Bracing for sustainable agriculture: the development and function of brace roots in members of *Poaceae*

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Highlights

- Aerial nodal roots (brace roots) are important in plant anchorage and consequently lodging resistance
- In a meta-analysis, more than half of the genes known to influence brace roots in maize are also characterized to alter the juvenile to adult phase change
- Agriculturally important C4 crops such as sorghum, sugarcane, and setaria develop aerial nodal roots, however, the anatomical descriptions remain limited

Abstract

Optimization of crop production requires root systems to function in water uptake, nutrient use, and anchorage. In maize, there are two types of post-embryonic roots – subterranean nodal roots (crown roots) and aerial nodal roots (brace roots). Recent research in maize demonstrates that aerial nodal roots play an important role in crop anchorage. However, most studies have been unsuccessful at teasing apart the relationship between phase change, flowering time, and brace root development. Though less is known about function, other members of the Poaceae have documented aerial nodal roots including sorghum, sugarcane, setaria. To meet the demands of a growing population, the development and function of aerial nodal roots in crops must be better characterized. Here, we summarize recent research demonstrating 1) the importance aerial nodal roots in maize anchorage, 2) the fundamental association between phase change, flowering time, and brace roots in maize, and 3) the limited knowledge of brace root development and function in other members of Poaceae. This work highlights the critical knowledge gap of aerial nodal root development and function and suggests new focus areas for breeding resilient crops.

Importance of Aerial Roots for Agriculture

Plant adaptation to changing environments requires the dynamic response of root systems to optimize water uptake, nutrient use, and anchorage under variable environments (Lynch, 1995; Aiken and Smucker, 1996; Hochholdinger, Park, et al., 2004; Hodge et al., 2009). Root systems are often thought of as the subterranean part of the plant, but recent work highlights the importance of aerial roots in plant adaptation. Exploring the development and function of aerial roots can fill a critical knowledge gap in root system optimization and identify new targets for breeding resilient crops.

One important function of aerial roots is anchorage, which contributes to lodging resistance. Lodging, defined by the vertical displacement of stalks, can be a result of stem (stalk lodging) and/or root (root lodging) failure (Berry et al., 2004; Rajkumara, 2008; Fedenko et al., 2015; Brune et al., 2018). This failure can be exacerbated by various factors, including groundwater accumulation, wind, drought, soil type, topographical variation, management practices, and plant growth stage (Pinthus, 1974; Sterling et al., 2003; Rajkumara, 2008). In large plants such as maize, stalk lodging typically results from internode buckling, whereas root lodging occurs when roots break or pull out of the soil (Erndwein et al., 2019). Efforts to understand root lodging resistance in maize have suggested that the aerial roots play an important role in anchorage (Liu
et al., 2012; Sharma and Carena, 2016; Shi et al., 2019) and this has now been demonstrated directly (Reneau et al., 2020).

Although poorly understood in general, crop aerial roots are best defined in maize. The maize root system consists of different root types including the subterranean embryonic primary and seminal roots, as well as postembryonic subterranean and aerial nodal roots (Feldman, 1994; Hochholdinger, Park, et al., 2004; Hochholdinger, Woll, et al., 2004; Hochholdinger, 2009; Blizard and Sparks, 2020) (Figure 1). Early in development, the primary and seminal roots provide nutritional and structural support, however once maize plants reach the vegetative leaf 6 (V6) stage of development, nodal roots become the essential root type (Hochholdinger, Park, et al., 2004). Nodal roots, also referred to as adventitious roots, develop from shoot nodes rather than other root tissues. Two classifications of nodal roots exist in maize – subterranean nodal roots, referred to as crown roots, and aerial nodal roots, referred to as brace roots (Feldman, 1994; Hochholdinger, Park, et al., 2004; Hochholdinger, 2009; Blizard and Sparks, 2020). Nodal roots develop sequentially beginning at the coleoptile node and continue from each consecutive node for seven or eight nodes. Typically, five of the nodes become crown roots and the remainder initiate above the soil line as brace root whorls (Hoppe et al., 1986).

The term brace root was coined because these aerial roots were historically observed to prop (or ‘brace’) the plant in the event of lodging. This idea has been supported by recent studies that identify a correlation between brace root traits and root lodging-resistance (Liu et al., 2012; Sharma and Carena, 2016; Shi et al., 2019). In addition to these correlations, a direct test confirms the importance of brace roots for plant anchorage (Reneau et al., 2020). Several of these studies have identified the number of brace root whorls that hit the soil as an important trait for anchorage (Sharma and Carena, 2016; Shi et al., 2019; Reneau et al., 2020). Since brace root whorls form sequentially, the timing and regulation of development is likely an important regulator of brace root traits and consequently function.

Phase Change, Flowering Time, and Brace Roots in Maize

A major transition in plant development that alters vegetative growth is the phase change from juvenile to adult. Brace roots have been classified as a juvenile trait because prolonging the juvenile phase results in more brace roots (Evans and Poethig, 1995). In maize, the phase change from juvenile to adult phase is influenced by the interplay of microRNAs (miR) and flowering time regulators. Previous work on the genetic mechanisms of brace root development has linked the number of nodes containing brace roots to flowering time (Zhang et al., 2018). Brace root developmental signaling mechanisms have been characterized through screening mutants for root defects. The maize *rootless concerning crown and seminal roots* (*rtcs*) mutant does not initiate seminal, crown, or brace roots, which demonstrates a shared signaling mechanism for root development (Hetz et al., 1996). The expression of RTCS is induced by auxin (Taramino et al., 2007), a plant hormone which is essential for initiation of stem-born roots (Tyburons and Tretyan, 2004; Xu et al., 2005; Morita and Kyozuka, 2007; Kitomi et al., 2008, 2011; Liu et al., 2009; Negi et al., 2010; Vidoz et al., 2010; Lavenus et al., 2013; Pacurar et al.,
In contrast to **rtcs**, the basic helix-loop-helix (bHLH) transcription factor mutant **rootless1 (rt1)** affects brace root development more than crown root development (Jenkins, 1930; Sakai and Taramino, 2009).

Beyond these two mutants, brace root developmental defects have also been identified as secondary phenotypes in mutant analyses of phase change and flowering time. We have curated a list of genes with mutant phenotypes known to influence brace roots in the maize genetics literature, the Identified Annotated Modulators of Brace Roots (IAMBROOT; **Table 1** and **Supplemental Table 1**). Out of the 20 IAMBROOT genes, 14 are also characterized to alter the juvenile to adult phase change. For example, overexpression of CONSTANS CONSTANS-LIKE TIMING OF CAB1 protein domain1 (ZMCCT1), a major photoperiod response regulator (Stephenson et al., 2019), or overexpression of miR156b (Corngrass1; Chuck et al., 2007) delays phase change and promotes brace root formation. In contrast, loss of early phase change (**epc**) hastens the transition from juvenile to adult phase and results in fewer nodes with brace roots (Moose and Sisco, 1994; Vega et al., 2002). In addition, the flowering time regulator *vegetative to generative transition1 (vgt1/zmrap2.7)*, was recently identified as a determinant of the number of nodes containing brace roots (Li et al., 2019). These results highlight the importance of phase change and flowering time for the timing and regulation of brace root development.

In addition to individual gene analyses, nodal root traits have been mapped as quantitative trait loci (QTL) in maize (**Supplemental Table 2**). Analysis of the overlap between nodal root QTL and known IAMBROOT genes identifies candidates for 34 of the 161 QTL (**Table 2**; **Supplemental Table 3**). These brace root QTL map to **rt1** and **rtcll**, as well as a number of genes known to regulate flowering time and phase change. Included in this latter group are **cct1** and **zmrap2.7**, alleles of which comprise flowering time QTL in maize (Salvi et al., 2007; Hung et al., 2012). The over-representation of flowering and phase change regulators in IAMBROOT suggests that additional candidates for brace root QTL may be found among known flowering time regulators. We expanded upon existing lists of proposed flowering time regulators in maize to include recent findings (**Supplemental Table 4**; **Supplemental Table 5**). The overlap between these (**Supplemental Table 5**; 26 QTL includes **indeterminate1 (id1)**, **phosphatidylethanolamine-binding protein8/zeacentroradialis8 (pebp8/zcn8)**, **delayed flowering1 (dlf1)**, and **elongated mesocotyl1 (elm1)**), which encode **bona fide** flowering time QTL in maize (Hung et al., 2012; Liu et al., 2015) but have not been investigated for brace root phenotypes.

In order to optimize brace root function in maize, the association between phase change, flowering time, and brace roots must be further defined. This relationship may be fundamental to resource allocation in angiosperms (Coleman et al., 1994), as suggested by a common garden experiment using 20 divergent dry-grassland species that found flowering time predicted shootroot biomass allocation better than phylogenetic relationships (Husáková et al., 2018). The fundamental association between these three traits has confounded the genetic analysis of brace root development. However, these traits are genetically separable (Vega et al., 2002; Shi et al., 2019; Reneau et al., 2020), which suggests that there are independent control mechanisms that remain to be defined.
Prevalence of Brace Roots in the Poaceae

Brace roots have been defined in maize by their assumed function in structural stability and location on a vertical stem, instead of evolutionary origin. Using the narrow definition of “brace root”, these structures are present only in the Andropogoneae and Paniceae tribe. Other Poaceae form aerial roots, but these roots may not constitutively contribute to anchorage (Figure 2). Comparative studies between genera will be critical to define the shared regulation and function of aerial roots.

The research highlighted in this review focuses on maize, but the mechanisms that control the development and function of brace roots are likely to be shared across the Poaceae. This family also includes a number of taxa widely utilized as experimental systems including sorghum (Sorghum), sugarcane (Saccharum), miscanthus (Miscanthus), foxtail millet (Setaria), pearl millet (Pennisetum), switchgrass (Panicum), rice (Oryza), oats (Avena), barley (Hordeum), rye (Secale), and wheat (Triticum) (Figure 2) (Sebastian and Dinneny, 2017). Within Poaceae, maize belongs to the Andropogoneae tribe along with other C4 photosynthetic crops including sorghum, miscanthus, and sugarcane (Paterson et al., 2013).

The closest maize relative, Sorghum, shared a common ancestor 12-16 million years ago (MYA) (Swigoňová et al., 2004; Zhang et al., 2017). Although there has been scant research on sorghum brace root development, there is variation in sorghum brace root formation (Supplemental Table 2)(Li et al., 2014; Harris-Shultz et al., 2019). For example, the parents of a sorghum recombinant inbred line (RIL) population were shown to have a dramatically different number of brace root whorls - Sansui, has 6-8 nodes containing brace roots, whereas Jiliang 2, only contains brace roots on the lowest node (Li et al., 2014). Two QTL located on chromosome 6 and 7 explained 7.6% and 52.5% of the phenotypic variation, respectively (Li et al., 2014). A similar candidate gene search for sorghum brace root QTL indicates that the locus on chromosome 6 overlaps with the flowering time regulator Maturity1 (Murphy et al., 2011). Therefore, the shared control of brace roots and flowering time (Supplemental Table 5) may not be unique to maize. Sugarcane diverged from sorghum 6-9 MYA and is more closely related to sorghum than maize, (Paterson et al., 2004; Janno et al., 2007; Thirugnanasambandam et al., 2018). Sugarcane differs from other members of Andropogoneae in that it is primarily propagated vegetatively, which means that the root system is entirely nodal-derived. Thus, the ability to form aerial roots (i.e. brace roots) is important for propagation as well as anchorage. Setaria, commonly referred to as foxtail millet, is a C4 photosynthetic plant in the Paniceae tribe, which is sister to the Andropogoneae. Like sorghum, maize, and sugarcane, Setaria also develops aerial roots (unpublished personal observation).

Conclusion

Aerial roots have been identified in several genera of Poaceae. In maize, these roots are called brace roots based on their critical function in maize anchorage, but this function has been poorly defined in the other genera. Defining the regulation of brace root development and function in maize provides a critical foundation for future comparative studies. Therefore, separating the regulation of brace root traits, phase change, and flowering time is essential.
The future of breeding resilient crops must consider dynamic responses of plant development and function to environmental stressors. One breeding target is root system improvement, given its central function in water uptake, nutrient use, and anchorage. Despite recent work highlighting a role in anchorage, the aerial portion of the root system has been poorly studied. Aerial roots likely function in water uptake, nutrient use, vegetative propagation, and stress resistance as well. Thus, it is of critical importance to explore these functions in members of Poaceae.

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Literature Cited

Aiken, R. M., and A. J. M. Smucker. 1996. Root System Regulation of Whole Plant Growth. *Annual Review of Phytopathology* 34: 325–346.

Berry, P. M., M. Sterling, J. H. Spink, C. J. Baker, R. Sylvester-Bradley, S. J. Mooney, A. R. Tams, and A. R. Ennos. 2004. Understanding and reducing lodging in cereals. Advances in Agronomy, 217–271. Elsevier.

Blizard, S., and E. E. Sparks. 2020. Maize Nodal Roots. *Annual Plant Reviews online*, 281–304. American Cancer Society.

Brune, P. F., A. Baumgarten, S. J. McKay, F. Technow, and J. J. Podhiny. 2018. A biomechanical model for maize root lodging. *Plant and Soil* 422: 397–408.

Chuck, G., A. M. Cigan, K. Saeteurn, and S. Hake. 2007. The heterochronic maize mutant Corngrass1 results from overexpression of a tandem microRNA. *Nature Genetics* 39: 544–549.

Coleman, J. S., K. D. McConnaughay, and D. D. Ackerly. 1994. Interpreting phenotypic variation in plants. *Trends in ecology & evolution* 9: 187–191.

Erndwein, L., D. D. Cook, D. J. Robertson, and E. E. Sparks. 2019. Field-based mechanical phenotyping of cereal crops to assess lodging resistance. *arXiv*: arXiv: 1909.08555.

Evans, M. M., and R. S. Poethig. 1995. Gibberellins promote vegetative phase change and reproductive maturity in maize. *Plant physiology* 108: 475–487.

Evans, M. M., and R. S. Poethig. 1997. The viviparous8 mutation delays vegetative phase change and accelerates the rate of seedling growth in maize. *The Plant Journal* 12: 769–779.

Fedenko, J. R., J. E. Erickson, and M. P. Singh. 2015. Root lodging affects biomass yield and carbohydrate composition in sweet sorghum. *Industrial Crops and Products* 74: 933–938.

Feldman, L. 1994. The maize root. The maize handbook, 29–37. Springer.

Gu, D., X. Mei, T. Yu, N. Sun, D. Xu, C. Liu, and Y. Cai. 2017. QTL identification for brace-root traits of maize in different generations and environments. *Crop Science* 57: 13–21.

Harris-Shultz, K. R., C. M. Hayes, and J. E. Knoll. 2019. Mapping QTLs and identification of genes associated with drought resistance in sorghum. *Sorghum*, 11–40. Springer.

Hetz, W., F. Hochholdinger, M. Schwall, and G. Feix. 1996. Isolation and characterization of rtics, a maize mutant deficient in the formation of nodal roots. *The Plant Journal* 10: 845–857.

Hochholdinger, F. 2009. The maize root system: Morphology, anatomy, and genetics. *In J. L. Bennetzen, and S. C. Hake [eds.], Handbook of maize: Its biology*, 145–160. Springer New York, New York, NY.

Hochholdinger, F., and G. Feix. 1998. Early post-embryonic root formation is specifically affected in the maize mutant lrt1. *The Plant Journal* 16: 247–255.

Hochholdinger, F., W. J. Park, M. Sauer, and K. Woll. 2004. From weeds to crops: genetic analysis of root development in cereals. *Trends in Plant Science* 9: 42–48.

Hochholdinger, F., K. Woll, M. Sauer, and D. Dembinsky. 2004. Genetic dissection of root formation in maize (*Zea mays*) reveals root-type specific developmental programmes. *Annals of Botany* 93: 359–368.

Hodge, A., G. Berta, C. Doussan, F. Merchán, and M. Crespi. 2009. Plant root growth, architecture and function. *Plant and Soil* 321: 153–187.

Hoppe, D. C., M. E. McCully, and C. L. Wenzel. 1986. The nodal roots of Zea: their development in relation to structural features of the stem. *Canadian Journal of Botany* 64: 2524–2537.

Hung, H.-Y., L. M. Shannon, F. Tian, P. J. Bradbury, C. Chen, S. A. Flint-Garcia, M. D. McMullen, et al. 2012. ZmCCT and the genetic basis of day-length adaptation underlying the
postdomestication spread of maize. *Proceedings of the National Academy of Sciences* 109: E1913–E1921.

Husáková, I., J. Weiner, and Z. Münzbergová. 2018. Species traits and shoot–root biomass allocation in 20 dry-grassland species. *Journal of Plant Ecology* 11: 273–285.

Jannoo, N., L. Grivet, N. Chantret, O. Garsmeur, J. C. Glaszmann, P. Arruda, and A. D’Hont. 2007. Orthologous comparison in a gene-rich region among grasses reveals stability in the sugarcane polyploid genome. *The Plant Journal* 50: 574–585.

Jenkins, M. T. 1930. Heritable characters of maize. XXXTV. Rootless. *Journal of Heredity* 21.

Kitomi, Y., H. Ito, T. Hobo, K. Aya, H. Kitano, and Y. Inukai. 2011. The auxin responsive AP2/ERF transcription factor CROWN ROOTLESS5 is involved in crown root initiation in rice through the induction of OsRR1, a type-A response regulator of cytokinin signaling. *The Plant Journal* 67: 472–484.

Kitomi, Y., A. Ogawa, H. Kitano, and Y. Inukai. 2008. CRL4 regulates crown root formation through auxin transport in rice. *Plant Root* 2: 19–28.

Ku, L. X., Z. H. Sun, C. L. Wang, J. Zhang, R. F. Zhao, H. Y. Liu, G. Q. Tai, and Y. H. Chen. 2012. QTL mapping and epistasis analysis of brace root traits in maize. *Molecular Breeding* 30: 697–708.

Lavenus, J., T. Goh, I. Roberts, S. Guyomarc’h, M. Lucas, I. De Smet, H. Fukaki, et al. 2013. Lateral root development in Arabidopsis: fifty shades of auxin. *Trends in plant science* 18: 450–458.

Li, J., F. Chen, Y. Li, P. Li, Y. Wang, G. Mi, and L. Yuan. 2019. ZmRAP2.7, an AP2 transcription factor, is involved in maize brace roots development. *Frontiers in Plant Science* 10.

Li, R., Y. Han, P. Lv, R. Du, and G. Liu. 2014. Molecular mapping of the brace root traits in sorghum (Sorghum bicolor L. Moench). *Breeding science* 64: 193–198.

Liu, H., Y. Niu, P. J. Gonzalez-Portilla, H. Zhou, L. Wang, T. Zuo, C. Qin, et al. 2015. An ultra-high-density map as a community resource for discerning the genetic basis of quantitative traits in maize. *BMC genomics* 16: 1078.

Liu, S., F. Song, F. Liu, X. Zhu, and H. Xu. 2012. Effect of planting density on root lodging resistance and its relationship to nodal root growth characteristics in maize (Zea mays L.). *Journal of Agricultural Science* 4: 182.

Liu, S., J. Wang, L. Wang, X. Wang, Y. Xue, P. Wu, and H. Shou. 2009. Adventitious root formation in rice requires OsGNOM1 and is mediated by the OsPINs family. *Cell research* 19: 1110–1119.

Lynch, J. 1995. Root architecture and plant productivity. *Plant Physiology* 109: 7–13.

Minow, M. A., L. M. Ávila, K. Turner, E. Ponzoni, I. Mascheretti, F. M. Dussault, L. Lukens, et al. 2018. Distinct gene networks modulate floral induction of autonomous maize and photoperiod-dependent teosinte. *Journal of experimental botany* 69: 2937–2952.

Moose, S. P., and P. H. Sisco. 1994. Glossy15 controls the epidermal juvenile-to-adult phase transition in maize. *The Plant Cell* 6: 1343–1355.

Morita, Y., and J. Kyozuka. 2007. Characterization of OsPID, the rice ortholog of PINOID, and its possible involvement in the control of polar auxin transport. *Plant and Cell Physiology* 48: 540–549.

Murphy, R. L., R. R. Klein, D. T. Morishige, J. A. Brady, W. L. Rooney, F. R. Miller, D. V. Dugas, et al. 2011. Coincident light and clock regulation of pseudoresponse regulator protein 37 (PRR37) controls photoperiodic flowering in sorghum. *Proceedings of the National Academy of Sciences* 108: 16469–16474.
Negi, S., P. Sukumar, X. Liu, J. D. Cohen, and G. K. Munday. 2010. Genetic dissection of the role of ethylene in regulating auxin-dependent lateral and adventitious root formation in tomato. *The Plant Journal* 61: 3–15.

Pacurar, D. I., I. Perrone, and C. Bellini. 2014. Auxin is a central player in the hormone cross-talks that control adventitious rooting. *Physiologia Plantarum* 151: 83–96.

Paterson, A. H., J. E. Bowers, and B. A. Chapman. 2004. Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. *Proceedings of the National Academy of Sciences* 101: 9903–9908.

Paterson, A. H., P. H. Moore, and T. L. Tew. 2013. The gene pool of Saccharum species and their improvement. Genomics of the Saccharinae, 43–71. Springer.

Pinthus, M. J. 1974. Lodging in Wheat, Barley, and Oats: The Phenomenon, its Causes, and Preventive Measures. In N. C. Brady [ed.], Advances in Agronomy, 209–263. Academic Press.

Poethig, S. 1988. A non–cell–autonomous mutation regulating juvenility in maize. *Nature* 336: 82–83.

Rajkumara, S. 2008. Lodging in cereals – a review. *Agricultural Reviews* 29: 55–60.

Reneau, J. W., R. S. Khangura, A. Stager, L. C. Erndwein, T. Weldekidan, D. D. Cook, B. P. Dilkes, and E. E. Sparks. 2020. Maize brace roots provide stalk anchorage. *bioRxiv* : 2020.07.28.225656.

Sakai, H., and G. Taramino. 2009. Plants with altered root architecture, involving the rt1 gene, related constructs and methods.

Salvi, S., G. Sponza, M. Morgante, D. Tomes, X. Niu, K. A. Fengler, R. Meeley, et al. 2007. Conserved noncoding genomic sequences associated with a flowering-time quantitative trait locus in maize. *Proceedings of the National Academy of Sciences* 104: 11376–11381.

Sebastian, J., and J. R. Dinneny. 2017. *Setaria viridis*: A Model for Understanding Panicoid Grass Root Systems A. Doust, and X. Diao [eds.]. *Genetics and Genomics of Setaria*: 177–193.

Sharma, S., and M. J. Carena. 2016. BRACE: A Method for High Throughput Maize Phenotyping of Root Traits for Short-Season Drought Tolerance. *Crop Science* 56: 2996–3004.

Shi, J., B. J. Drummond, J. E. Habben, N. Brugire, B. P. Weers, S. M. Hakimi, H. R. Lafitte, et al. 2019. Ectopic expression of ARGOS8 reveals a role for ethylene in root-lodging resistance in maize. *The Plant Journal* 97: 378–390.

Stephenson, E., S. Estrada, X. Meng, J. Ourada, M. G. Muszynski, J. E. Habben, and O. N. Danilevskaya. 2019. Over-expression of the photoperiod response regulator ZmCCT10 modifies plant architecture, flowering time and inflorescence morphology in maize. *Plos one* 14: e0203728.

Sterling, M., C. J. Baker, P. M. Berry, and A. Wade. 2003. An experimental investigation of the lodging of wheat. *Agricultural and Forest Meteorology* 119: 149–165.

Sun, N., C. Liu, X. Mei, D. Jiang, X. Wang, E. Dong, J. Zhang, and Y. Cai. 2020. QTL identification in backcross population for brace-root-related traits in maize. *Euphytica* 216: 32.

Suzuki, M., Y. Sato, S. Wu, B.-H. Kang, and D. R. McCarty. 2015. Conserved Functions of the MATE Transporter BIG EMBRYO1 in Regulation of Lateral Organ Size and Initiation Rate. *The Plant Cell* 27: 2288–2300.

Swigoňová, Z., J. Lai, J. Ma, W. Ramakrishna, V. Llaca, J. L. Bennetzen, and J. Messing. 2004. Close Split of Sorghum and Maize Genome Progenitors. *Genome Research* 14: 1916–1923.

Taramino, G., M. Sauer, J. L. Stauffer, D. Multani, X. Niu, H. Sakai, and F. Hochholdinger. 2007. The maize (*Zea mays L.*) RTCS gene encodes a LOB domain protein that is a key regulator of embryonic seminal and post-embryonic shoot-borne root initiation. *The Plant Journal* 50: 649–659.
Thirugnanasambandam, P. P., N. V. Hoang, and R. J. Henry. 2018. The challenge of analyzing the sugarcane genome. Frontiers in plant science 9: 616.

Tyburski, J., and A. Tretyn. 2004. The role of light and polar auxin transport in root regeneration from hypocotyls of tomato seedling cuttings. Plant growth regulation 42: 39–48.

Vega, S. H., M. Sauer, J. A. Orkwiszewski, and R. S. Poethig. 2002. The early phase change gene in maize. The Plant Cell 14: 133–147.

Vidoz, M. L., E. Loreti, A. Mensuali, A. Alpi, and P. Perata. 2010. Hormonal interplay during adventitious root formation in flooded tomato plants. The Plant Journal 63: 551–562.

Xu, M., L. Zhu, H. Shou, and P. Wu. 2005. A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. Plant and cell physiology 46: 1674–1681.

Zhang, A., Z. Cui, C. Li, J. Luo, Y. Guan, L. Liu, Z. Zhang, et al. 2018. Identification of maize brace-root quantitative trait loci in a recombinant inbred line population. Euphytica 214: 168.

Zhang, Y., D. W. Ngu, D. Carvalho, Z. Liang, Y. Qiu, R. L. Roston, and J. C. Schnable. 2017. Differentially regulated orthologs in sorghum and the subgenomes of maize. The Plant Cell 29: 1938–1951.

Zhang, Z., X. Zhang, Z. Lin, J. Wang, M. Xu, J. Lai, J. Yu, and Z. Lin. 2018. The genetic architecture of nodal root number in maize. The Plant Journal 93: 1032–1044.
### Table 1. Identified Annotated Modulators of Brace Roots in maize (IAMBROOT). Maize genes with mutant phenotypes known to influence brace roots.

| Locus name                              | Gene Model (B73v4)                  | Root Phenotypes                                      | Reference                                |
|-----------------------------------------|-------------------------------------|------------------------------------------------------|------------------------------------------|
| rootless1 (rt1)                         | GRMZM2G163975                       | Reduced number of nodal roots                         | Jenkins 1930                             |
| Teopod1 (Tp1)                           |                                     | Increase in number of brace root whorls               | Poethig 1988                             |
| Teopod2 (Tp2)                           |                                     | Increase in number of brace root whorls               | Poethig 1988                             |
| Teopod3 (Tp3)                           |                                     | Increase in number of brace root whorls               | Poethig 1988                             |
| Hairy sheath frayed1 (Hsf1)             | GRMZM2G151223                       | Increase in number of brace root whorls               | Moose and Sisco 1994                     |
| dwarf plant1 (d1)                       | GRMZM2G036340                       | Increase in number of brace root whorls               | Evans et al., 1995                       |
| dwarf plant3 (d3)                       | GRMZM2G093195                       | Increase in number of brace root whorls               | Evans et al., 1995                       |
| dwarf plant5 (d5)                       | GRMZM2G093603                       | Increase in number of brace root whorls               | Evans et al., 1995                       |
| anther ear1 (an1)                        | GRMZM2G081554                       | Increase in number of brace root whorls               | Evans et al., 1995                       |
| lateral rootless1 (lrl1)                |                                     | Shorter nodal roots at the seedling stage             | Hochholdinger and Feix 1998             |
| early phase change (epc)                |                                     | Reduced number of nodal roots                         | Vega et al., 2002                        |
| Corngrass1 (Cg1)                        | GRMZM5G838324/zmaMIR156b            | Increase in number of brace root whorls               | Chuck et al., 2007                       |
| rootless concerning crown and seminal roots (rtcs1) | GRMZM2G092542                  | No brace roots                                       | Taramino et al., 2007                    |
| teosinte glume architecture1 (tga1) and neighbor of tga1 (not1) | GRMZM2G101511/tga1; AC233751.1_FG002/not1 | Increase in number of brace root whorls               | Wang et al., 2015                        |
| rtcs-like1 (rtcl1)                      | AC149818.2_FG009                    | Reduced elongation of shoot-borne crown roots         | Xu et al., 2015                          |
| big embryo1 (bigel1)                    | GRMZM2G148937                       | Increase in number of brace root whorls               | Suzuki et al., 2015                      |
| zmcc1                                   | GRMZM2G381691                       | Increase in number of brace root whorls               | Stephenson et al., 2019                  |
| zmrap2.7                                | GRMZM2G700665                       | Reduced number of nodal roots                         | Li et al., 2019                          |
| argos8                                  | GRMZM2G354338                       | Reduced number of nodal roots                         | Shi et al., 2019                         |
| vivparous8 (vp8)                        | GRMZM2G010353                       | Increase in number of brace root whorls               | Evans and Poethig 1997                   |
Table 2. Summary of the overlap between IAMBROOT candidate gene list and nodal root traits mapped in QTL analysis. Candidate genes from the IAMBROOT curated list were overlaid with known nodal root QTL. QTL were compiled from Gu et al., 2016; Ku et al., 2012; and Zhang et al., 2018. A full list can be found in Supplemental Table 3.

| Annotation | Candidate Gene Model (B73 RefGenV4) | Root Traits | No. of QTL | IAMBROOT References |
|------------|-------------------------------------|-------------|------------|---------------------|
| an1        | Zm00001d032961                      | BRN         | 2          | Evans et al., 1995  |
|            |                                     | RNPL        | 1          |                     |
| argos8     | Zm00001d038075                      | BRWN        | 1          | Shi et al., 2019    |
|            |                                     | TNWN        | 1          |                     |
| cct1       | Zm00001d024909                      | BRWN        | 2          | Minow et al., 2018  |
|            |                                     | EBRWN       | 1          |                     |
|            |                                     | ARNPL       | 1          |                     |
|            |                                     | CRN         | 1          |                     |
|            |                                     | CRNPL       | 1          |                     |
|            |                                     | CRWN        | 1          |                     |
|            |                                     | RNPL        | 1          |                     |
|            |                                     | TNRN        | 1          |                     |
|            |                                     | TNWN        | 1          |                     |
| d1         | Zm00001d039634                      | BRWN        | 1          | Evans et al., 1995  |
|            |                                     | TNWN        | 1          |                     |
| d5         | Zm00001d002349                      | TNWN        | 1          | Evans et al., 1995  |
| rap2       | Zm00001d010987                      | BRWN        | 1          | Minow et al., 2018  |
|            |                                     | BRN         | 2          |                     |
|            |                                     | CRN         | 2          |                     |
|            |                                     | CRNPL       | 1          |                     |
|            |                                     | TNWN        | 1          |                     |
| rt1        | Zm00001d040186                      | BRN         | 1          | Jenkins 1930        |
|            |                                     | BRWN        | 1          |                     |
|            |                                     | TNRN        | 1          |                     |
|            |                                     | TNWN        | 1          |                     |
| rtcl1      | Zm00001d048401                      | BRWN        | 1          | Xu et al., 2015     |
|            |                                     | EBRWN       | 1          |                     |
| rtcl1      | Zm00001d048401                      | BRN         | 1          | Xu et al., 2015     |
Evans and Poethig, 1997

| vp8 | Zm00001d034383 | Evans and Poethig, 1997 |
|-----|----------------|-------------------------|
| BRN | 1              |                         |
| TNRN| 1              |                         |
| TNWN| 1              |                         |

**Figure Legends**

**Figure 1. The maize root system.** The maize root system consists of primary embryonic roots and secondary roots. The primary embryonic roots include the primary root (red) and the seminal root (green). The secondary post-embryonic roots include the lateral roots (white), crown roots (blue), and brace roots (yellow).

**Figure 2. Phylogenetic distribution of members in the Poaceae family.** Aerial roots have been observed within sugarcane (purple), sorghum (teal), maize (yellow), and foxtail millet (green), all of which are members of the Andropogoneae and Paniceae tribe. However, with the exception of maize, the development and function have been poorly defined.
Supplementary Tables

1. Identified Annotated Modulators of Brace Roots (IAMBROOT)

2. Summary of all the nodal root QTL detected in various maize and sorghum studies

3. Summary of the overlap between the 161 QTLs reported in maize for nodal root trait variation and IAMBROOT gene list

4. Putative candidate gene list of maize genes that influence flowering time

5. Summary of the overlap between the 161 QTLs reported in maize for nodal root trait variation and bona fide flowering QTL in maize
