

# BUZZ: an essential gene for postinitiation root hair growth and a mediator of root architecture in *Brachypodium distachyon*

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## Summary

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Received: 9 March 2023  
Accepted: 10 May 2023

New Phytologist (2023)  
doi: 10.1111/nph.19079

**Key words:** Arabidopsis, Brachypodium, BUZZ, kinase, nitrate, root architecture, root hair, RSLs.

- Here, we discover a player in root development. Recovered from a forward-genetic screen in *Brachypodium distachyon*, the *buzz* mutant initiates root hairs but they fail to elongate. In addition, *buzz* roots grow twice as fast as wild-type roots. Also, lateral roots show increased sensitivity to nitrate, whereas primary roots are less sensitive to nitrate.
- Using whole-genome resequencing, we identified the causal single nucleotide polymorphism as occurring in a conserved but previously uncharacterized cyclin-dependent kinase (CDK)-like gene. The *buzz* mutant phenotypes are rescued by the wild-type *B. distachyon* BUZZ coding sequence and by an apparent homolog in *Arabidopsis thaliana*. Moreover, T-DNA mutants in *A. thaliana* BUZZ have shorter root hairs.
- BUZZ mRNA localizes to epidermal cells and develops root hairs and, in the latter, partially colocalizes with the *NRT1.1A* nitrate transporter. Based on qPCR and RNA-Seq, *buzz* overexpresses ROOT HAIRLESS LIKE SIX-1 and -2 and misregulates genes related to hormone signaling, RNA processing, cytoskeletal, and cell wall organization, and to the assimilation of nitrate.
- Overall, these data demonstrate that BUZZ is required for tip growth after root hair initiation and root architectural responses to nitrate.

## Introduction

Root hairs develop distinctly from many other plant cell types. Generally, root hair development can be divided into two phases: cell-fate determination and growth. In the first, epidermal cells differentiate either to become a root hair cell (*trichoblast*) or to remain a rank-and-file epidermal cell (*atrachoblast*). In the growth phase, a conventionally parenchymatous epidermal cell forms a localized bulge that subsequently elongates by tip growth. Typically, soon after tip-growth rate becomes steady, the diffuse growth of the rest of the epidermal cell stops. Both phases of the underlying developmental program are influenced by physiology so that the numbers of root hairs made by a given root at a given time, as well their length and character, are a sensitive function of the environment (Gilroy & Jones, 2000; Salazar-Henao *et al.*, 2016).

The process of cell-fate determination has been particularly well elucidated in *Arabidopsis thaliana* (Grierson *et al.*, 2014).

Briefly, root hair cell fate is negatively regulated by a transcriptional complex including the WEREWOLF (WER), GLABRA3, and TRANSPARENT TESTA GLABRA1 (TTG1), a complex that promotes expression of GLABRA2 (GL2; Grebe, 2012; Schiefelbein *et al.*, 2014; Salazar-Henao *et al.*, 2016). In hair cells, interaction between WER and the transcriptional complex is inhibited. This activates a trio of transcription factors: ROOT HAIR DEFECTIVE 6 (RHD6), RHD6-LIKE1 (RSL1), and RHD6-LIKE 4. Finally, this trio turns on the genes needed to build and manage a root hair (Masucci & Schiefelbein, 1994; Yi *et al.*, 2010; Balcerowicz *et al.*, 2015; Datta *et al.*, 2015).

Once the alternating cell fate pattern has been decided, hair formation is considered to have two developmental phases – initiation and tip growth (Salazar-Henao *et al.*, 2016). Root hair growth then proceeds in distinct steps: polarization of the cell wall at sites of GEF3/ROP2 accumulation, localized loosening of the cell wall to form a bulge, reorganization of microtubule and actin cytoskeleton into arrays constructed to ship convoys of vesicles for tip growth, and finally, rapid elongation growth via polarized exocytosis (Dolan *et al.*, 1994; Bascom Jr. *et al.*, 2018;

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Denninger *et al.*, 2019). Plants govern both the rate and extent of root hair growth in response to the environment, including the availability of water, nitrate, or phosphate (Gilroy & Jones, 2000; Salazar-Henao *et al.*, 2016).

The genetic bases of root hair development are revealed by identifying root hair mutants with aberrant cell-fate determination or defective root hair growth. These mutants have also laid the groundwork for transcriptomic and proteomic studies on whole roots and single cells to define the 'so-called' trophomorphome (Bruex *et al.*, 2012; Salazar-Henao & Schmidt, 2016; Ryu *et al.*, 2019).

The above outline of root hair development is based almost wholly on *A. thaliana*. To what extent are these pathways conserved? For example, grasses synthesize a cell wall that differs notably from that of nongrasses (Carpita & Gibeau, 1993), prompting the question of whether grasses share the adaptations found in eudicot tip-growing cells to orchestrate cell wall assembly. Furthermore, in Pooidae grasses, root hairs are not determined in cell files but rather on a cell-by-cell basis; in this program, the smaller product of an asymmetric cell division results in a root hair (Salazar-Henao *et al.*, 2016).

In grasses, several root hairless mutants have been described (Klamer *et al.*, 2019). In many cases, the identified gene points to mechanistic conservation. For example, the root hairless mutants *rth6* and *rth2* in both maize and rice harbor mutations in the same class of cellulose synthase (CSLD) that supports tip growth in *A. thaliana* (Kim *et al.*, 2007; Yuo *et al.*, 2011; Li *et al.*, 2016). Likewise in *Brachypodium distachyon*, knocking down a *ROOT HAIR DEFECTIVE 6 (RHD6)-like (RSL)* gene generates roots with shorter root hairs, while overexpressing the gene increases the number and length of root hairs (Kim & Dolan, 2016; Zhang *et al.*, 2018).

Along with development, root hair mutants have been useful for uncovering function. Surprisingly in field trials, maize *rth1*, *rth2*, and *rth3* plants grow well (Wen & Schnable, 1994), a vigor that challenged long-held assumptions about root hair function. Since then, studies of nutrient acquisition have taken advantage of root hairless mutants in both cereals and crop eudicots (Gahoonia & Nielsen, 2004; Brown *et al.*, 2013; Zhang *et al.*, 2018). For example, *rth2* showed that root architecture was regulated by low-phosphorus soils (Klamer *et al.*, 2019). While the results certainly confirm a role for root hairs in absorbing nutrients, the role is complex, and studies are often confounded by additional phenotypes, such as dwarfism. For example, *RSL* overexpression did not improve phosphate uptake and content (Zhang *et al.*, 2018). Thus, new root hair mutants in grasses have the potential to illuminate not only mechanisms of root hair differentiation and growth but also mechanisms of plant nutrition.

As an unbiased means of gene discovery and to elucidate the pathway governing root hair development in grasses, we performed a forward-genetic screen in *B. distachyon* and identified a root hair mutant, named *buzz*. In *buzz* compared with wild-type, primary and lateral roots grow faster and lateral roots are more dense. Whole-genome resequencing pinpoints the causal mutation to a putative cyclin-dependent kinase (CDK)-like gene. Here, we define a new player in both tip growth and nitrate-responsive root architecture that is evolutionarily conserved.

## Materials and Methods

### Plant material, EMS mutagenesis, and growth conditions

*Brachypodium distachyon* L. seeds were surface sterilized as described (Smertenko *et al.*, 2020). Seeds were placed on ½ strength Murashige and Skoog medium (MS; PhytoTech Laboratories, Lenexa, KS, USA), 3% Gelzan (PhytoTech Laboratories) plates, stored at 4°C for 2 d in the dark, and then grown in a 16 h : 8 h, day : night cycle for 5 d at a light intensity of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 22°C. EMS mutagenesis was performed as described (<https://jgi.doe.gov/our-science/science-programs/plant-genomics/brachypodium/>). Approximately 20 000 M<sub>0</sub> seeds in the Bd21 background were EMS mutagenized and germinated for 2 wk at 4°C in the dark. 5000 M<sub>1</sub> plants were transferred to soil and self-pollinated. Over 4600 families were screened in the M<sub>2</sub> generation for seedling root phenotypes. The *buzz*<sup>TDNA</sup> allele is in Bd21-3 (Bragg *et al.*, 2012). For nitrate assays, plants were grown on nitrate medium as described previously (David *et al.*, 2019) and lateral root density and length were measured in 8-d-old roots. *Arabidopsis thaliana* L. (Heynh) ecotype Columbia-0 and T-DNA lines (031719C and 027496C) were obtained from the ABRC (<https://abrc.osu.edu/>). Seeds were sterilized as described previously (Lehman & Sanguinet, 2019). Seedlings were grown on ½ MS, 1% sucrose (PhytoTechnology Laboratories), and 1% Bacto Agar (BD, Franklin Lakes, NJ, USA) medium in a 16 h : 8 h, day : night cycle for 7 d at a light intensity of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 22°C.

### Root measurements and kinematic analysis

Images of *B. distachyon* roots and *A. thaliana* seedlings were captured using a M205 FA Leica Stereo microscope, measured using IMAGEJ64 (<https://imagej.nih.gov/ij/>). Statistical analysis was performed using a two-sided *t*-test. For kinematic analysis, roots were imaged through a horizontal microscope with a 4× objective. A pair of images were captured at 30-s intervals and used as input for STRIPFLOW software to obtain the spatial profile of velocity (Baskin & Zelinsky, 2019). Then, velocity profiles were fitted by a piecewise polynomial and differentiated to obtain the profile of elemental elongation rate (Yang *et al.*, 2017).

### SEM analysis

SEM analysis was performed on roots as previously described (Kim & Dolan, 2011). Roots were placed in a slurry of liquid nitrogen and then transferred to a cold stage for cryo-SEM (JEOL JSM-6510 LA, Iwate University, College of Engineering, Morioka, Japan).

### Isolation and mapping of *buzz* using whole-genome resequencing

The original M<sub>2</sub> segregating family had *c.* 25% of individuals with a *buzz* phenotype, which were allowed to self-pollinate. M<sub>3</sub> plants were crossed to Bd3-1 for traditional mapping and self-pollinated, yielding homozygous M<sub>4</sub> *buzz* vs homozygous M<sub>4</sub> wild-type lines.

Whole-genome resequencing was performed following established techniques (Abe *et al.*, 2012; Woods *et al.*, 2014). Genomic DNA from 30 individuals was pooled into 10 replicates from three populations: Bd21 parental line pre-EMS mutagenesis for reference genome comparison, M4 wild-type siblings, and M4 *buzz* mutants. Genomic DNA was isolated using the FastDNA Green Spin Kit from MP Biomedicals according to the manufacturer's instructions. Individual libraries were generated from each pool and sequenced using an Illumina Hi-Seq 2500 at the WSU-Spokane Genomics Core Facility. Raw reads were trimmed, compared, and analyzed for quality control measures. Each read was mapped to the reference Bd21 genome using BWA (Li & Durbin, 2009). Single nucleotide polymorphism (SNP) calling was performed using the GATK pipeline (<https://www.broadinstitute.org/gatk/>). Specific PERL scripts were developed to map differential SNPs.

### Protein structure analysis

The protein structure model of wild-type BUZZ and BUZZ<sup>EMS</sup> was generated by SWISS-MODEL (<https://swissmodel.expasy.org/>) using the query found in Supporting Information Table S1.

### Cloning and transformation

A 2200-bp fragment of the *B. distachyon* BUZZ promoter and coding sequences of BUZZ (*Bradi3g46007*) and AtBUZZ (*At1g71530*) were amplified using Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) from Bd21 genomic DNA, Bd21 cDNA, and Col-0 cDNA and cloned into the pENTR p1p5r or pENTR p5p2 vectors, respectively. LR reactions were performed to generate *pBUZZ::BUZZ* or *pBUZZ::AtBUZZ* in the pIPKb001 expression vector (Himmelbach *et al.*, 2007) and transfected into *Agrobacterium tumefaciens* strain AGL1. Plant transformation was performed as described (Vogel & Hill, 2008). Additionally, a 1500-bp fragment of the *B. distachyon* BUZZ promoter along with the BUZZ CDS and VENUS YFP either on the N-terminal of BUZZ (N-terminally tagged) or C-terminal of BUZZ was synthesized (Twist Biosciences, South San Francisco, CA, USA) in the pTwist ENTR cloning vector and transformed as described above in the pIPKb001 vector.

### Phylogenetic analysis

Sixty-seven amino acid sequences of putative BUZZ proteins (Table S2) were aligned using MUSCLE (Edgar, 2004). The amino acid alignment was analyzed using maximum likelihood (ML) with RAxML-NG (v.0.9.0; Kozlov *et al.*, 2019) implementing the LG + G4m model with 10 random starting trees and 100 bootstrap replicates. Trees were rooted on the branch between *Physcomitrella patens* sequences and the rest of the sequences (all flowering plants).

### Transient transformation in *Nicotiana benthamiana* and stable transformation of *A. thaliana*

PCR products were cloned via a BP clonase reaction (Invitrogen) from Bd21 or Col-0 root cDNA into pDONR221 (Table S3)

using the manufacturer's specifications (Thermo Fisher, Waltham, MA, USA) and then transferred into pMDC43 or pMDC83 (Curtis & Grossniklaus, 2003) via LR clonase (Invitrogen) reaction. *Nicotiana benthamiana* leaf infiltrations were performed using a modified protocol (Romeis *et al.*, 2001) with p19 in a 2:1 ratio. Two days after infiltration, leaves were imaged with a Leica Confocal SP-8 (IPN Imaging Core, WSU, Pullman, WA). The pMDC43 and pMDC83 vectors harboring the AtBUZZ-GFP tags were used to transform the *Atbuzz* mutant as described previously (Clough & Bent, 1998). Transgenic lines were assayed for single insertions and analyzed in the T3 generation.

### RNA isolation

RNA was isolated from root tips (bottom 0.5 cm) and mesophyll of 5-d-old *B. distachyon* seedlings and from 7-wk-old panicles. Tissues were harvested, flash frozen in liquid nitrogen, and homogenized using an MP Biomedical Fastprep-24 5G Matrix A. RNA was extracted using a modified TRIzol protocol (Thermo Fisher) for qRT-PCR and using a Zymo Research Quick-RNA Plant Miniprep Kit (#R2404) and measured for concentration using a BioPhotometer (Eppendorf, Enfield, CT, USA).

### Gene expression analysis

DNaseI treatment of RNA was performed according to the manufacturer's specifications (Thermo Fisher). RNA was reverse transcribed using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). The expression levels of *RSL1*, *RSL2*, and *RSL3* (Kim & Dolan, 2016) were determined based on quantitative PCR (qPCR) and standardized relative to the housekeeping gene *SAMDC*. The reactions were performed using the iTaq Universal SYBR GREEN Supermix (Bio-Rad) with a specific primer designed to generate qPCR products (Table S4). Reactions were performed in a CFX 96 Touch (Bio-Rad) using the comparative  $C_T$  method for relative quantitation of expression levels (Livak & Schmittgen, 2001). Each data point represents the average mean of three biological replicates performed in technical triplicate.

### RNAscope *in situ* hybridization

*Brachypodium distachyon* BUZZ (*Bradi3g46007*), *NRT1.1A* (*Bradi3g16670*), and *CSLD4* (*Bradi4g05027*) mRNA-specific probes were custom synthesized (Advanced Cell Diagnostics, Newark, CA, USA), and their spatial expression was determined simultaneously using RNAscope *in situ* hybridization as described (Solanki *et al.*, 2020). *CSLD4* and *NRT1.1A* were used as highly expressed positive controls along with a 3-plex RNAscope negative control. Bd21 seeds were grown on 1/2 MS as described above. Individual roots were placed in cryomolds filled with semi-frozen Tissue-Tek optimal cutting temperature (OCT) compound and frozen at  $-80^{\circ}\text{C}$ . 10–15  $\mu\text{m}$  thick sections were cut on a Leica CM3050 S cryostat, and 16 sample slides were processed for RNAscope assay and visualization. Images were captured on Leica TCS SP8 X point scanning confocal microscope (IPN Imaging Core, WSU, Pullman, WA) and images were processed on LAS X software (Leica).

## RNA-Seq analysis

RNA-Seq analysis was performed in triplicate on root tips (0.5 cm) from 20 seedling roots per biological replicate. RNA was isolated as above and sent to Lexogen for QuantSeq 3' mRNA sequencing using their library preparation kit and then run on an Illumina sequencing platform (<https://www.lexogen.com/quantseq-3mrna-sequencing/>). The data were processed according to an established workflow (Table S5). Fold changes values of  $\geq |2|$  and an FDR-adjusted  $P$ -value  $\leq 0.05$  were used as minimum values to be considered as possible genes of interest.

## String analysis

Both *BUZZ* in *B. distachyon* (Bradi3g46007) and *A. thaliana* (Atlg71530) were used to query the string database (<https://string-db.org/>).

## Results

### A recessive root hair mutant that increases primary root growth rate: *buzz*

In *B. distachyon*, we identified a root hair mutant that we name *buzz*. The mutation was induced by EMS and mutant phenotypes segregated as expected from a single recessive allele. The *buzz* mutant initiates root hairs that fail to elongate (Fig. 1a–d). In soil, *buzz* roots are hairless with only root bulges along the root epidermis. This phenotype is seen not only in the primary root but also in seminal and lateral roots (Fig. S1). Interestingly, the primary root of *buzz* grows notably faster than that of Bd21 wild-type (Fig. 1e,g). To evaluate growth parameters underlying faster root growth, we ran a kinematic analysis revealing increases in both maximal elemental elongation rate and the length of the growth zone (Fig. 1h,i). To evaluate cell division, we measured the length of mature epidermal cells. The ratio of growth rate to mature cell length gives the rate of cell production by the meristem, per cell file (Baskin, 2013). Mature epidermal cell length in the mutant is slightly, albeit significantly, increased in *buzz* (Fig. S2a); however, the increase is far smaller than the increase in root growth rate, demonstrating that the *buzz* meristem produces cells far more rapidly than Bd21. According to visual inspection, the length of the meristem in *buzz* is the same as that of Bd21 (Fig. S2b), implying that the increased rate of cell production reflects an increased rate of cell division (rather than an increased number of dividing cells). Taken together, *buzz* eliminates root hair elongation but stimulates cell division and elemental elongation in the root body.

However, increased root growth in *buzz* is accompanied by neither increased shoot height nor yield (Fig. S2c,d); in fact, shoot biomass decreases notably in the mutant and there is a trend toward less mass even in the root (Fig. S2e,f). In addition, the initial asymmetric cell division generating trichoblast vs atrichoblast cells in the epidermis appears normal, with no evident difference in root hair density between Bd21 and *buzz* (Figs 1f, S3).

### Neither auxin nor ethylene rescue root hair elongation in *buzz*

Root hair initiation and elongation are regulated by the hormones auxin and ethylene (Pickett *et al.*, 1990; Masucci & Schiefelbein, 1994; Muday *et al.*, 2012). We therefore treated the *buzz* mutant with IAA or ACC. Intriguingly, neither hormone rescues the *buzz* root hair phenotype (Fig. S4a–l). However, they do inhibit primary root growth as expected, indicating that *buzz* roots are hormone responsive (Fig. S4m,n). These unique phenotypes prompted us to hypothesize that BUZZ mediates an essential yet previously uncharacterized tip growth pathway in *B. distachyon* root hairs.

### Genome sequencing revealed a telltale SNP in the *buzz*<sup>EMS</sup> background

To map *buzz*, we outcrossed the *buzz* mutant in the Bd21 background to Bd3-1. Segregating F2 individuals were genotyped for linked markers and *buzz* individuals co-segregated with a marker on chromosome 3 (data not shown), thus providing a rough chromosomal location for *buzz*. To identify the causal mutation underlying the *buzz* phenotype, we performed whole-genome resequencing (Abe *et al.*, 2012; Woods *et al.*, 2014). We sequenced the Bd21 parental line for genome reference, Bd21 post-EMS wild-type M4 pooled siblings (not harboring the *buzz* allele), and *buzz* M4 pooled seedlings. Over 1000 differential SNPs were identified between wild-type and *buzz* M4 siblings; of these, four tandem SNPs were found on chromosome 3 in the *buzz* population but not in wild type Bd21, with a single SNP present in the coding sequence of a gene (Fig. 2a; bottom panel). The SNP is a G-to-A base pair transition, typical of mutations induced by EMS, and results in a single amino acid substitution of glycine<sup>268</sup> with arginine.

The locus thus identified contains a gene model for a hitherto uncharacterized cyclin-dependent kinase (CDK) in a clade belonging to the super-family of CDKs but sufficiently distinct that they are usually considered to be CDK-like. Along with the kinase domain, the gene contains a cyclin-binding domain that overlaps with the kinase domain. In *A. thaliana*, the closest BUZZ ortholog, previously described as a CDK-like 12 (CDKL12; Menges *et al.*, 2005), is part of a group of root-specific genes specifically upregulated in response to salt stress (Ma *et al.*, 2006) and part of a co-transcriptional network implicated in root epidermal patterning (Bruex *et al.*, 2012). The SNP identified above occurs in the kinase catalytic domain. Among sequences, glycine<sup>268</sup> is conserved in other grass species as well as in more distantly related kinases, such as the putative ortholog in *A. thaliana* (AtBUZZ; Fig. 2b). However, modeling protein conformation finds no distinct change between wild-type and *buzz* allele (Fig. S5).

### A *buzz*<sup>T-DNA</sup> allele confirms the identity of BUZZ

To further test whether the correct gene was identified, we obtained an allele of BUZZ, in the Bd21-3 background, with a

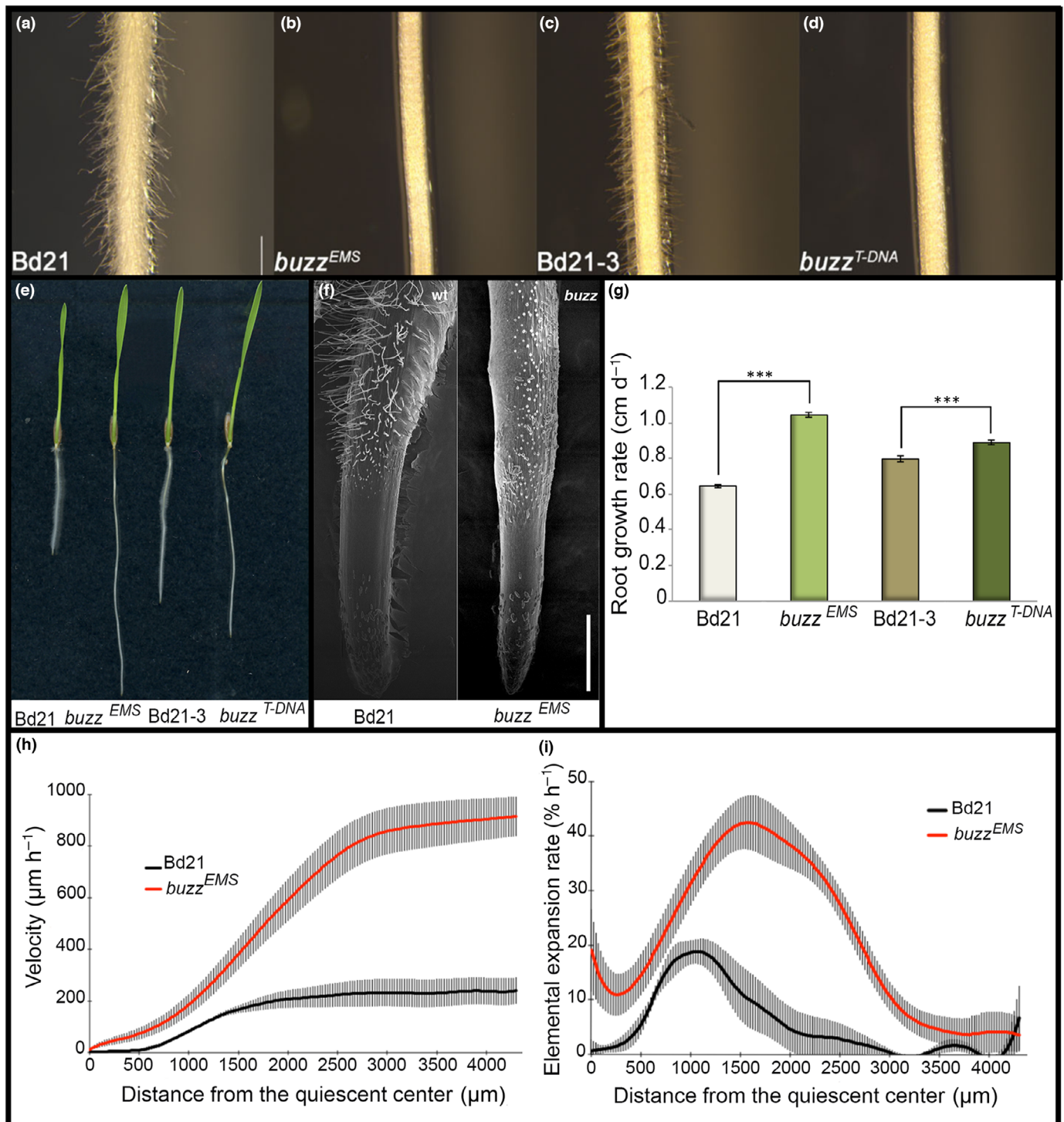


**Fig. 1** Phenotypic characterization of *Brachypodium distachyon* *buzz* mutants at 5-d-old. (a) Bd21; (b) *buzz*<sup>EMS</sup>; (c) Bd21-3; (d) *buzz*<sup>T-DNA</sup> seedling roots in the mature root zone; and (e) seedlings of the indicated genotypes showing increased root length in *buzz* mutants. (f) Cryo-SEM images of Bd21 and *buzz* roots. Bar, 500 μm. (g) Root growth rate measured between Day 1 and Day 5. Bar plots means ± SE for at least 29 individuals. The \*\*\* indicate equivalence of means rejected with  $P < 0.001$  by a two-sided  $t$ -test. (h, i) Kinematic analysis. Line plots means of 8–11 seedlings and the bars show the SE.

T-DNA insertion in the first exon (Fig. 3a; Bragg *et al.*, 2012). In the T2 generation, we observe a 1 : 3 segregation of the *buzz* root hair phenotype (Figs 1d, 3c,f). In addition, root growth rate is increased in *buzz*<sup>T-DNA</sup> compared with wild-type

(Fig. 3h), although not as much as for the *buzz*<sup>EMS</sup> allele. Furthermore, in homozygous *buzz*<sup>T-DNA</sup> individuals, a full-length BUZZ transcript is undetectable based on RT-PCR (Fig. S6).

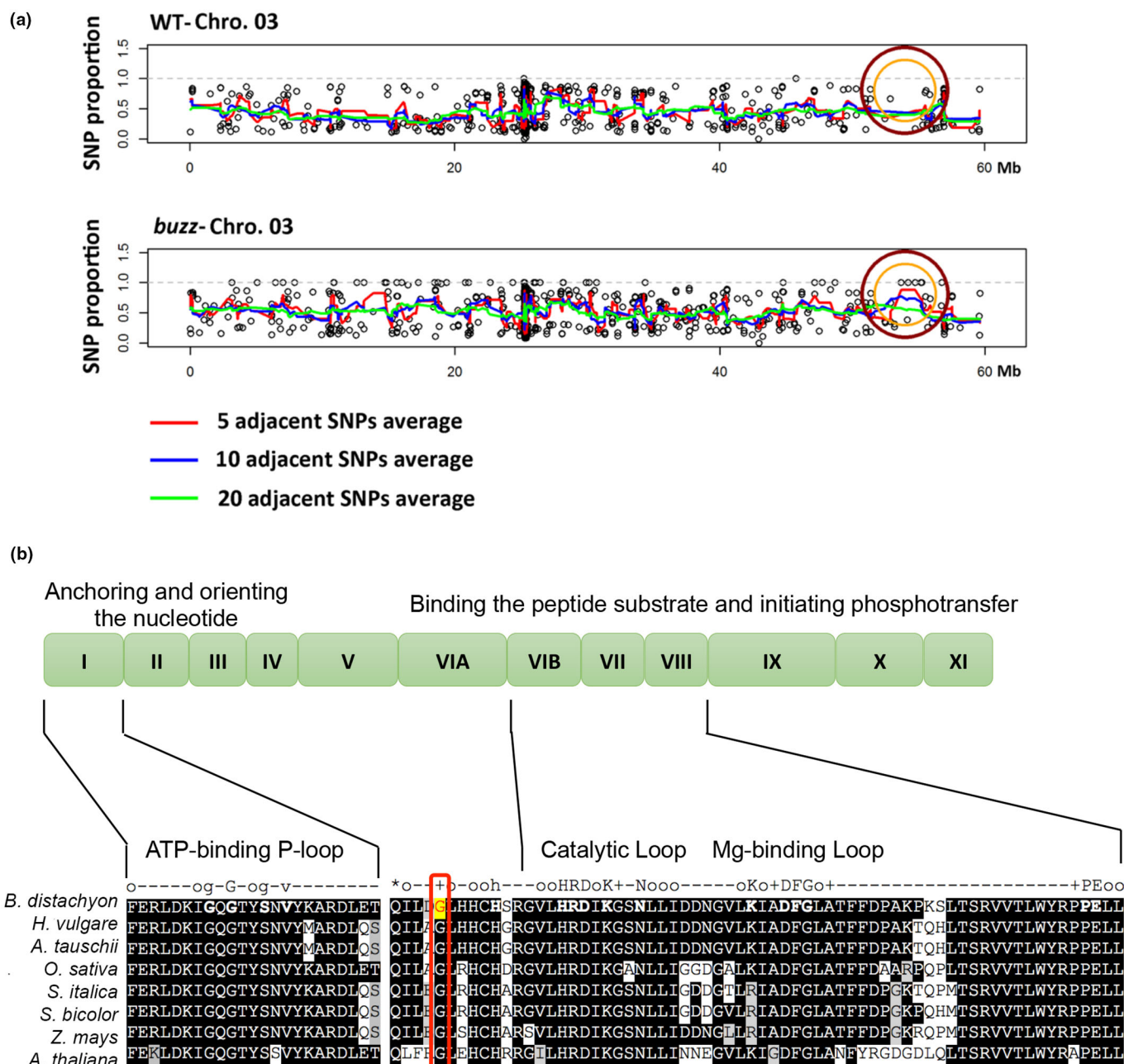


**Fig. 2** Identification of the *Brachypodium distachyon* BUZZ locus and schematic of the conserved kinase domain. (a) Differential single nucleotide polymorphism (SNP) analysis from whole-genome sequencing for chromosome 3. The concentric orange and red circles show the location of homozygous SNPs that are present in *buzz* but not Bd21. (b) Schematic of the 12 highly conserved domains of eukaryotic protein kinases depicted in BUZZ (top) and protein alignments from the indicated regions (below). The Gly residue mutated to an Arg in the *buzz*<sup>EMS</sup> allele is boxed in red and highlighted in yellow. Identical residues highlighted in black, similar residues highlighted in gray. o, conserved nonpolar; \*, conserved polar; +, conserved small, neutral.

## Complementation of *buzz*

Next, we transformed *buzz*<sup>EMS</sup> with the native promoter driving the wild-type BUZZ coding sequence. T<sub>0</sub> plants were genotyped for the presence of the hygromycin resistance marker. Hygromycin-positive T<sub>0</sub> plants were transplanted to soil and allowed to self-pollinate (T<sub>1</sub>). Germinated T<sub>1</sub> seedling roots were imaged for the presence or absence of root hairs and then confirmed in the T<sub>2</sub> and T<sub>3</sub> generations. As predicted for

complementation, *buzz*<sup>EMS</sup> plants express *pBUZZ::BUZZ* grow root hairs luxuriously (Fig. 3d). Likewise, the coding sequence of the ortholog from *A. thaliana* (i.e. *AtBUZZ*), driven under the native *B. distachyon* BUZZ promoter, also rescues the root hair phenotype of *buzz*<sup>EMS</sup> (Fig. 3g). Additionally, both the *B. distachyon* BUZZ and *AtBUZZ* return root growth rate of *buzz*<sup>EMS</sup> to wild-type levels (Fig. 3h). These data confirm the identification of the BUZZ gene and reveal that the function of the gene is conserved between *B. distachyon* and *A. thaliana*.

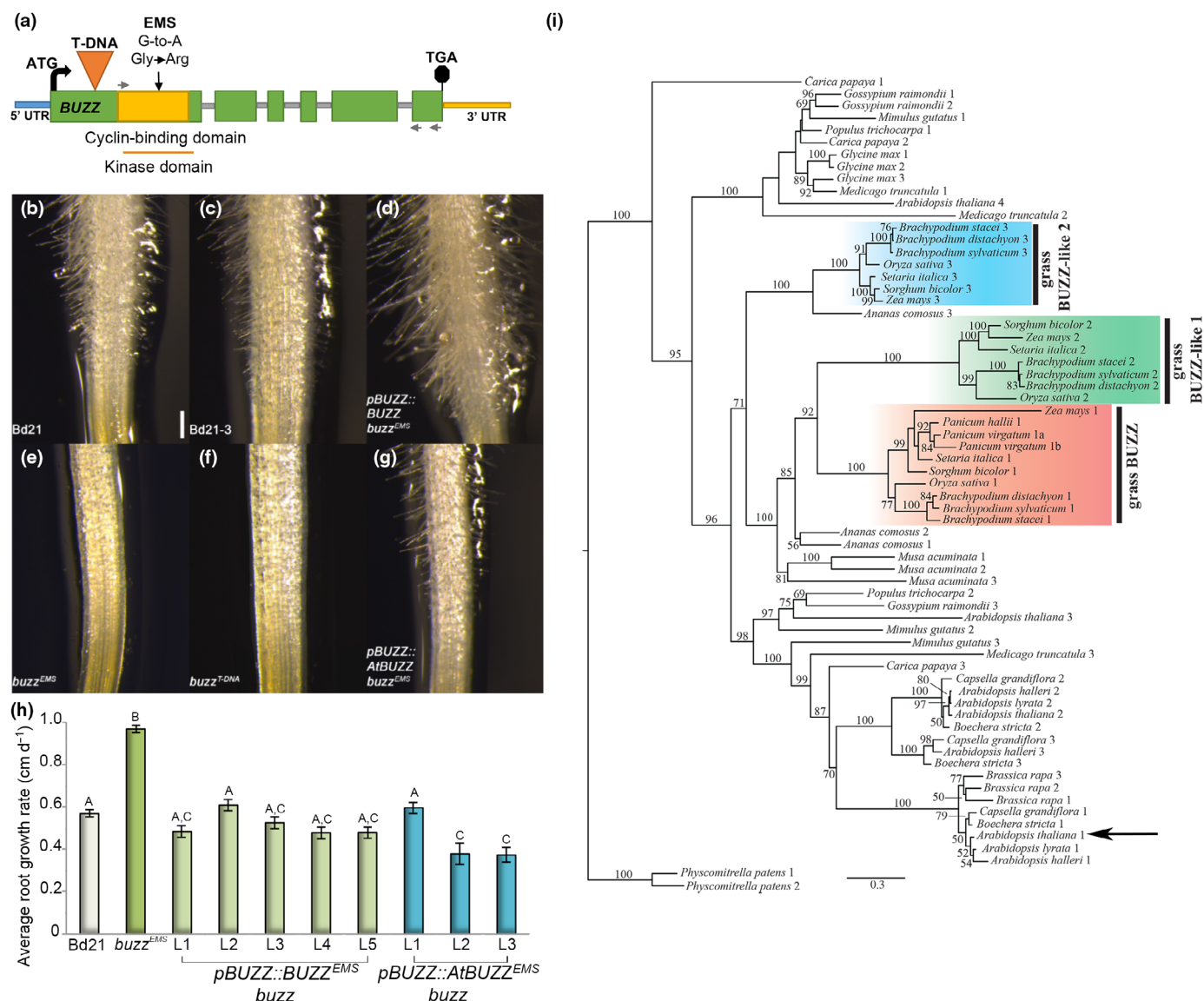


**Fig. 3** Genetic complementation of *buzz* in *Brachypodium distachyon* and phylogenetic analysis *BUZZ*. (a) Schematic of the *BUZZ* gene with localization of the EMS and T-DNA mutations. The 5' UTR is shown in blue, the 3' UTR in yellow, exons as green boxes and grey arrows represent primer binding sites for qRT-PCR. (b–g) Micrographs of 5 dag roots of (b) Bd21 (c) Bd21-3 (d) *pBUZZ::BUZZ buzz<sup>EMS</sup>* (e) *buzz<sup>EMS</sup>* (f) *buzz<sup>T-DNA</sup>* and (g) *pBUZZ::AtBUZZ buzz<sup>EMS</sup>*. Bar (in c), 500 µm. (h) Root growth rate in the indicated genotypes (each bar in the graph represents independently transformed lines). The rate was measured between Days 1 and 5. Bar plots mean of 25 to 30 individuals ± SE. Letters above the bars indicate statistical significance with *P*-value = 0.05 as determined by a two-sided *t*-test. (i) Phylogenetic tree of *BUZZ* protein sequences from a selection of plant lineages. Numbers above the nodes show bootstrap support values. The arrow indicates the *AtBUZZ* protein.

## Phylogenetic analysis

To provide insight into the evolution of *BUZZ*, we performed maximum-likelihood phylogenetic analysis (Fig. 3i). For the grass sequences, the overall branching structure of the tree has three paralog sets; 'grass *BUZZ*' and 'grass *BUZZ*-like 1' are more closely related to each other than they are to 'grass *BUZZ*-like 2' (Fig. 3i). Apparently, grass *BUZZ* and *BUZZ*-like 1 clades arose from a duplication that happened before the grass BOP and PACMAD clades diverged (Soreng *et al.*, 2015), but after the graminoid monocots diverged from the lineages related to bananas. By contrast, the *BUZZ*/*BUZZ*-like 1 lineage diverged from the

grass *BUZZ*-like 2 lineage before grasses diverged from bananas and all of the related monocot lineages.

## *Atbuzz* has a short root hair phenotype

To examine the conservation of *BUZZ* further, we characterized two independent T-DNA lines with insertions in *AtBUZZ* (Fig. 4a). *AtBUZZ* is specifically expressed in trichoblast cells within elongation and maturation zones (Winter *et al.*, 2007; Waese *et al.*, 2017). Both *Atbuzz* T-DNA alleles have decreased root hair length (62% or 45%) compared with wild-type and the full-length transcript is detected in neither T-DNA line

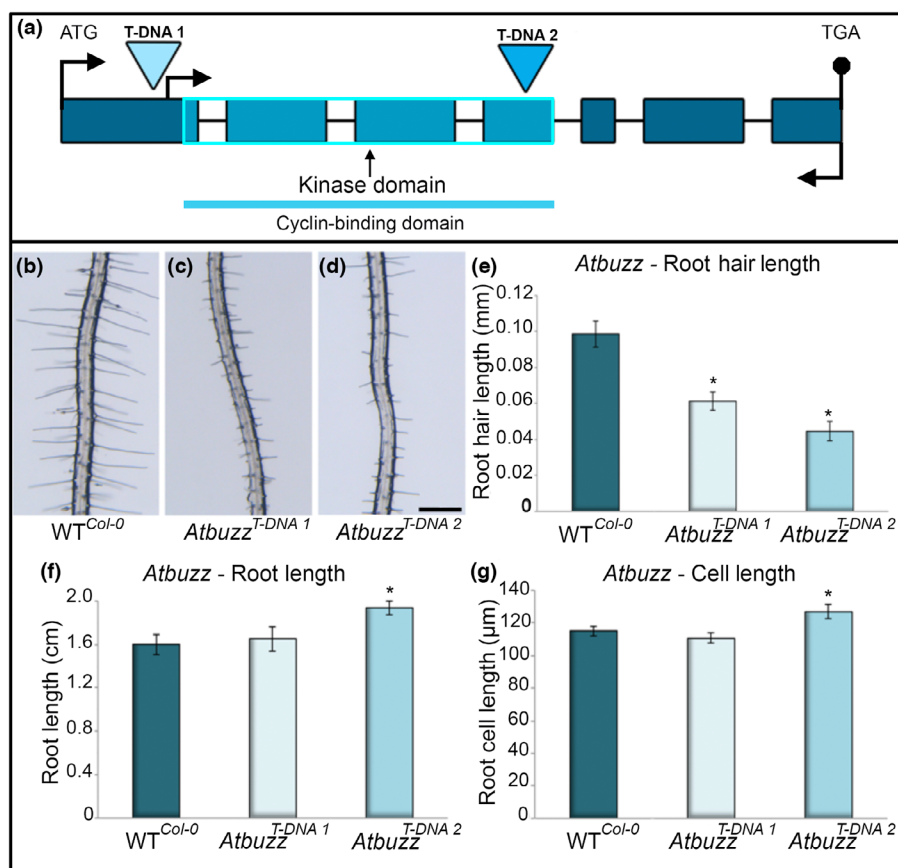


**Fig. 4** Phenotypic characterization of the *Arabidopsis thaliana* *buzz* mutant (*Atbuzz*). (a) Schematic of the *AtBUZZ* gene with localization of the insertions with black directional arrow indicating the ATG start site and location of primers for RT-PCR. (b–d) Micrographs of the mature region of the root of the indicated genotypes. Bar (in d), 200 μm. (e) Root hair length. Bar plots mean ± SE of 60 individuals. (f) Root length. Bar plots mean ± SE of 7-d-old seedlings for 24–37 individuals. (g) Cell length. Bar plots mean ± SE of 29–38 epidermal cells in the mature zone. The \* indicates equivalence with the wild-type mean is rejected with  $P < 0.05$  by using a two-sided  $t$ -test (\*,  $P < 0.05$ ).

(Fig. 4b–e, S6). Next, we determined whether *Atbuzz* also has a faster primary root growth rate. One insertion line does not affect root growth detectably; however, the other (insertion in the kinase domain) stimulates modestly both primary growth and epidermal cell length (Fig. 4f,g). Evidently, *AtBUZZ* supports root hair growth and limits primary root growth but less strongly than *BUZZ* does in *B. distachyon*. *Atbuzz* mutants establish tip-growing root hairs, but they are short. To determine whether the root hair length of *Atbuzz* mutants could be increased, we treated them with auxin or ACC. Strikingly, either hormone vastly increases root hair length, in both wild-type and *Atbuzz* (Fig. S7). These results, taken together with the inability of auxin or ACC to increase root hair length in *B. distachyon* (Fig. S4), suggest the hormones in grasses and eudicots act through partially distinct pathways.

### Expression of *BUZZ* via qRT-PCR

To determine the organs and tissues in which *BUZZ* is expressed, we performed qRT-PCR analysis. We compared expression of *BUZZ* in both Bd21 and the *buzz*<sup>EMS</sup> mutant. Notably, we could not detect full-length transcript in the *buzz*<sup>EMS</sup> allele, but qPCR primers designed to be gene-specific at the 3' end of the gene detected a partial transcript (Fig. S6). Overall, *BUZZ* is expressed at very low levels relative to the reference gene (Fig. 5a). *BUZZ* is expressed mainly in the root tip; however, the gene is also

expressed above background in the panicle, particularly in the mutant. While the reason for panicle expression remains to be discovered, the root tip expression is consistent with *BUZZ* functioning in root hairs.

### Subcellular localization of *BUZZ*

To determine subcellular localization, we transiently expressed *B. distachyon* or *A. thaliana* *BUZZ* coding sequences, tagged with green fluorescent protein (GFP) at either N- or C-terminus, in *N. benthamiana* leaf pavement cells. When driven by the cauliflower mosaic virus (CaMV) 35S promoter, the *BUZZ* protein with an N-terminal tag localizes to the nucleus, whereas *BUZZ* with a C-terminal tag localizes to the plasma membrane and, sometimes, to the cytosol (Fig. 5b–i). Neither 35S construct is fully functional insofar as neither fully rescues the *A. thaliana* mutant (Fig. 5j,k) although both versions show partial rescue. Next, we transformed *B. distachyon* with the native promoter driving either *BUZZ*-YFP or YFP-*BUZZ* in the *buzz* mutant background and both the N and C-terminal fusions fully rescue both the root hair phenotype (Fig. S8a–c). When the *BUZZ* sequence is driven by the native promoter, no signal is detected, presumably owing to the low expression (Figs 5a, S8d–l). Moreover, in the rescued *buzz* lines, fluorescence from the tag is undetectable. Thus, determining where *BUZZ* resides in the cell will require more sensitive methodology.

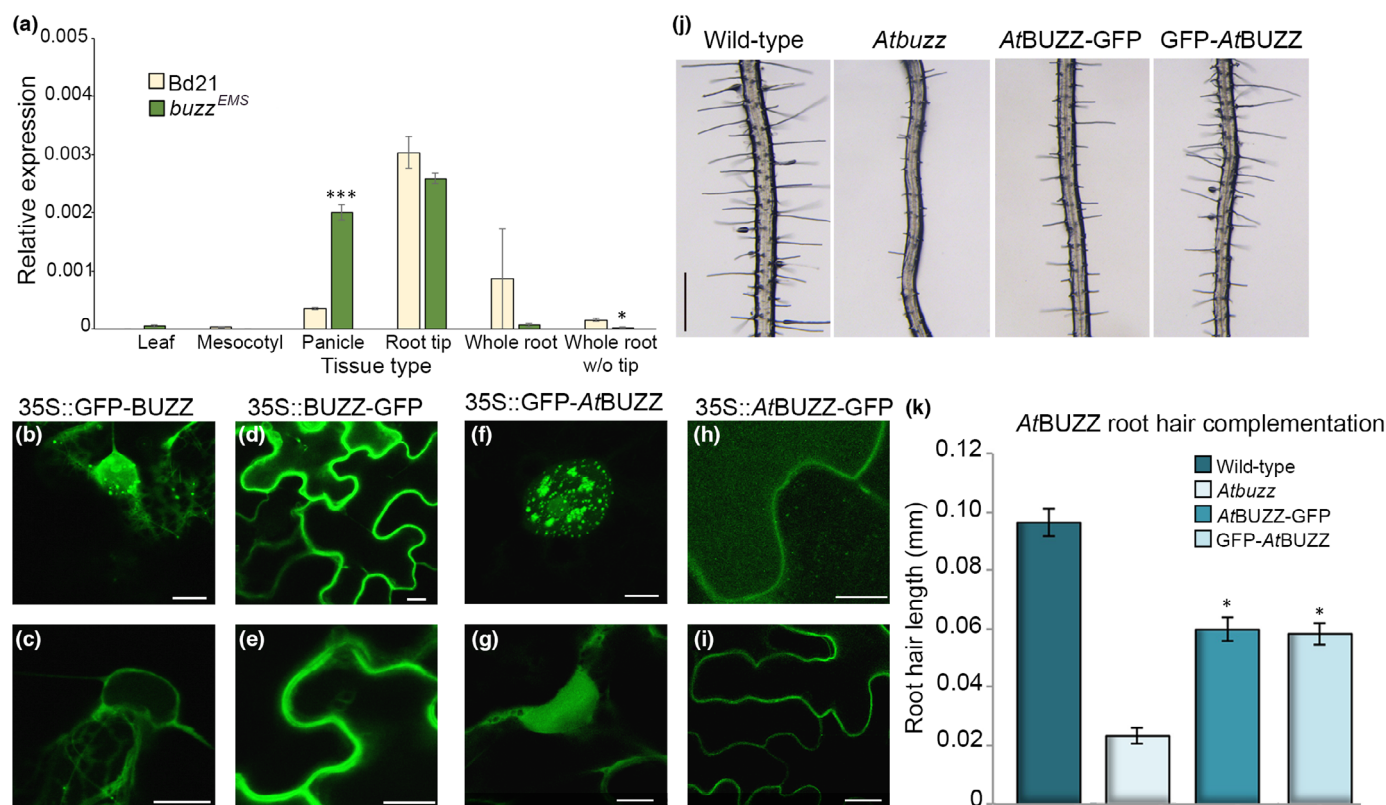


**Fig. 5** *BUZZ* expression in *Brachypodium distachyon* and subcellular localization in *Nicotiana benthamiana* pavement cells. (a) RNA abundance measured by qRT-PCR. Bar plots mean  $\pm$  SE of three biological replicates. Expression is measured relative to the housekeeping gene, *SAMDC*, the \*\*\*, indicates equivalence with the *Bd21* control mean is rejected with  $P < 0.001$  two-sided *t*-test. (b–i) Subcellular localization in pavement cells. Micrographs showing fluorescence from the indicated constructs transiently expressed in *N. benthamiana* leaves. Bars, 10  $\mu$ m. (j) Micrographs of the mature region of 7-d-old *Arabidopsis thaliana* roots of wild-type Col-0, *Atbuzz*, 35S::AtBUZZ-GFP in *Atbuzz*, and 35S::GFP-AtBUZZ in *Atbuzz*. Bar, 500  $\mu$ m. (k) Root hair length of wild-type Col-0, *Atbuzz*, 35S::AtBUZZ-GFP in *Atbuzz*, and 35S::GFP-AtBUZZ in *Atbuzz* in 7-d-old seedling roots. Bar plots mean  $\pm$  SE of five to seven individuals. The \*, indicates equivalence with the *Atbuzz* mean is rejected with  $P < 0.05$  by using a two-sided *t*-test (\*,  $P < 0.05$ ).

## *BUZZ* mRNA is predominately localized in epidermal cells and root hair tips

Given the inability to localize tagged versions of *BUZZ* protein, we localized *BUZZ* mRNA using RNAscope, which uses a methodology for signal amplification, background suppression, and enables simultaneous detection of multiple target RNAs at the single molecule level in individual cells (Solanki *et al.*, 2020). As positive controls, we used two highly expressed genes: a nitrate transporter *NRT1.1A* and the cellulose synthase-like gene, *CSLD4*. As expected in *B. distachyon* roots, *CSLD4* is expressed mainly, if not exclusively, in the meristem and appears absent from the root cap and epidermis (Figs 6a–f, S9, Video S1). Likewise, *NRT1.1A* is expressed as expected in the cortex of the meristem and elongation zone and in the developing xylem. By contrast, *BUZZ* mRNA is largely confined to the epidermis and is present in the meristem, elongation zone, and growing root hairs of the early maturation zone. Interestingly, *BUZZ* and *NRT1.1A* are both expressed in actively growing root hairs, co-localized as puncta at the root hair tips (Fig. 6g–n).

## *RSL* class I genes are upregulated in *buzz*

In *B. distachyon*, root hair development requires *RSL* class I genes (Kim & Dolan, 2016). Therefore, we measured the relative transcript abundance for *RSL1*, *RSL2*, and *RSL3* in root tips. Surprisingly, although the level of *RSL3* is slightly lowered in *buzz*, levels of the other two genes are upregulated, particularly *RSL1* (Fig. 7a). Perhaps, the upregulated expression reflects a response to the absence of root hairs, but in any case, the lack of root hair growth in *buzz* does not reflect a downregulation of these *RSL1* genes. Next, we examined the *A. thaliana buzz* mutant and found that *RHD6* and *RSL4* were expressed normally, but found that other root hair genes including *TRH1*, *GL2*, and *WER* were overexpressed whereas *RSL3* was repressed (Fig. S7k).

## RNA-Seq suggests putative roles for *BUZZ*

RNA-Seq analysis was performed in triplicate on 5-d-old root tips – specifically, the rootward *c.* 0.5 cm of the root. We found 478 transcripts are upregulated in *buzz* root tips and 184

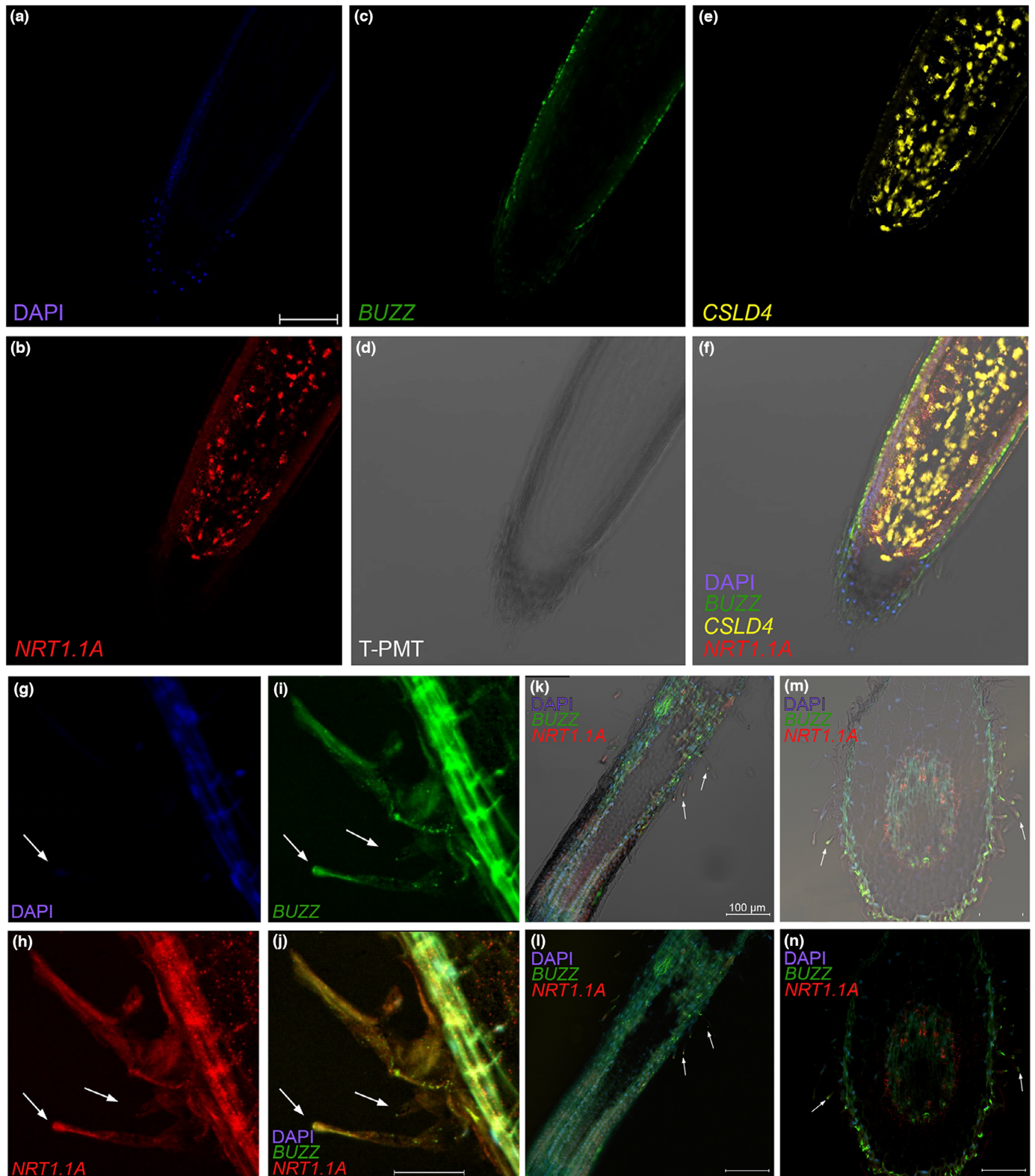


**Fig. 6** Localization of *BUZZ* mRNA with *in situ* hybridization by using RNAscope in *Brachypodium distachyon* roots at 5-d-old. Confocal fluorescence micrographs of roots labeled with the indicated probes or imaged in transmitted light ('T-PMT'). (a–f) Root meristem. Bar (in a), 100  $\mu$ m. (g–j) Elongating root hairs. Arrows on the left side of the panel mark growing root hairs; the bright band on the right side is neighboring epidermis. Bar (in j), 50  $\mu$ m. (k, l). Early maturation zone. Note *BUZZ* expression in the root hair is in the form of puncta near the tip (arrows). Bar (in k), 100  $\mu$ m. (m, n) Early maturation zone. *BUZZ* expression in epidermis generally and as puncta in developing root hairs (white arrows) while *NRT1.1A* is confined to the stele. Bar (in n), 100  $\mu$ m.

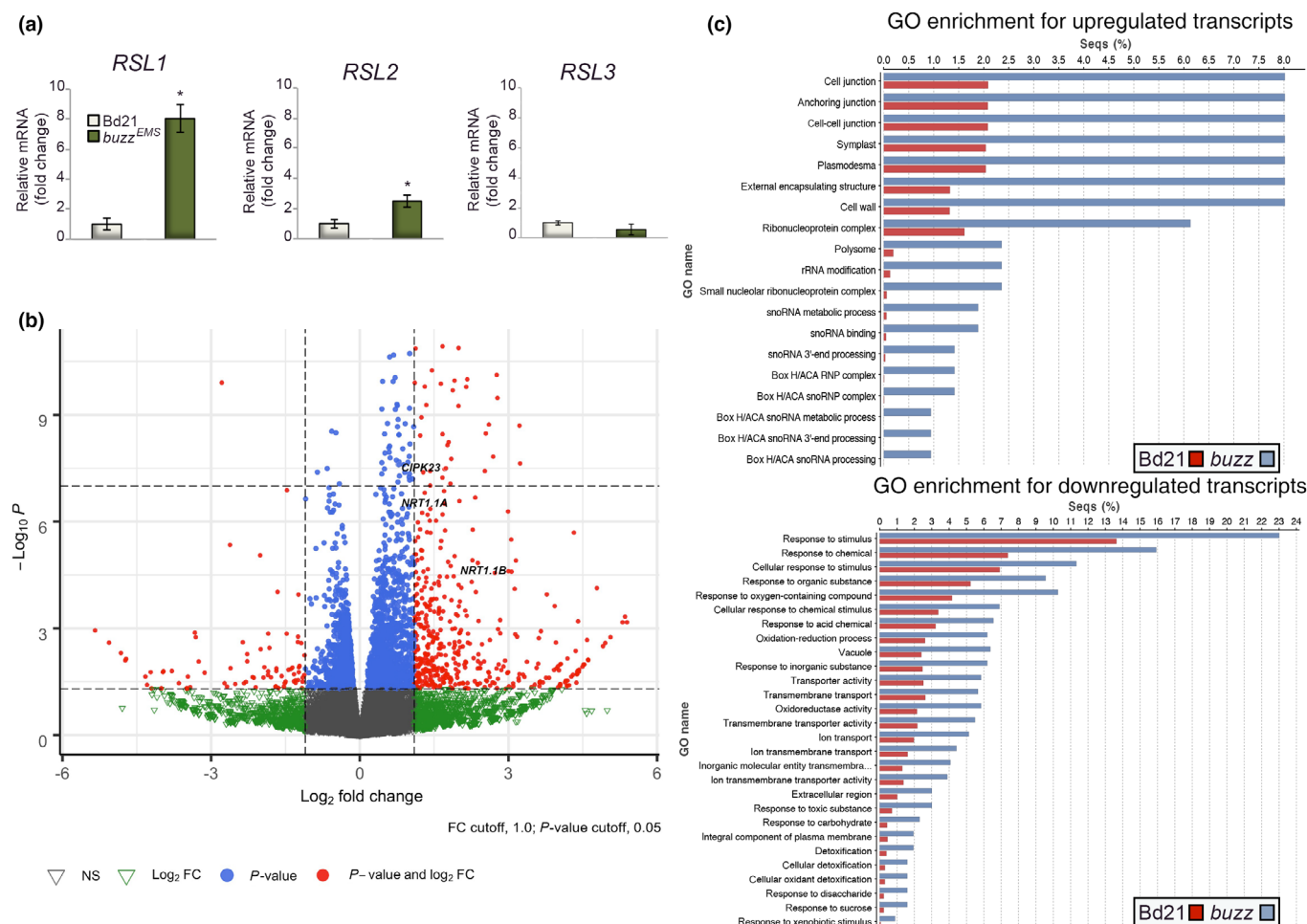


**Fig. 7** Analysis of *RSL* gene expression and RNA-Seq in *Brachypodium distachyon* *Bd21* and *buzz*. (a) Relative RNA abundance measured by qRT-PCR. Bar plots mean  $\pm$  SE for three biological replicates of the fold increase in the mutant compared with the wild-type. Statistical analysis performed using a two-sided *t*-test, the \*, indicates  $P < 0.05$ . (b) RNA-Seq analysis represented by a volcano plot. Red dots show sequences differing by more than twofold and passing a statistical reference of 0.05. Blue dots show sequences whose abundance differed significantly but by less than twofold and green triangles show sequences whose abundance was statistically the same in the two genotypes. (c) Gene ontology (GO) term enrichment analysis for upregulated (top) and downregulated (bottom) transcripts in *buzz* versus *Bd21*. For (b, c), the *buzz*<sup>EMS</sup> mutant was used. NS, not significant.

transcripts are downregulated (Fig. 7b; Table S6). Based on GO analysis, 22 downregulated transcripts are predicted to be involved in RNA biogenesis and ribosome functioning. Also, downregulated are five transcripts involved in either actin or tubulin binding as well as 18 transcripts involved in cell wall processes, consistent with root hair morphogenesis demanding these activities (Salazar-Henao *et al.*, 2016). Among transcripts that are upregulated are groups of transcripts associated with the following activities: kinases (21), transcription factors (18), hormone signaling (15), and nutrient sensing and signaling (28) half of which are specific to nitrate. Notably, overexpressed in *buzz* are two nitrate transporters, *NRT1.1A*, *NRT1.1B*, and *CIPK23* (Fig. 7c). The latter encodes a serine/threonine protein kinase that phosphorylates *NRT1.1*, switching it from low affinity to high-affinity transport (Ho *et al.*, 2009). Upregulation is surprising because nitrate transport and signaling transcripts are rarely upregulated in root hairless mutants. In fact, a root hairless mutant in *A. thaliana* represses nitrate transport genes including *NRT1.1*, *NRT2.1*, *NRT2.2*, and *NRT3.1* (Ehrhry *et al.*, 2020).

In addition to transporting nitrate, *NRT1.1* helps generate signals that convey nitrate status to the nucleus, signals that condition not only genetic responses related directly to nitrate assimilation (Maghiaooui *et al.*, 2020) but also responses involved in drafting blueprints for root system architecture (Remans *et al.*, 2006; Krouk *et al.*, 2010).

### BUZZ response to nitrate

To investigate the link between *BUZZ* and nitrate, we first examined overall root architecture. Notably, the *buzz* mutant has twice the lateral root density compared with wild-type *Bd21* (Fig. 8a). In the presence of no or little nitrate, lateral root initiation is suppressed, but in  $\frac{1}{2}$  MS (control) or high nitrate conditions, both lateral root density and length increases (Fig. 8b,c). In addition, primary roots do not change their growth rate in a step-wise response to differential nitrate levels in *buzz* vs *Bd21*, thus displaying an increased nitrate foraging phenotype (Fig. 8d). Then, we quantified total nitrogen in shoots and roots and found no

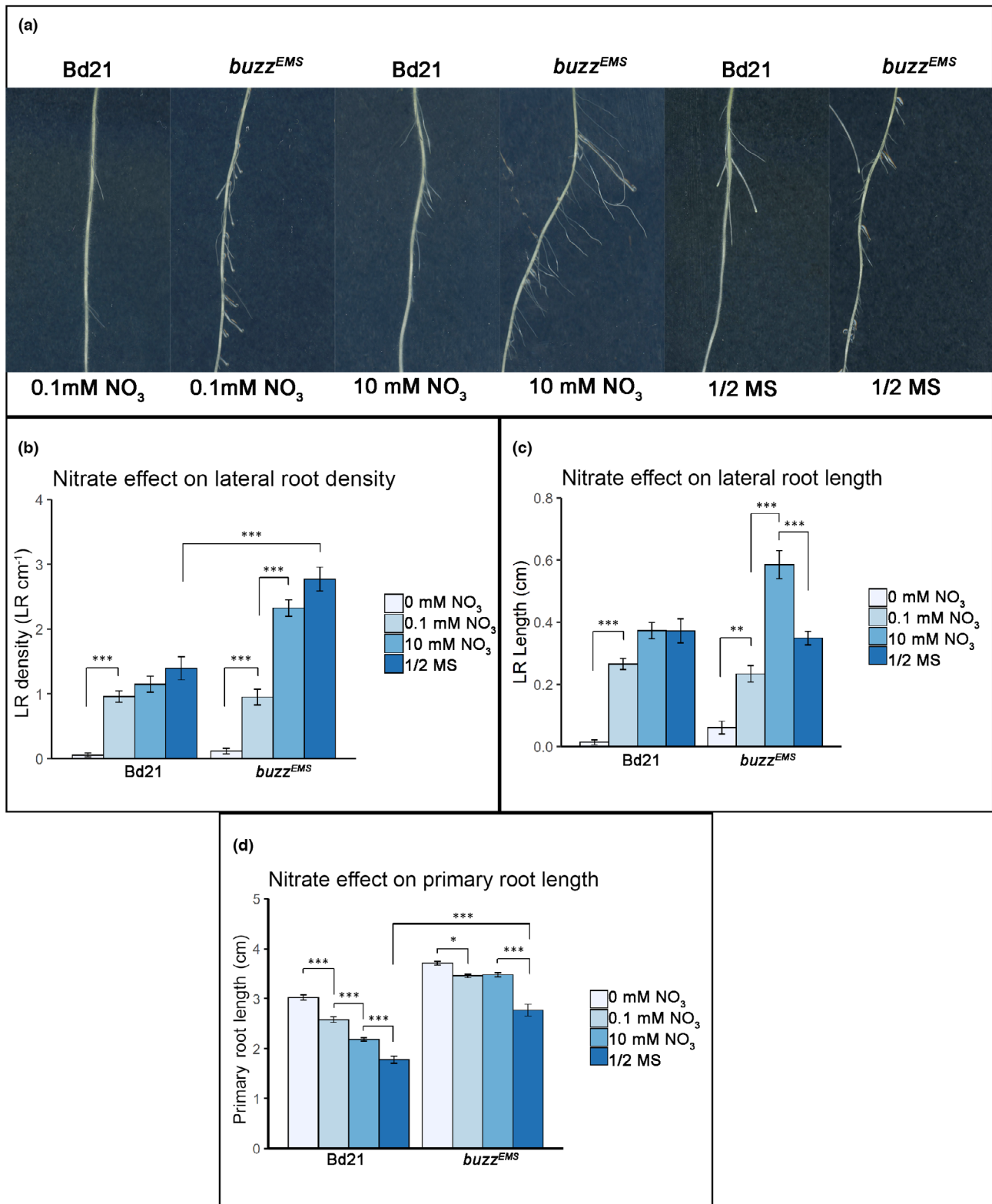


**Fig. 8** Nitrate effects on lateral and primary roots in *Brachypodium distachyon* Bd21 and *buzz*. (a) Micrographs of Bd21 and *buzz* roots grown on 0.1 mM nitrate, 10 mM nitrate, and the 1/2 Murashige and Skoog (MS) control. 1/2 strength MS contains c. 20 mM ammonium nitrate. (b) Nitrate effect on lateral root density. (c) Nitrate effect on lateral root length. (d) Nitrate effect on primary root length. Bar plots means  $\pm$  SE of 30–40 individuals. In (b–d), equivalence of the indicated means is rejected by a two-sided *t*-test: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P \leq 0.001$ . LR, lateral root.

statistically significant difference between Bd21 and *buzz* (Fig. S10).

### STRING analysis reveals putative BUZZ protein–protein interactors

To provide insight into a potential mechanism for BUZZ function, we performed STRING analysis with the BUZZ and AtBUZZ protein sequences. Overall, 10 proteins are predicted to interact with BUZZ including four cyclins (CYCH;1, CYCT1;1, CYCT1;2, and CYCT1;3), three DNA-directed RNA polymerases (NRPD1A, NRPD1B, and NRPB1), and KTF1, an effector of RNA-mediated DNA methylation triggered by small RNAs (Fig. S11). These results suggest that BUZZ is localized to the nucleus at least during some part of the cell cycle.

### Discussion

Here, we investigated the root hair mutant *buzz* in *B. distachyon*, a model for temperate grasses. In the literature, the *buzz* phenotype of increased root growth and lack of root hair elongation is rare. To our knowledge, none of the myriad mutants with short root hairs in *A. thaliana* have been reported to increase root growth rate. However, in *B. distachyon*, increased root growth rate and shorter root hairs occur in at least two other mutants, namely in loss-of-function alleles of *TAR2-LIKE* and *ETHYLENE INSENSITIVE 2-like 1* (Pacheco-Villalobos *et al.*, 2013). Interestingly, in these two mutants, both phenotypes are rescued by ACC treatment, whereas in *buzz*, that treatment is ineffective. That several mutants exist in *B. distachyon* with slow root hairs and fast roots, possibly reflecting distinct pathways, suggests that the processes could be linked through some sort of metabolite partitioning (e.g., flow of sucrose) such as is the case for the TOR pathway that affects root and root hair growth in *A. thaliana* (Malinovskiy *et al.*, 2017; Salem *et al.*, 2018). Nevertheless, we favor the more straightforward interpretation that BUZZ promotes tip growth in root hairs while repressing growth of the root body.

Regardless of any partitioning scheme linking the root hairs to the main root, BUZZ is necessary for a root hair to elongate. In *buzz*, the earlier stages of root hair development, namely pattern formation and bulging (Dolan *et al.*, 1994), proceed evidently as in the wild-type. However, after bulge formation, root hair expansion fails to occur, suggesting BUZZ is required for the bulge-to-tip transition.

Given the pivotal role of BUZZ for growth – positive for root hairs, negative for the root – we were surprised it was revealed to be a CDK-like gene. These genes are well studied as regulators of cell division (Menges *et al.*, 2005). In the primary root, *buzz* plants grew faster than Bd21 not only because elemental elongation rate (aka ‘cell’ elongation) was stimulated and the elongation zone was lengthened, but also because cell production rate was increased. Producing cells faster is expected to lengthen the elongation zone (Beemster & Baskin, 1998) but as a rule, cell expansion rates in the elongation zone are not tied closely to cell production rates (Baskin, 2013). Nevertheless, as far as BUZZ activity in the root goes, holding back the cell cycle could be its

function. However, for root hairs, while division machinery is certainly involved in fate determination (Caro *et al.*, 2007), the process of tip growth itself is long past cytokinesis and, to our knowledge, has not been shown to be regulated by components of the cell cycle. Given that BUZZ is on a branch of the CDK family tree located somewhat removed from the familiar division-regulating members and is considered CDK-like (Menges *et al.*, 2005), we hypothesize that, for the root hair, BUZZ has evolved to regulate new targets.

The question remains, however, of BUZZ CDK activity. The *buzz*<sup>EMS</sup> phenotype results from a nonsynonymous mutation in a highly conserved glycine residue in the presumptive catalytic domain. Although modeling suggests the substitution does not change the overall protein structure (Fig. S5), given the change from neutral to charged, it might affect protein–protein interactions or catalytic properties (i.e. kinase activity). Not unexpectedly, STRING analysis of BUZZ predicts that it interacts with the H and T cyclins CYCH;1 and CYCT1;1, CYCT1;2, and CYCT1;3, which have been implicated in stomatal opening and drought stress and viral resistance, respectively (Cui *et al.*, 2007; Zhou *et al.*, 2013). Future research will focus on confirming putative cyclin-binding partners for BUZZ in addition to potential substrates.

Previously, class I *RSL* genes have been shown to be sufficient for root hair development with double knockdowns exhibiting decreased root hair length (Kim & Dolan, 2016). Therefore, we hypothesized the *RSL* pathway might be perturbed in *buzz*. Indeed, we discovered overexpression of *RSL1* and *RSL2* in *buzz* (Fig. 7a; Kim & Dolan, 2016). These results suggest that the *buzz* defect inhibits root tip growth, specifically the bulge-to-tip transition independently of *RSL* genes. We envision a model in which *RSL* proteins work upstream of BUZZ to transmit a signal for root hair tip growth. When the signal pathway is interrupted, as in the *buzz* mutant, root-specific *RSL* genes are overexpressed as a consequence of positive feedback acting on the root hair pathway. By contrast, *RHD6* and *RSL4* are not misexpressed in the *A. thaliana buzz* mutant but other root hair genes including *TRH1*, *WER*, and *GL2* are overexpressed (Fig. S7k), indicating some divergence of the root hair signaling pathway between monocots and eudicots.

To gain further insight into pathways regulated by BUZZ, we used RNA-Seq, comparing Bd21 and *buzz* root tips (Fig. 7b). We found differentially expressed transcripts involved in hormone signaling, cell wall synthesis, and the cytoskeleton. Of particular interest, one of the cytoskeletal genes strongly overexpressed in *buzz* is an  $\alpha$ -tubulin gene, *TUA1*. In many species, *TUA1* is expressed only in the male gametophyte and is alleged to have isoform-specific functions (Yu *et al.*, 2009). Nevertheless, *TUA1* is expressed in *B. distachyon* root tips (Fig. 7b), suggesting that the isoform is used by grasses for tip growth not only of pollen tubes but also of root hairs. Another strongly upregulated transcript in *buzz* encodes the calcium sensor CIPK10, which in *A. thaliana*, when overexpressed leads to shorter but wider pollen tubes (Zhou *et al.*, 2015). This is not surprising given that calcium signaling is an essential component in tip growth (Mendrinna & Persson, 2015).

In addition, analysis of upregulated transcripts in *buzz* reveals an overrepresentation of transcripts associated with small nucleolar ribonucleoproteins (snoRNPs; Fig. 7c), proteins that promote the assembly of ribosomes via maturation of ribosomal RNA (Ojha *et al.*, 2020). These data suggest that ribosomal RNA processing and mature ribosome assembly might play a disproportionate role in the formation and elongation of root hairs. This is in line with a recent study that found an *A. thaliana* *pimilio23* (*Atpum23*) allele of a ribosome biogenesis gene caused root hairs to adopt the nonhair cell fate (Wang *et al.*, 2020). In line with these data, STRING analysis with AtBUZZ (Fig. S11) predicts protein–protein interactions with several RNA polymerase subunits as well as components of the RNA-dependent DNA methylation (RdDM) pathway, including NRDP1, NRDP2, KTF1, and GTA2. In *A. thaliana*, RdDM mutants closely resemble the wild-type, but genes in question have been implicated in tolerance to various stresses (Popova *et al.*, 2013; Erdmann & Picard, 2020). Therefore, it is intriguing to consider that BUZZ may be at the crux of an RdDM pathway mediating nitrate status.

Another intriguing finding from RNA-Seq was that nutrient transporters were upregulated. In *A. thaliana*, ectopic expression of *AtGRXS8*, a plant-specific glutaredoxin regulated by nitrate (Patterson *et al.*, 2016), represses primary nitrate response genes including nitrate transporters, a repression that eliminates nearly all lateral roots and eliminates or shortens root hairs (Jung *et al.*, 2018; Ehrary *et al.*, 2020). However, while the short or missing root hair phenotype of *AtGRXS8* overexpression lines resembles the root hairless mutant *buzz*, the concomitant suppression of lateral roots is distinct. Further distinguishing short or missing root hairs from BUZZ loss of function and *AtGRXS8* overexpression, in *buzz* according to our RNA-Seq, the nitrate transporters *NRT1.1A* and *NRT1.1B* are among its most highly upregulated transcripts (Fig. 7). This transporter is well known to be pivotal for enhancing nitrate responses in roots (Munos *et al.*, 2004; Remans *et al.*, 2006), including stimulating lateral root development in response to nitrate availability (Krouk *et al.*, 2010; Bouguyon *et al.*, 2015; Riveras *et al.*, 2015). Consistently, *buzz* root system architecture appears to respond vigorously to nitrate (Fig. 8). Additionally, BUZZ is linked to nitrate assimilation through the *buzz* mutant having another highly upregulated transcript, *CIPK23*, which encodes a so-called ‘calcineurin B-like protein-interacting protein kinase’. This kinase switches NRT1.1 between low- and high-affinity nitrate binding, in response to calcium signals (Ho *et al.*, 2009). Taken together, we suggest that BUZZ mediates nitrate-responsive growth both in the primary and lateral roots.

Consistent with BUZZ being active in nitrate pathways, the mRNAs for *BUZZ* and *NRT1.1A* overlap spatially, strikingly so at the root hair tip (Fig. 6). *NRT1.1* in *A. thaliana* has been reported to be expressed in root hairs (Lan *et al.*, 2013), and two tomato nitrate transporters, *LeNrt1-1* and *LeNrt1-2*, are expressed in root hairs (Lauter *et al.*, 1996). Presumably, for nitrate assimilation, BUZZ acts after the root hair has started to elongate.

On a whole plant level, root hairs massively increase the surface area of root systems and facilitate nutrient and water

acquisition, especially during periods of stress. For example, the involvement of root hairs for phosphorus acquisition has been characterized in barley, wheat, and maize, with low phosphorus availability increasing root hair length presumably to increase phosphorus uptake (Gahoonia *et al.*, 1997; Gahoonia & Nielsen, 2004; Zhu *et al.*, 2010; Brown *et al.*, 2013; Haling *et al.*, 2013). Furthermore, mutants lacking root hairs tend to assimilate fewer nutrients and have decreased biomass and yield (Hochholdinger & Zimmermann, 2008). However, in *B. distachyon*, *rs1* root hair mutants grow poorly on low phosphorus but ectopic expression of *RSLs*, while consistently increasing root hair length, affected phosphorus acquisition inconsistently (Zhang *et al.*, 2018), findings that suggest no simple relation between root hair length and assimilation. Root hairs, given their prowess for organized exocytosis, might also play a substantial role in the plant’s delivery of material to the rhizosphere; this potential role of root hairs has received scant attention. While the idea is commonplace that root hairs are crucial for plant success particularly under stressful conditions, we have a great deal more to learn about how root hairs function in the life of the plant.

Here, we discover a gene, BUZZ, that drives root hair tip growth and represses cell division, elemental elongation, lateral root formation, and apparently nitrate responsiveness. BUZZ appears to play a role in the developmental plasticity of the root system in response to changes in nutrient status. Future research will focus on elucidating connections between these phenotypes as well as how BUZZ engages, in both root hair and root body, with the machinery for cell growth and division.

## Acknowledgements

We thank Lynne Brenner (WSU-IPN) for generously letting us use the cryostat and RNAscope incubator. We thank John Vogel and Jennifer Bragg for sharing T-DNA lines from *Brachypodium distachyon*. We thank Daniel Woods for crossing the *buzz* line to Bd3-1 for initial mapping. Funding was provided by the Washington State University CAHNRS Emerging Research Initiative grant 3019-0100 (PG00012803), USDA/NIFA Hatch projects 1014527, and 1015621 as well as start-up funds from WSU, CAHNRS, and CSS to KAS. We thank Dr Xiaoli Yang (China Agriculture University) for root imaging for kinematics. Early stages of this work were supported by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the US Department of Energy (grant no. DE-FG-03ER15421) to the laboratory of TIB.

## Competing interests

None declared.

## Author contributions

TAL, MAR, RATBA, TIB and KAS planned and designed the research. TAL, MAR, RATBA, SS, ZBY, RD, YW, ER, PZ, DM and KAS performed research and analysis. TAL, MAR, RATBA, TIB and KAS wrote the manuscript. All authors read

and edited the manuscript. TAL and MAR contributed equally to this work.

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## Data availability

Sequence data for *Brachypodium distachyon* and *Arabidopsis thaliana* BUZZ and related sequences are publicly available. The raw data generated from RNA sequencing of Bd21 and buzz 5-d-old root tips presented in Fig. 7 has been deposited in NCBI and can be accessed at the following link: <https://www.ncbi.nlm.nih.gov/sra/PRJNA973671>. Any reasonable request for germplasm or other information will be happily provided by the corresponding author.

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## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** *Brachypodium distachyon* buzz phenotype in soil compared with Bd21 wild-type.

**Fig. S2** Analysis of root cell length, meristem length, yield and plant height, and root biomass and shoot biomass in *Brachypodium distachyon* buzz compared with Bd21 wild-type.

**Fig. S3** Confocal and bright field images of the *Brachypodium distachyon* root epidermis in Bd21 and buzz.

**Fig. S4** Auxin and ACC fail to rescue the *Brachypodium distachyon* buzz phenotype.

**Fig. S5** Structural comparison between the wild-type BUZZ protein and BUZZ<sup>EMS</sup> protein.

**Fig. S6** RT-PCR validation of the buzz and Atbuzz alleles in *Brachypodium distachyon* and *Arabidopsis thaliana*.

**Fig. S7** Auxin and ACC rescue the Atbuzz phenotype in *Arabidopsis thaliana*.

**Fig. S8** Complementation of the buzz root hair phenotype in *Brachypodium distachyon*.

**Fig. S9** RNAscope in greyscale.

**Fig. S10** TruSpec analysis in *Brachypodium distachyon* Bd21 and buzz shoots and roots.

**Fig. S11** STRING analysis of BUZZ and Atbuzz.

**Table S1** Protein sequences used for structural analysis.

**Table S2** Protein sequences used to generate the BUZZ phylogenetic tree.

**Table S3** Primer list for cloning *Brachypodium distachyon* BUZZ, *Arabidopsis thaliana* BUZZ, and GFP.

**Table S4** qRT-PCR and RT-PCR primer list.

**Table S5** Workflow for RNA-Seq analysis.

**Table S6** List of 478 transcripts upregulated in buzz vs wild-type Bd21 and 184 transcripts downregulated in buzz vs wild-type Bd21.

**Video S1** Expression of BUZZ, NRT1.1A, and CSLD4/KOJAK with DAPI in the longitudinal section of *Brachypodium distachyon* root.

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