture by structural-functional modelling. C, Seminal root defects of the *rtcs*  
 409 mutant cause highly branched lateral roots emerging from the primary root. Reconstruction of root  
 410 architecture and carbon allocation by MRI combined with PET. Intensity of carbon deposition by  
 411 radiolabelled  $^{11}\text{C}$  is visualized by colour code. Note that when  $^{11}\text{C}$  was supplied to leaves for the first  
 412 time, the first two seminal roots were already formed. As MRI images were taken after the PET images,

413 growing root tips are not at the same position. **D**, Standard uptake fraction of seminal roots and lateral  
414 roots as a function of SRN. For each SRN the average proportion of water uptake per root type is  
415 expressed as a ratio relative to overall water uptake. The relative contribution to water uptake is  
416 considered separately for the primary root, lateral roots initiated from the primary root, total seminal  
417 roots and lateral roots initiated from seminal roots. Note that some of the traditional varieties with lower  
418 SRN already formed very short crown roots, but their contribution to water uptake is not considered. **E**,  
419 Simulation of transpiration rates of representative traditional varieties ( $n = 76$ ) from a subset of 218  
420 traditional varieties. A maize traditional variety with one seminal root requires larger gradients in soil  
421 water potential than a traditional variety with five seminal roots to sustain the same transpiration rate.  
422 Hence stress onset limit (SOL) occurs at a lower negative leaf water potential for plants with lower SRN.



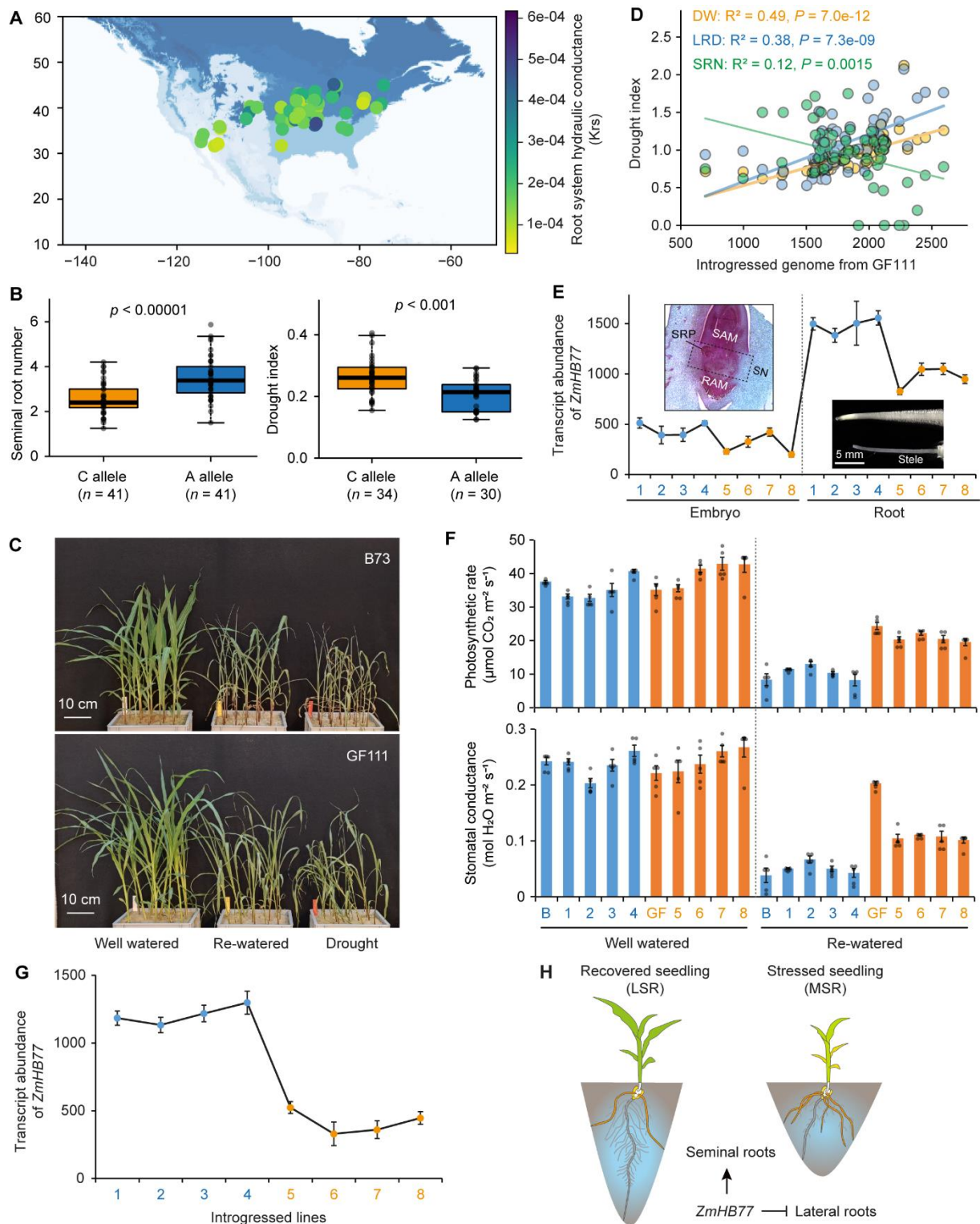
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424 **Main figure 5. Functional characterization of *ZmHb77* controlling root traits and drought**  
 425 **tolerance. A**, Manhattan plot from GWA mapping of SRN in 1,604 diverse modern inbred lines. The  
 426 dashed horizontal line represents the suggestive threshold ( $p = 1.0e-05$ ). The known gene *RTCS* and  
 427 five novel candidate genes controlling SRN are indicated by arrows. **B**, Sequence of *ZmHb77* and the  
 428 target sites of mutation by CRISPAR/Cas9. PAM, protospacer-adjacent motif. CRISPR-knockout (KO#1  
 429 and KO#3) plants of *ZmHb77* display lower SRN (**C, D**) but higher lateral root density than the wild type

430 (WT) (**E, F**). Root phenotyping was performed for two-week-old maize plants grown in the paper rolls.  
431 SRN was counted and lateral root density was obtained from the number of lateral roots per cm of  
432 primary root. At least ten individual plants were examined for root traits. **G**, Haplotype analysis of maize  
433 inbred lines. Comparison of drought tolerance between WT and plants of the two *ZmHb77* CRISPR  
434 knockout lines grown under well-watered (**H**), drought (**I**) and drought followed by re-watering (**J**). **K**,  
435 Photosynthetic rate of mutants and wild type under well-watered and drought conditions. (**L**) Survival  
436 rate of WT and *ZmHb77* knockout lines after exposure to drought stress. Wild type and mutant seeds  
437 were precultured under well-watered conditions until three-leaf stage, and then either adequately  
438 supplied with water or not watered for another twelve days. Drought-stressed plants were re-watered  
439 and the survival rate was recorded after seven days. Three biological replicates were performed and  
440 each replicate included twelve individual plants. Significant differences between WT and KO lines are  
441 indicated by indicated *p* values (one-sided Student's *t*-test). ns, not significant.

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**Main figure 6. Natural variation of the *ZmHb77* allele and its contribution to root architecture and drought tolerance in maize seedlings.** **A**, Geographical distribution of root system hydraulic conductance. Each data point corresponds to the structural-functional model outcome. **B**, Haplotype analysis for traditional maize varieties. Boxes span from the first to the third quartile, lines represent the median and whiskers include data within the 1.5 $\times$  interquartile range of the lower and upper quartiles. Data points outside of whiskers represent outliers. **C**, Seedling performance of B73 and GF111 (inbred line developed by repeated selfing and selected from Gaspé Flint) grown under well-watered, drought and drought followed by re-watering. **D**, Correlation between drought index and the proportion of introgressed genome from GF111. SRN, seminal root number; LRD, lateral root density; DW, dry weight. **E**, Tissue specific expression of *ZmHb77* in the embryo and root stele between different introgressed

454 lines from B73 (1–4) and GF111 (5–8) donors. SAM, shoot apical meristem; RAM, root apical meristem;  
455 SN, scutellar node; SRP, seminal root primordia. **F**, Photosynthetic rate and stomatal conductance of  
456 different introgression lines from B73 and GF111 donors under well-watered and re-watered conditions,  
457 respectively. **G**, Expression of *ZmHb77* in the root stele tissue after re-watering among different  
458 introgression lines. **H**, Working model of a potential function of *ZmHb77* on the formation of seminal  
459 roots and lateral roots in contribution to maize seedling drought tolerance. MSR, more seminal roots;  
460 LSR, less seminal roots.

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506 **Acknowledgements**

507 We thank Jeffrey Ross-Ibarra (University of California–Davis, USA) for reading and comments on the  
508 final version of the manuscript. We are grateful to the North Central Regional Plant Introduction Station  
509 (NCRPIS, USDA-ARS and Iowa State University, Ames, Iowa, USA) and the International Maize and  
510 Wheat Improvement Center (CIMMYT) for providing seeds for this work. NCRPIS is part of the USDA-  
511 ARS National Plant Germplasm System. We thank Alain Charcosset (GQE-Le Moulon, INRAE, Univ.  
512 Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Gif-sur-Yvette, France) and Manuel López  
513 Luaces (Centro Investigaci3n Agrarias Mabegondo, Spain) for contributing European maize  
514 germplasm. We thank Simon Mayer and Steffen Wagner (Leibniz Institute of Plant Genetics and Crop  
515 Plant Research, Gatersleben, Germany) for technical support for NMR and Stefan Ortleb, Laura Kalms,  
516 Philipp Hinrichs (Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany)  
517 for image segmentation. R.K., D.v.D, R.M. and D.P. acknowledge support from the Helmholtz  
518 Association for the Forschungszentrum J3lich GmbH and thank Antonia Chlubek and Jonas B3hler for  
519 technical support by MRI and PET. We thank Antonia Chlubek, Gregor Huber and Jonas B3hler  
520 (Forschungszentrum Juelich GmbH, IBG-2) for technical support by MRI and PET. We thank Selina  
521 Siemens, Alexa Brox and Helmut Rehkopf (Crop Functional Genomics, INRES, University of Bonn,  
522 Germany) for root phenotyping and DNA extraction. This work is supported by the Deutsche  
523 Forschungsgemeinschaft (DFG) grants HO2249/22-1 to F.H. and YU272/1-1 to P.Y., 444755415  
524 (Emmy Noether Programme) to P.Y., 403671039 (SPP2089 “Rhizosphere spatiotemporal organisation  
525 - a key to rhizosphere functions”) to F.H. and P.Y., 403641034 (SPP2089 “Rhizosphere spatiotemporal  
526 organisation—a key to rhizosphere functions”) to A.S., and Bundesministerium für Bildung und  
527 Forschung (BMBF) grant 031B195C to F.H. This work was partially funded by the Deutsche  
528 Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy-  
529 EXC 2070 – 390732324. R.J.H.S. is supported by the USDA National Institute of Food and Agriculture  
530 and Hatch Appropriations under Project number PEN04734 and Accession number 1021929, Consejo  
531 Nacional de Ciencia Tecnologia (FOINS-2016-01) and National Science Foundation (No. 1546719).  
532 T.W. is supported by the National Key Research and Development Program of China (No.  
533 2021YFD1200700, No. 2016 YFD0100103, No. 2020YFE0202300) and Innovation Program of Chinese  
534 Academy of Agricultural Sciences). The germplasm propagation is funded by the TRA Sustainable  
535 Futures (University of Bonn) as part of the Excellence Strategy of the federal and state governments.  
536 X.C. is supported by the Innovation Research 2035 Pilot Plan of Southwest University (No. SWU-  
537 XDZD22001). This work was also supported in part by the U.S. Department of Agriculture, Agricultural  
538 Research Service (USDA-ARS) to V.B.

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546 I.P., M.F.R.R.J., S.S., A.A., M.A., M.A.A., F.M.B., A.S., G.L., A.H., K.S., L.S., C.M.M.

547 Funding acquisition: P.Y., F.H., X.C., T.W., R.J.H.S and A.S.

548 Writing – original draft: P.Y. and F.H.

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550 **Competing interests:**

551 The authors declare no competing interests.