# Abscisic acid mediates barley rhizosheath formation under mild soil drying by promoting root hair growth and auxin response

**Running title:** Barley rhizosheath under mild soil drying

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**Highlight:** Mild soil drying induces barley rhizosheath formation by modulating abscisic acid and auxin responses for root elongation and root hair growth.

**ABSTRACT**

Soil drying enhances root ABA accumulation and rhizosheath formation, but whether ABA mediates rhizosheath formation is unclear. Here, we used the ABA–deficient mutant *Az34* to investigate molecular and morphological changes by which ABA could affect rhizosheath formation. Mild soil drying with intermittent watering increased rhizosheath formation by promoting root and root hair elongation. Attenuated root ABA accumulation in *Az34* barley constrained the promotion of root length and root hair length by drying soil, such that *Az34* had a smaller rhizosheath. Pharmacological experiments of adding fluridone (an ABA biosynthesis inhibitor) and ABA to drying soil restricted and enhanced rhizosheath formation respectively in Az34 and wild–type Steptoe barley. RNA sequencing suggested that ABA accumulation mediates auxin synthesis and responses, root and root hair elongation in drying soil. In addition, adding indole–3–acetic acid (IAA) to drying soil increased rhizosheath formation by promoting root and root hair elongation in Steptoe and *Az34* barley. Together, these results show that ABA accumulation induced by mild soil drying enhance barley rhizosheath formation, which may be achieved through promoting auxin response.

Keywords: abscisic acid, auxin, rhizosheath formation, root hair, root length, soil drying

## INTRODUCTION

Decreased water availability for agricultural purposes threatens future global crop yields, exacerbated by climate change (Gupta, Rico–Medina, & Cano–Delgado, 2020; L. Xu et al., 2018). Roots play an important role in water uptake (Carminati et al., 2017), and enhancing root water uptake can improve plant performance in drying soil. Root hairs (single celled projections from the roots) constitute up to 77% of the root surface area in crops (Parker, Cavell, Dolan, Roberts, & Grierson, 2000) and for a root of 1 mm diameter, root hairs (0.5 or 1 mm average length) will improve water uptake by 30 to 55% compared to roots lacking root hairs (Segal, Kushnir, Mualem, & Shani, 2008; Wang et al., 2017). Nevertheless, the impact of root hairs on plant water uptake seems to depend on prevailing evaporative demand, as they seem redundant (Dodd & Diatloff, 2016) or essential (Carminati et al., 2017) at low and higher evaporative demand respectively. They may also enhance rhizosheath formation (L.K. Brown et al., 2012), especially in drying soil.

The rhizosheath is the soil that adheres to the root surface as a consequence of root hair penetration between soil particles and mucilage secretion from roots or microbes that bind the particles together. The rhizosheath is beneficial for water acquisition as it has higher water content than bulk soil (Young, 1995) thereby increasing water uptake (Zhang et al., 2020a). Drought–tolerant plants generate a greater and more porous rhizosheath mass than drought–sensitive plants (Rabbi et al., 2018; Zhang et al., 2020a). Root hairs are one of the major determinants of rhizosheath formation and might confer associated rhizosheath benefits (George et al., 2014; Haling et al., 2014; Robertson–Albertyn et al., 2017; Watt, McCully, & Canny, 1994). They permeate the rhizosheath (McCully, 1999) and provide a physical framework for extending the rhizosheath (Watt et al., 1994). Moderate soil drying can stimulate both root hair growth and rhizosheath formation in some crops, such as maize (*Zea mays*, Watt et al., 1994), foxtail millet (*Setaria italica*, Liu et al., 2019) and rice (*Oryza sativa*, Zhang et al., 2020ab). Combining longer root hairs and larger rhizosheaths may protect agricultural yields despite decreased water availability.

Abscisic acid (ABA) is the main plant stress hormone regulating plant responses to soil drying (McAdam, Brodribb, & Ross, 2016). Under moderate water deficit, ABA establishes and maintains root meristem function and stimulates root elongation (Liang, Mitchell, & Harris, 2007; Rosales, Maurel, & Nacry, 2019; Yang et al., 2014). High exogenous ABA concentrations or ABA accumulation in well–watered conditions can inhibit plant growth (Rowe, Topping, Liu, & Lindsey, 2016; Shi et al., 2015; Tardieu, Parent, & Simonneau, 2010). However, low concentrations of applied ABA or ABA accumulation can promote root growth (X. Li, Chen, Forde, & Davies, 2017; Sharp, 2002; W. Xu et al., 2013) and root hair elongation (Wang et al., 2017). ABA controls root hair growth by regulating the root hair transcription factor Root Hair Defective 6–Like2 and transcriptional regulator OBF BINDING PROTEIN4 in Arabidopsis (Rymen et al., 2017). Exogenous ABA (0.1–2 μM) can promote root hair elongation via the conserved PYR/PP2C/SnRK2 ABA signalling module and crosstalk with auxin biosynthesis in rice (Wang et al., 2017). Conversely, higher ABA concentrations (0.25–10 μM) in agar plates reduced root hair growth in another study (Rymen et al., 2017). Despite these observations, the role of ABA in regulating root hair growth in drying soil is not clear.

Recently, rice rhizosheath formation was associated with ABA accumulation under moderate water stress (Zhang et al., 2020a), however, how ABA in regulating rhizosheath formation remains largely unknown. The application of ABA–deficient mutants (Humplík, Bergougnoux, & Van Volkenburgh, 2017) have been widely used to investigate the physiological role of ABA in regulating root growth, but not to address whether ABA–mediated root (hair) elongation affects rhizosheath formation. We hypothesized that ABA could mediate rhizosheath formation by promoting both root and root hair elongation, and used transcriptomics to address the underlying molecular mechanisms. Thus, we used wild–type (*Hordeum vulgare* cv. Steptoe) and ABA–deficient mutant *Az34* barley plants (Martin–Vertedor & Dodd, 2011) to investigate whether ABA affected rhizosheath formation.

## MATERIALS AND METHODS

### Plant materials and experimental design

All experiments were conducted in a controlled greenhouse with light–dark cycle of 14/10 h from LED light with photosynthetic photon flux density was 300 μmol photons $m^{-2}$ $s^{-1}$, day–night temperature cycle of 26°C/22°C, and relative humidity was 40%. Soil used in pot experiments was collected from a paddy rice field (0–20 cm depth) located near the town of Huayang, Jiangxi Province, China (115°09′32″E, 28°32′29″N). The physical and chemical properties of soil are summarized in Supplementary Table S1.

Two barley (*Hordeum vulgare*) cultivars, Steptoe and Optic were used to assess the root growth and rhizosheath formation under difference watering treatments. Optic was often used in studies of barley rhizosheath formation (Brown et al., 2012; George et al., 2014). Steptoe was the wild type barley of ABA–deficient mutant *Az34* (Martin–Vertedor & Dodd, 2011) which was also utilized in this study. *Az34* mutant was originally selected based on nitrate reductase deficiency after sodium azide mutagenesis (Kleinhofs, Warner, Murhlbauer, & Nilan, 1978). The F1 seedlings produced from the cross *Az34* by Steptoe were normal in NADH–nitrate reductase activity; and the segregation of the F2 seedlings grown in the field indicate a single, recessive Mendelian trait (Kleinhofs, Kuo, & Warner, 1980). Then, it was also found to be deficient in the molybdenum cofactor–requiring enzyme aldehyde oxidase required for oxidation of ABA precursor (Walker–Simmons, Kudrna, & Warner, 1989). Thus *Az34* has reduced ABA concentrations under well–watered conditions and cannot produce as much extra ABA as the wild type under soil drying (Bacon, Wilkinson, & Davies, 1998).

Seeds were surface–sterilized using 1.5% (v/v) sodium hypochlorite for 15 min and rinsed with double–distilled water for 5 times, then were placed on moistened filter paper in the dark for 2 days. After that, seeds were placed on moistened filter paper in a 14/10 h light–dark cycle for 3 days prior to planting. Seedlings of uniform size were transplanted to pots (12 cm diameter, 18 cm height) with 2.6 kg dry soil. Each pot had

a single seedling. In the first week, each pot was irrigated with 200 ml each day, then irrigation treatments were started and lasted for 14 days. Well–watered plants received 200 ml water every 2 days. Soil drying (SD) treatment Ⅰ (SD1) plants were irrigated 400 ml water every 6 days. Soil drying treatment Ⅱ (SD2) plants were irrigated 200 ml water every 6 days. Pots were weighed daily to estimate soil water content.

In the pharmacological experiments, 1.5 mL solution was uniformly sprayed on leaves of each barley plant for 14 days under SD1. Total root length and average root hair length in Steptoe barley was studied after application of different concentrations of ABA (0, 1, 5, 10, 20 μM) and indole-3-acetic acid (IAA, 0, 0.5, 1, 2, 3, 5 and10 nM) was used under soil drying. Further, ABA (1 μM), ABA inhibitor fluridone (10 μM, Zhang et al., 2020a), ABA(1 μM) + fluridone (10 μM), and IAA (1 nM) treatments were applied to evaluate plant phenotypes and rhizosheath formation under SD1.

**Plant traits and rhizosheath weight**

Pots were tipped out, and roots gently shaken to remove bulk soil; leaving the rhizosheath soil. Root fresh weight with adhering rhizosheath was recorded. The root system was washed, then rhizosheath soil with water was collected and dried at 105°C for 3 days, and soil weighed to determine rhizosheath weight. Root images were scanned using an Epson scanner (Epson, Herts, UK) and total root length analysed using a winRHIZO software (Regent Instruments, Quebec, Canada). After that, root fresh weight was measured. Specific rhizosheath weight was calculated as rhizosheath weight divided by total root length. Root hair length was determined as previously described (Zhang et al., 2020b). Briefly, root fragments excised from crown roots were photographed under a SMZ18 stereomicroscope using a DS–U3 camera (Nikon, Tokyo, Japan). Then root hair length was measured using Image J software (US National Institutes of Health, Maryland, USA). Roots and shoot dry weights of each plant were recorded after samples dried at 80°C for 2 days. The water content of rhizosheath soil was calculated as follows: (fresh weight of root with rhizosheath soil

168 − fresh weight of root– dry weight of rhizosheath soil) ÷ dry weight of rhizosheath
169 soil. Bulk soil water content was also calculated.
170
171 **Determination of leaf and root ABA concentration**
172 The youngest fully expanded leaf was harvested, then frozen in liquid nitrogen. The
173 pot was disassembled and the total root was collected and quickly cleaned, frozen in
174 liquid nitrogen. Leaf and root samples were stored at –80°C until ABA determination.
175 For ABA determination, samples were freeze dried, finely ground and weighed. ABA
176 in sample was dissolved using deionized water with 1:50 weight ratio (sample: water)
177 in tube. Then the tube was shaken for 24 h at 4°C for ABA fully extraction. The
178 supernatant was analysed by a radioimmunoassay method as described (Quarrie et al.,
179 1988).
180
181 **Root collection, RNA isolation, RNA sequencing and data analysis**
182 For RNA–seq, roots of Steptoe and *Az34* barley under WW and SD treatments were
183 rapidly harvested, washed, dried with paper towel, frozen in liquid nitrogen
184 immediately. Each treatment had three replicates. Samples were stored at –80 °C
185 until subsequent RNA extraction and sequencing. Root total RNA extraction, quality
186 control and quantification was perfomed as described previously (Zhang et al., 2016).
187 Sequencing libraries were generated using NEBNext Ultra (NEB, MA, USA) and
188 sequenced using the BGISEQ–500 sequencer (BGI, Shenzhen, China) as described
189 previously (Zhang et al., 2020a). Raw reads were pre–processed using SOAPnuk
190 (version 1.4.0) and trimmomatic (version 0.36). Adapter sequences, each read
191 containing ≥5% poly–N, and low–quality reads (Q–score ≤ 10 for ≥ 20% of
192 nucleotides per read) were filtered. The clean data were deposited to NCBI in SRA
193 (SRR13559037–SRR13559048). The resulting clean data were mapped to the
194 reference genome (*Hordeum_vulgare*_L., release–39) using Hierarchical Indexing
195 for Spliced Alignment of Transcripts (HISAT, version 2.1.0). Read alignment was
196 used Bowtie 2 (version 2.2.5), and RSEM was used to calculate the expression of

genes and transcripts. DEGseq (Fold Change ≥ 2, and adjusted *P* value ≤ 0.001) was used for identifying differentially expressed genes (DEGs). Gene Ontology (GO) classifications and pathway analysis was used GO (http://www.geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg), respectively. Pathway enrichment was used R package "phyper".

**RT–qPCR**

For reverse–transcription quantitative polymerase chain reaction (RT–qPCR) analysis, total RNA extracted as above was reverse–transcribed into cDNA using Strand cDNA Synthesis kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. RT–qPCR was performed as described previously (Zhang et al., 2020a). The transcript levels were normalized to that of Ubi1 as an endogenous control. The primers used were listed in Supplementary Table S2. Each of three biological replicates was represented by three technical replicates.

**Statistical analysis**

Statistical analyses were performed using SPSS (version 17.0, IBM). Two–way ANOVA determined the effects of irrigation treatment, cultivar, and their interaction. Statistically different groups were determined by a one–way ANOVA at $p < 0.05$. Datasets were checked for normality using homogeneity of variance test. If variances were homogeneous equal, *post hoc* Duncan's test was applied for multiple comparisons. If variances were homogeneous unequal, *post hoc* Tamhane's T2 test was used for analysis. Graphs were generated using SigmaPlot12.5 (Systat Software Inc. Chicago, IL, USA), or R package.

**RESULTS**

**Soil drying increased rhizosheath formation of barley**

Well–watered (WW) barley plants were irrigated every two days, so that soil water

content did not drop below 30%, with slightly higher values for Optic than Steptoe throughout much of the experiment (Fig. 1a). Soil water content of both soil drying treatments (SD1 and SD2) declined to 17% on Day 5, whereupon different irrigation volumes recovered values to 29% and 21% respectively on Day 6. Soil drying occurred to 16% and 13% respectively on Day 11, with recovery to 27% and 17% on Day 12, respectively. At the end of the experiment (Day 14), soil water contents of the WW, SD1 and SD2 treatments were about 30%, 24% and 15%, respectively, with similar values in both genotypes when grown under the same treatment.

Both cultivar and irrigation treatment significantly affected shoot dry weight, total weight of whole plant and root to shoot ratio (Fig. 1b, Fig. S1a–b), but both cultivars responded similarly to soil drying (no cultivar x treatment interaction). Well–watered Optic plants had 19% more shoot biomass than WW Steptoe plants. Soil drying decreased shoot biomass of Optic plants by 17% and 28% in the SD1 and SD2 treatments respectively, and of Steptoe by 10% and 44% in the comparable treatments. Total biomass of Optic was relatively unresponsive to the watering treatments while the SD2 treatments decreased total biomass of Steptoe by 38% (Fig. S1a). Both soil drying treatments increased root to shoot ratio of both genotypes similarly (Fig. S1b). Thus soil drying significantly decreased shoot biomass (and to a lesser extent total biomass), but increased root to shoot ratio in both barley cultivars.

Soil drying significantly increased total root length, but the two cultivars responded differently to the SD2 treatment. Whereas the SD1 treatment increased total root length by about 69% (compared to well–watered plants) in both cultivars, the SD2 treatment increased total root length by 105% and 53% in the Optic and Steptoe cultivars, respectively (Fig. 1c). Average root hair length of both cultivars responded similarly to the treatments (Fig. 1d), with the SD1 and SD2 treatments increasing root hair length by 41% and 22% compared to well–watered plants in Optic, and by 66% and 27% in Steptoe. Absolute rhizosheath weight increased with the severity of soil drying in Optic, with the SD1 and SD2 treatments increasing rhizosheath weight by 1– and 1.3–fold respectively compared to well–watered plants. In Steptoe, the SD1

255 treatment generated the greatest rhizosheath (96% higher than WW plants) whereas
256 the SD2 treatment had a smaller effect (37% higher than WW plants). Specific
257 rhizosheath weight (rhizosheath per unit weight length) changed comparatively little
258 in Optic (increased by 22% only in the SD1 treatment) but increased in Steptoe in the
259 SD1 treatment (by 12%) and decreased in the SD2 treatment (by 14%). While mild
260 soil drying (SD1) stimulated root and root hair length, and absolute and specific
261 rhizosheath weight in both cultivars, more severe drying (SD2) had less stimulative
262 effect or even negative effect in the two cultivars.

263 Although bulk soil water content (when harvested) decreased in the SD1 and SD2
264 treatments (Fig. 1a), the ratio of rhizosheath to bulk soil water content increased by
265 54% and 70% respectively, implying maintenance of rhizosheath water content (Fig.
266 S1c). Root ABA concentrations in well–watered Optic plants 41% higher than Steptoe
267 plants (Fig. S1d). Root ABA concentrations increased with soil drying, with both
268 cultivars having similar root ABA concentrations in the SD2 treatment. In the SD1
269 treatment, root ABA concentrations of Optic plants were 9% higher than Steptoe
270 plants. Overall, root ABA concentration was correlated with bulk soil (and
271 rhizosheath) water content in SD1, with the SD1 treatment selected for further
272 experiments as it increased root (and root hair) length and rhizosheath development in
273 both cultivars.
274

275 **ABA increased rhizosheath formation by promoting root and root hair growth**

276 The ABA–deficient *Az34* barley mutant and wild–type (WT) Steptoe barley were
277 used to investigate the effects of endogenous ABA concentration on rhizosheath
278 formation. Both leaf and root ABA concentrations showed similar effects of genotype
279 and soil drying, with attenuated ABA accumulation in the *Az34* mutant (as indicated
280 by significant genotype x treatment interactions Fig. 2a–b). Under well–watered
281 conditions, leaf ABA concentrations of Steptoe were 12% higher than *Az34*. Soil
282 drying increased leaf ABA concentration WT Steptoe by 14%, but had no significant
283 effect in *Az34*. Under well–watered conditions, root ABA concentrations of Steptoe

were 56% higher than *Az34*. Soil drying increased root ABA concentration by about 24% for both genotypes. Genotypic differences in endogenous ABA concentrations were greater in roots than in leaves.

Under WW conditions, both genotypes had a similar total root length, but soil drying increased total root length more in WT (by 89%) than that in *Az34* (by 40%), as indicated by a significant genotype x treatment interaction (Fig. 2c). Under WW conditions, WT root hairs were 79% longer than *Az34* root hairs. Soil drying increased average root hair length of both genotypes similarly by 72% (Fig. 2d). Both absolute rhizosheath and specific rhizosheath dry weight of WT plants was greater than *Az34* plants irrespective of soil moisture (Fig. 2e–f). While soil drying increased specific rhizosheath dry weight of both genotypes similarly by 102% (no genotype x treatment interaction), absolute rhizosheath increment in response to soil drying was greater in WT than *Az34* plants. Thus the ABA–deficient barley mutant had shorter root hairs, and less rhizosheath mass independently of soil drying.

To further understand the role of ABA in root hair growth and rhizosheath formation, ABA and its biosynthesis inhibitor fluridone were applied to both genotypes grown in drying soil. Total root length and average root hair length were both significantly increased after using 1 μM of exogenous ABA compared with 0 μM ABA treatment in Steptoe barley under soil drying (Fig. S2). Therefore, we selected exogenous 1 μM ABA for our later experiments. The expression of ABA response gene (HORVU7Hr1G035500: ABA responsive element binding factor 2, *ABF2*) was also assessed in Steptoe and *Az34* barley under soil drying and soil drying with exogenous ABA (1 μM) treatments for 14 days. Results showed that exogenous ABA could increase the expression of gene *ABF2* in both two barley genotypes (Fig. S3). Genotype, irrigation treatment and their interaction all significantly affected total root length, rhizosheath and specific rhizosheath dry weight of barley plants under soil drying and Genotype, irrigation treatment significantly affected average root hair length (Fig. 3). Total root length, average root hair length, rhizosheath and specific rhizosheath dry weight were significantly larger in Steptoe than *Az34* plants, with

ABA application significantly increasing all phenotypes consistently across both genotypes. Also, fluridone application decreased all these phenotypes and ABA application could partly reverse the effect of fluridone (Fig. 3). Thus, exogenous ABA application to drying soil enhanced average root hair length, total root length and rhizosheath formation in both *Az34* and WT barley.

**RNA–seq global analysis**

Roots of Steptoe WT and *Az34* barley under WW and SD treatments were used for RNA–seq. Table S3 indicates total raw reads, total clean reads, total clean bases, clean reads Q20, clean reads Q30 and clean reads ratio from RNA–seq. Over 1 G clean bases were obtained and the clean reads ratio was more than 96% from each sample (Table S3). Over 90% of the clean reads were aligned to the barley reference genome (Table S3). Compared with WW plants, soil drying resulted in 2338 DEGs (824 up–regulated and 1514 down–regulated genes) and 1778 DEGs (707 up–regulated and 1071 down–regulated genes) in WT (Steptoe) and *Az34* plants respectively, and 855 DEGs overlapped in both genetypes (Fig. S4).

**DEGs related to ABA, auxin, and root and root hair growth**

From GO and KEGG pathway analysis, there were many DEGs related to the ABA biosynthetic process, ABA receptors and response to abscisic acid (Table S4). For example, a gene encoded a 9–cis–epoxycarotenoid dioxygenase (NCED), a key enzyme in ABA biosynthesis, was down–regulated in *Az34* compared to Steptoe. Soil drying up–regulated genes of the Tryptophan metabolism pathway (an important auxin biosynthesis substrate) in Steptoe WT plants (21 DEGs) but did not induce these genes in *Az34* barley (14 DEGs) compared to WW plants (Fig. S5). Nine of these genes (including aromatic–L–amino–acid decarboxylase–like and tryptophan decarboxylase) were overlapping among these two groups. Soil drying up–regulated 21 genes in WT plants that included aromatic–L–amino–acid decarboxylase–like and tryptophan decarboxylase in the Tryptophan metabolism pathway, while 9 of these 21

genes were less induced in *Az34*. A further 15 DEGs were related to auxin synthesis, 3 were related to auxin transport and 13 related to auxin response were compared in the different genotype x irrigation treatment combinations (Fig. 4). This indicated that *Az34* barley had a weaker induction of auxin synthesis under soil dying than WT barley.

Soil drying also resulted in a further 6 DEGs related to root and root hair growth (Fig. 4). For example, soil drying increased expression of a gene encoding a root–specific lectin by 4.2– and 2.1–fold in Steptoe and *Az34* plants respectively; while this gene had 3–fold more expression in Steptoe than *Az34* roots under well–watered conditions. Furthermore, soil drying down–regulated a gene encoding glutamine synthetase root isozyme 5 by 4.3– and 4.5–fold in Steptoe and *Az34* respectively, even though expression of this gene was similar under WW conditions. Soil drying up–regulated a gene encoding extensin–1–like by 2.4 and 2.6–fold in Steptoe and *Az34* respectively, compared to WW plants. In *Az34,* soil drying up–regulated a gene encoding ROOT HAIR DEFECTIVE 3 homolog 2–like protein by 3.2–fold compared to WW plants; although there was no significant effect in Steptoe plants. Thus, genes related to root and root hair growth were regulated by ABA and soil drying.

**Auxin increased rhizosheath formation by promoting root and root hair growth**

To verify the role of auxin in ABA–mediated rhizosheath formation, exogenous IAA was applied to Steptoe WT and *Az34*. Total root length and average root hair length were significantly increased after application of 1 nM exogenous IAA compared with 0 nM IAA treatment in Steptoe barley under soil drying (Fig. S6). Therefore, we selected exogenous 1nM IAA for our later experiments. The expression of auxin response gene (HORVU2Hr1G110460: auxin–induced protein 6B–like, *AIP6B*) was tested in exogenous IAA treatments in Steptoe and *Az34* barley, the results showed that exogenous IAA could increase the expression of gene *AIP6B* in two barley genotypes under soil drying (Fig. S7). However, the expression of the gene *AIP6B*

was greatly up-regulated in *Az34* compared with the Steptoe under both SD and SD with exogenous IAA (Fig. S7), which show that low ABA content in *Az34* might require a higher IAA response. Genotype and auxin treatment significantly affected total root length, average root hair length, rhizosheath and specific rhizosheath dry weight (Fig. 5). Exogenous IAA application to drying soil enhanced total root length, average root hair length, total rhizosheath and specific rhizosheath dry weight of Steptoe and the *Az34* mutant (Fig. 5). Thus, auxin significantly enhanced rhizosheath development with root and root hair growth.

## DISCUSSION

Soil drying increased rhizosheath dry weight and specific rhizosheath dry weight of two barley cultivars (Fig. 1e and f), suggesting that the rhizosheath is an important trait for coping with drought stress (Lawrie K. Brown, George, Neugebauer, & White, 2017). Though there are conflicting findings on the relationship between rhizosheath size and root hair length as reviewed by Pang et al. (2017), our previous research showed that root hair length greatly influences specific rhizosheath weight in rice (Zhang et al., 2020a). Here, average root hair length influenced specific rhizosheath weight under different watering treatments in both cultivars (Fig. 1d and f). Since soil drying increases rhizosheath formation by promoting root length and root hair growth, and stimulates root ABA accumulation (Fig. S1d and Fig. 2b), we investigated the role of ABA in mediating these adaptive responses.

### ABA promotes rhizosheath formation under soil dying by influencing root and root hair growth

Soil dying induced ABA accumulation in both roots and shoots (Fig. 2a and b). In addition, moderate water stress in rice induces rhizosheath formation associated with abscisic acid (Zhang et al., 2020a). Thus, we hypothesized that soil drying–induced rhizosheath formation was related to ABA accumulation. In support of this hypothesis, the *Az34* (ABA–deficient mutant, Fig. 2a and b) had less rhizosheath and specific

rhizosheath dry weight than its WT under both WW and SD1 (Fig. 2e and f). Moreover, adding exogenous ABA to drying soil generally increased rhizosheath and specific rhizosheath dry weight of both genotypes; and Steptoe still had a higher rhizosheath and specific rhizosheath dry weight than *Az34* (Fig. 3c and d). However, adding exogenous fluridone, an ABA biosynthetic inhibitor, to drying soil sharply decreased rhizosheath and specific rhizosheath dry weight of both Steptoe and *Az34* (Fig. 3c). Thus, increased rhizosheath formation induced by soil drying may depend on ABA accumulation in roots.

Irrespective of soil moisture, the larger rhizosheath and specific rhizosheath weight of Steptoe than *Az34* was correlated with longer root hairs of Steptoe (Fig. 2d–f). While both genotypes had a similar total root length under WW conditions, soil drying greatly increased total root length of Steptoe but had a limited effect in *Az34* (Fig. 2c). In addition, soil drying up–regulated a gene encoding a root–specific lectin in both Steptoe and *Az34* barley plants, and this gene was also up–regulated in *Az34* compared with Steptoe under WW (Fig. 4). Plant lectins are carbohydrate–binding proteins and a plant lectin (named ArathEULS3) in Arabidopsis confers ABA hypersensitivity and enhanced drought tolerance (D. Li et al., 2014). These results suggest that root–specific lectin may be involved in regulating root growth under SD.

Moreover, soil drying up–regulated the root hair related genes extensin–1–like and ROOT HAIR DEFECTIVE 3 homolog 2–like (which act downstream of auxin in Arabidopsis) in *Az34* barley, while the extensin–1–like gene was also up–regulated in Steptoe barley (Fig. 4) (Wong, Mazumdar, Lau, & Harikrishna, 2018). Further, in both genotypes, adding exogenous ABA to drying soil increased root and root hair length (Fig. 3a and b). These results indicate the importance of ABA stimulation of root and root hair elongation in enhancing rhizosheath formation under soil drying.

**ABA accumulation mediates auxin responses to promote rhizosheath formation under soil dying**

Auxin response is also involved in regulating root and root hair growth, and ABA

involved in this regulation (W. Xu et al., 2013; Zhang et al., 2020a). Here, soil drying up–regulated DEGs in Tryptophan metabolism, such as genes encoding aromatic–L–amino–acid decarboxylase–like and tryptophan decarboxylase, while most of these genes were down–regulated in *Az34* (Fig. 4 and S5). Tryptophan–dependent pathways are main pathways for the biosynthesis of IAA, including the indole–3–acetamide pathway, the indole–3–pyruvic acid pathway, the tryptamine pathway, and the indole–3–acetaldoxime pathway (Mano & Nemoto, 2012). Further, many DEGs related to auxin synthesis, auxin transport, auxin response and root hair were also regulated in Steptoe or/and *Az34* under WW compared with SD (Fig. 4). Thus, we proposed that auxin also mediated rhizosheath formation. Adding exogenous IAA to drying soil increased rhizosheath weight and specific rhizosheath weight of Steptoe and *Az34*, along with root and root hair length (Fig. 5).These results indicate that auxin might enhance root and root hair growth to promote rhizosheath formation.

In conclusion, our results show that mild soil dying in barley increases rhizosheath formation. Use of the ABA–deficient *Az34* barley mutant and an inhibitor of ABA synthesis demonstrated that ABA regulates rhizosheath formation under mild soil dying by stimulating root and root hair growth. In addition, transcriptome data and exogenous hormone treatments suggested that root ABA accumulation mediated auxin responses to promote rhizosheath formation under mild soil dying. These findings extends our understanding of rhizosheath formation which may be used to breed drought resistant crop.

## ACKNOWLEDGEMENTS

We are grateful for grant support from the National Key R&D Program of China (2017YFE0118100), National Natural Science Foundation of China (31901428, 31872169), Newton Advanced Fellowship (NSFC–RS: NA160430). We thank Prof. Timothy S. George (The James Hutton Institute, Invergowrie Dundee, DD2 5DA, UK) for donating the *nrh* and Optic barley seeds used in this study, and Dr. Jaime

Puertolas (Lancaster University, Lancaster, UK) for training in measuring of ABA concentration.

## AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: WX, YZ. Performed the experiments: YZ, YD, HD, FX, QZ. Analyzed the data: YZ, XD, YC. Wrote the paper: YZ, FX, WX, ID.

## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available.

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**FIGURE LEGENDS:**

**Fig. 1** Soil drying increased rhizosheath formation of Steptoe and Optic barley cultivars. (a) Daily soil water content under different soil drying treatments. (b) Shoot dry weight, (c) total root length, (d) average root hair length, (e) total rhizosheath and (f) specific rhizosheath weight of Steptoe and Optic barley cultivars after 14 days of soil drying treatments. WW: Well–watered; SD1: Soil drying treatment Ⅰ; SD2: Soil drying treatment Ⅱ; In a–f, data are means ±SE, n=5. Bars with different letters were significantly different at $p<0.05$. Two–way ANOVA determined the effects of genotype (G), irrigation treatment (T), and their interaction (G*T).

**Fig. 2** Rhizosheath dry weight of *Az34* barley (ABA–deficient mutant) was lower than the wild–type Steptoe barley under both well–watered (WW) and soil drying (SD1) treatments. (a) Leaf ABA concentration, (b) root ABA concentration, (c) total root length, (d) average root hair length, (e) total rhizosheath and (f) specific rhizosheath weight of *Az34* barley and Steptoe barley after 14 days soil drying treatments. In a–f, data are means ±SE, n=5. Bars with different letters were significantly different at $p<0.05$. Two–way ANOVA determined the effects of genotype (G), irrigation treatment (T), and their interaction (G*T).

**Fig. 3** ABA increased rhizosheath formation of wild–type Steptoe barley and *Az34* barley (ABA–deficient mutant) by promoting root and root hair growth. (a) Total root length, (b) average root hair length, (c) total rhizosheath and (d) specific rhizosheath weight of Steptoe and *Az34* barley under soil drying (SD1) treatment, SD with exogenous ABA (1 μM), SD with exogenous fluridone (FLU, an ABA biosynthetic inhibitor, 10 μM) and SD with both ABA (1 μM) and FLU (10 μM) for 14 days. In a–d, data are means ±SE, n=5. Bars with different letters were significantly different at $p<0.05$. Two–way ANOVA determined the effects of genotype (G), irrigation treatment (T), and their interaction (G *T).

**Fig. 4** Differentially expressed genes (DEGs) related to auxin and root–hair growth in the roots of wild–type Steptoe barley and *Az34* mutant (ABA–deficient mutant) barley under well–watered (WW) and soil drying (SD1).

**Fig. 5** IAA increased rhizosheath formation of wild–type Steptoe barley and *Az34* barley (ABA–deficient mutant). (a) Total root length, (b) average root hair length, (c) total rhizosheath and (d) specific rhizosheath weight of Steptoe and *Az34* mutant barley under treatments of soil drying (SD1) and SD1 with exogenous indole-3-acetic acid (IAA, 1 nM) for 14 days. In a–d, data are means ±SE, n=5. Bars with different letters were significantly different at $p<0.05$. Two–way ANOVA determined the effects of genotype (G), irrigation treatment (T), and their interaction (G*T).

**SUPPORTING INFORMATION**

**Table S1.** Chemical and physical characteristic of the soil used in this study.

**Table S2** Primers used in this study.

**Table S3.** Summary of the RNA–seq data.

**Table S4.** Differentially expressed genes (DEGs) related to abscisic acid in the roots of wild–type Steptoe barley and Az34 mutant barley under well–watered (WW) and soil drying (SD1).

**Fig. S1.** Plant traits of Steptoe and Optic barley cultivars under different soil dring treatments.

**Fig. S2.** 1 μM ABA increased both total root length and average root hair length of Steptoe barley.

**Fig. S3.** Exogenous ABA (1 μM) increased the expression of ABA response gene ABF2 in both wild–type Steptoe barley and *Az34* barley (ABA–deficient mutant) under soil drying (SD1).

**Fig. S4.** Differentially expressed genes (DEGs) of roots in wild–type Steptoe barley and ABA–deficient mutant *Az34* barley that under soil drying (SD1) compared with

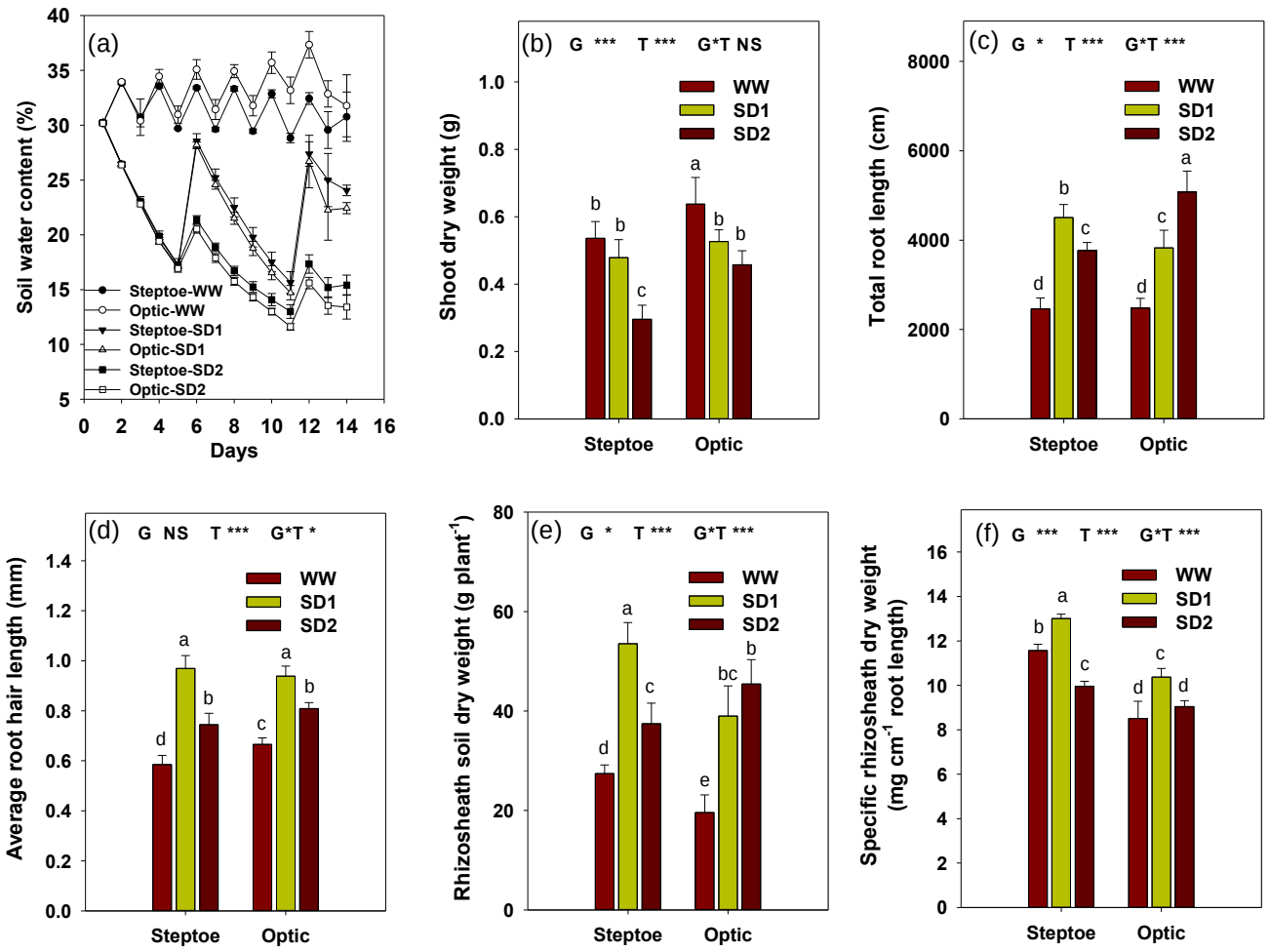
well–watered (WW).

**Fig. S5.** Tryptophan metabolism pathway was enriched in up–regulated genes of wild–type Steptoe and in down–regulated genes of ABA–deficient mutant *Az34* barley under soil drying (SD1) compared with well–watered (WW).

**Fig. S6.** 1nM IAA increased total root length and average root hair length of Steptoe barley.

**Fig. S7.** Exogenous indole-3-acetic acid (IAA, 1 nM) increased the expression of auxin response gene *AIP6B* in both wild–type Steptoe barley and *Az34* barley (ABA–deficient mutant) under soil drying (SD1).

Soil drying enhances root ABA accumulation and rhizosheath formation, but whether ABA mediates rhizosheath formation is unclear. Here, we show that ABA accumulation induced by mild soil drying enhance barley rhizosheath formation, which may be achieved through promoting auxin response.

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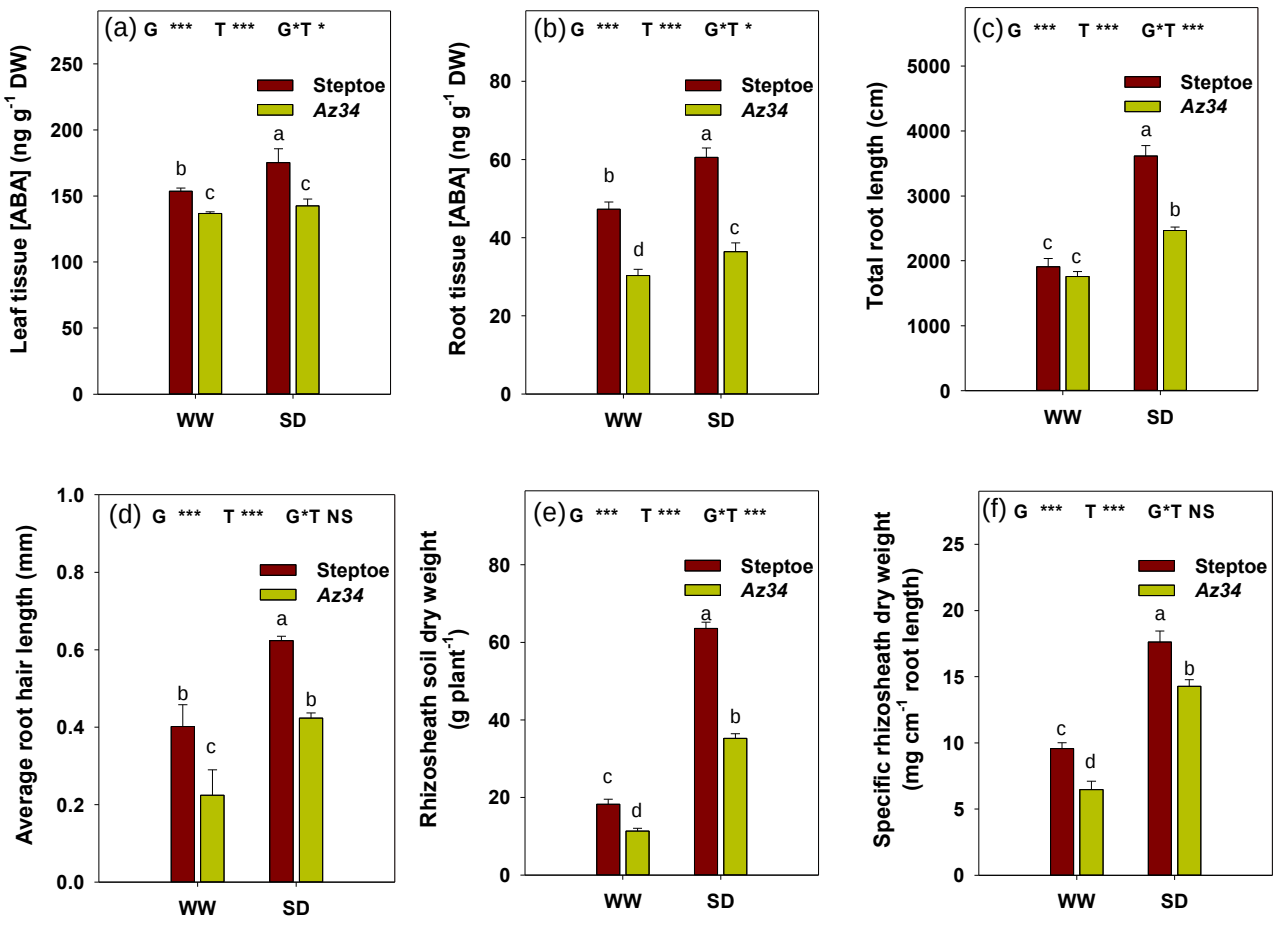


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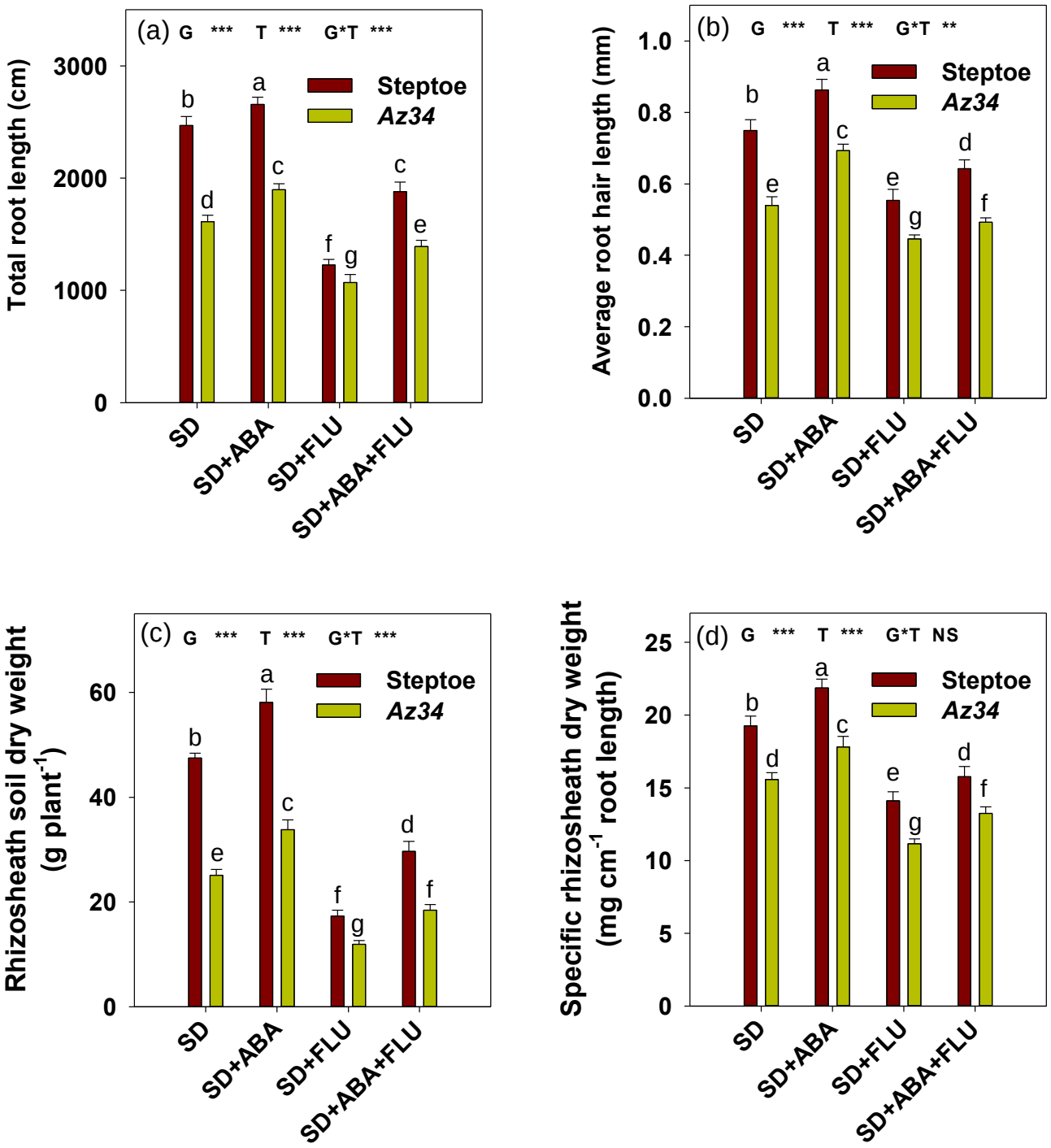


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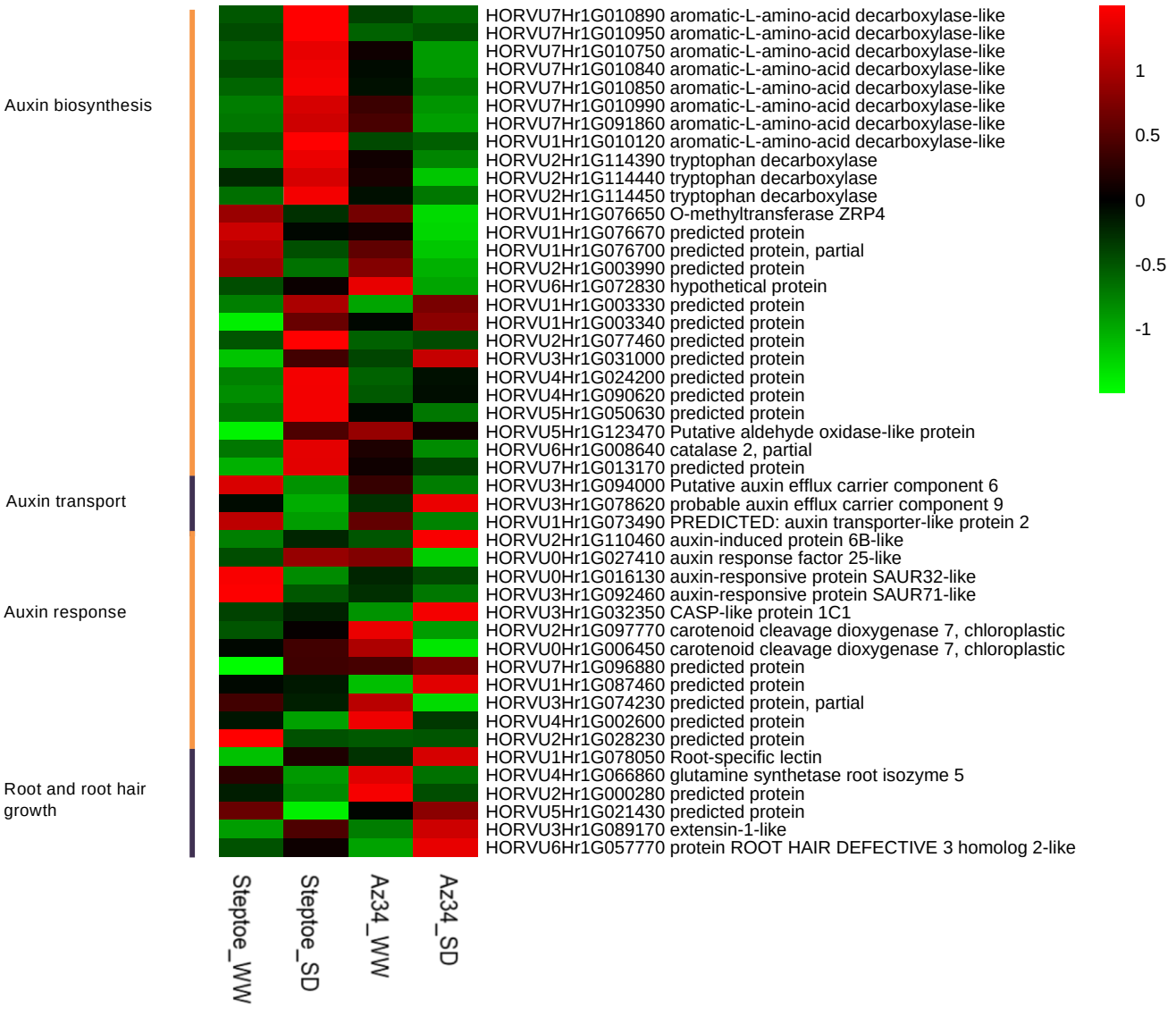


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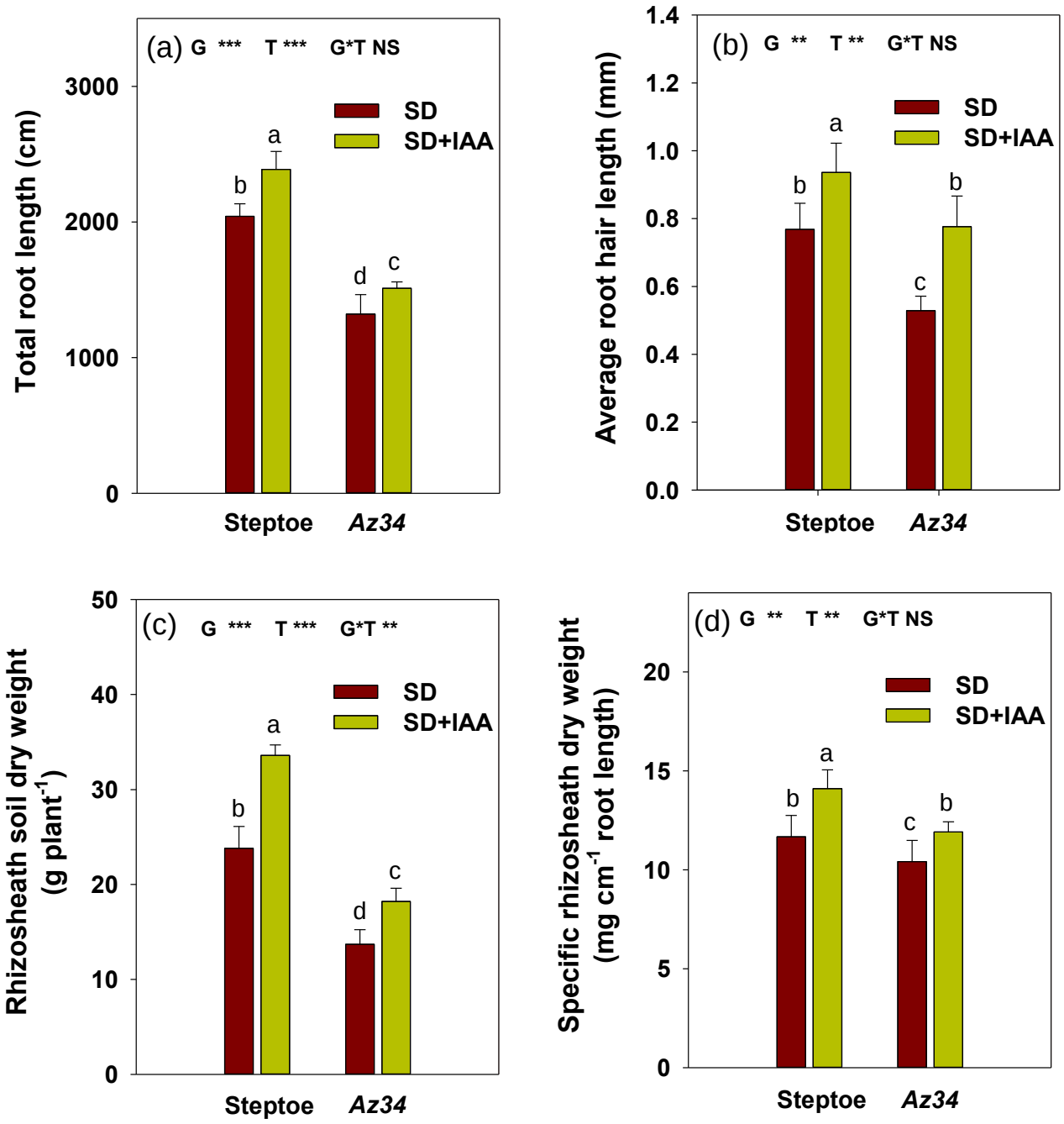


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