

Growth angles of distinct root classes in wheat are determined by a gradient of anti-gravitropic activity

Atish Bansod

University of Nottingham

James Simmonds

John Innes Centre <https://orcid.org/0000-0003-4936-1694>

Gizem Dimlioglu

University of Nottingham

Darren Wells

University of Nottingham <https://orcid.org/0000-0002-4246-4909>

Adam Binns

Fera Science Ltd <https://orcid.org/0000-0003-0441-1635>

Jonathan Atkinson

<https://orcid.org/0000-0003-2815-0812>

Brian Atkinson

University of Nottingham <https://orcid.org/0000-0001-8791-1613>

Craig Sturrock

University of Nottingham <https://orcid.org/0000-0002-5333-8502>

Cristobal Uauy

John Innes Centre <https://orcid.org/0000-0002-9814-1770>

Rahul Bhosale

`rahul.bhosale@nottingham.ac.uk`

University of Nottingham <https://orcid.org/0000-0001-6515-4922>

Article

Keywords:

Posted Date: September 19th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10495752/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

Abstract

The spatial arrangement of plant roots in soil is shaped by different root classes with distinct gravitropic setpoint angles (GSAs)^{1,2,3}. GSA is thought to arise from a balance between gravitropism and an opposing anti-gravitropic offset (AGO)²⁻⁵, but how this balance is established across root classes remains unclear. Here we show, using hexaploid wheat (*Triticum aestivum*), that root class-specific GSAs emerge from a gradient of AGO activity acting on a conserved gravitropic response. Primary and seminal roots differ quantitatively in AGO strength, with successive seminal root classes exhibiting progressively stronger AGO and correspondingly shallower GSAs. We demonstrate that this AGO gradient requires parallel pathways controlled by EGT1⁶ and EGT2⁷. Disruption of AGO abolishes root-class differences in gravitropic behaviour, producing uniformly steep root systems and altering root-class-specific transcriptional programmes associated with cell wall organisation and redox processes. These architectural changes are accompanied by reduced agronomic performance under both irrigated and non-irrigated field conditions. Our findings establish antigravitropic regulation as a quantitative mechanism underlying root system architecture and demonstrate its importance for crop performance.

Main text

Root system architecture (RSA) determines how plants acquire water and nutrients from the soil and is a major determinant of crop performance under environmental stress⁸. A defining feature of complex root systems is the presence of different root classes that grow at characteristic angles relative to gravity, termed gravitropic setpoint angles (GSAs), which enable coordinated exploration of different soil layers¹⁻³. Shallow roots enhance the capture of relatively immobile nutrients such as phosphorus concentrated in the topsoil, whereas steeper roots improve access to deeper water and mobile nutrients^{3,9-11}. Root growth angle is therefore a key trait for resource-use efficiency and resilience.

In cereals such as wheat, RSA consists of embryonic root classes (primary and seminal roots), followed by post-embryonic crown (nodal) roots^{12,13}. The primary root typically grows steeply along the gravity vector, whereas seminal roots adopt progressively shallower angles and crown roots are often the shallowest. Lateral roots arising from all classes further expand soil exploration through predominantly near-horizontal growth^{2,3}. Despite the functional importance of this hierarchical organisation, the mechanisms that generate and maintain different GSAs among root classes within a single root system remain poorly understood.

GSA is classically described as emerging from a balance between gravitropism and an opposing antigravitropic offset (AGO)²⁻⁵. Gravitropism is initiated in the root cap, where sedimentation of starch-filled amyloplasts in columella cells drives asymmetric auxin redistribution via PIN-mediated transport, leading to differential cell elongation and downward curvature¹⁴⁻¹⁹. In contrast, AGO counteracts gravitropic bending to stabilise non-vertical growth and has been linked to auxin-dependent and auxin-independent pathways^{2,4-7,20}. In cereals, *ENHANCED GRAVITROPISM 1* (EGT1) and *ENHANCED*

GRAVITROPISM 2 (EGT2) are proposed to function as components of an auxin-independent AGO pathway; loss of either gene results in steeper roots across classes^{6,7}. These findings suggest that variation in AGO, rather than gravitropism sensitivity alone, may underlie diversity in growth angles. However, how AGO activity is quantitatively regulated and coordinated across distinct root classes remains unknown.

Here, we show that root class-specific GSAs in hexaploid wheat are determined by a gradient of AGO activity acting on a conserved gravitropic response. Using cereal-specific gravistimulation assays, normalised growth analyses, and 3D clinorotation-based simulated microgravity experiments, together with genetic perturbation of AGO pathways, we demonstrate that seminal roots exhibit progressively stronger AGO across successive classes, whereas primary roots display comparatively weaker AGO. We further identify a genetic basis for this gradient involving parallel pathways mediated by *EGT1* and *EGT2*, which act as quantitative regulators of AGO activity. Simultaneous disruption of these pathways abolishes AGO, normalises gravitropic behaviour across root classes, and produces uniformly steep root systems. Together, these findings establish graded antigravitropic regulation as a unifying mechanism that generates diverse root growth angles from conserved gravitropic machinery.

Root class intrinsic GSA and growth rates influence gravitropic responses

Different root classes in complex root systems exhibit intrinsic differences in GSA and growth rate (Extended Data Fig. 1). We reasoned that these differences could confound quantitative comparisons of gravitropism in standard 90° reorientation assays by imposing unequal effective gravistimuli across root classes. To test this, we measured bending responses following 90° gravistimulation in hexaploid wheat (*Triticum aestivum* cv. Cadenza) using an improved cereal root assay that combines a growth pouch with an agar plate to maintain continuous root growth during imaging (Extended Data Fig. 2a-b and Supplementary Note 1). Root tip position, elongation rate, and bending dynamics were tracked over 18 h in primary and seminal roots.

Following 90° reorientation, bending responses varied systematically with the initial orientation of individual roots within and across root classes relative to the gravity vector (Extended Data Fig. 2c-f). Primary roots experienced a relatively uniform effective gravistimulus (~ 90°) and showed a classic bending response. In contrast, seminal roots exhibited a strong dependence on pre-existing orientation. Roots initially positioned closer to the gravity vector (< 90°) showed minimal bending and largely maintained their growth trajectory, whereas those positioned further away (> 90°) underwent pronounced downward bending. Consequently, bending magnitude scaled with the effective gravistimulus imposed by intrinsic GSAs, with seminal 2 roots experiencing the strongest stimulus, followed by seminal 1 and primary roots (Extended Data Fig. 2g).

In parallel, growth rates differed systematically across root classes, decreasing from primary to seminal 1 and seminal 2 roots (Extended Data Fig. 2h). Moreover, growth rate declined under stronger effective

gravistimuli, indicating that growth dynamics further influence observed gravitropic outputs (Supplementary Fig. 2a-b). Together, these results demonstrate that conventional 90° reorientation assays conflate intrinsic gravitropic response with angle-dependent stimulus strength and class-specific growth rates. To resolve intrinsic gravitropic responses, we therefore applied matched gravistimulus approach (Supplementary Fig. 3) and normalised for growth rate.

Normalisation of confounding factors reveal root class-specific gravitropic responses

To isolate intrinsic gravitropic responses, seedlings were reoriented at 45°, 90°, or 120°, generating a range of effective gravistimuli across root classes (Fig. 1a). Roots within each class were then grouped according to experienced gravistimulus (0–80°, aligned; 80–110°, intermediate; 110–200°, opposed), enabling comparisons under matched stimulus conditions. Whereas responses in aligned and opposed regimes remained influenced by variation in stimulus strength and elongation rate, the intermediate regime (80–110°) minimised these effects and best resolved intrinsic gravitropic responses (Extended Data Fig. 3).

Within this intermediate window, angle-based responses appeared broadly similar across root classes, whereas growth rate normalised kinetics revealed clear differences in response magnitude (Fig. 1b,c). Primary roots exhibited the strongest responses throughout the assay, seminal 1 roots showed intermediate responses, and seminal 2 roots consistently displayed the weakest responses. Together, these analyses defined a reproducible response hierarchy (primary > seminal 1 > seminal 2).

As AGO counteracts gravitropic machinery and GSAs progressively decrease across primary and seminal root classes, we reasoned that this hierarchy reflects quantitative differences in AGO activity across root classes. To test this, we removed sustained directional gravity input using 3D clinorotation. Under these conditions, primary roots exhibited minimal curvature, whereas seminal roots showed progressively stronger upward bending from seminal 1 to seminal 2 (Fig. 1d-g). Together, these results indicate that root class-specific gravitropic responses and thus GSAs are determined by graded AGO activity acting on a conserved gravitropic response.

Genetic disruption of AGO abolishes root class-specific GSAs and gravitropic hierarchy

To confirm that AGO underlies root class-specific gravitropic responses and GSAs, we generated near-isogenic wheat lines carrying loss-of-function mutations in known genetic regulators of AGO, *EGT1* and *EGT2*. Homoeologue specific TILLING mutant alleles (A, B, and D genomes) were combined and backcrossed into *Triticum aestivum* cv. Cadenza for four generations to minimise background effects.

Across growth-pouch assays and soil-grown plants imaged using X-ray Computed Tomography, *egt1* and *egt2* lines exhibited uniformly steeper root systems across all root classes relative to wild type,

indicating a shift in GSAs towards the gravity vector (Fig. 2a,c). The *egt1* phenotype was stronger than *egt2*, and the double mutant showed an additive effect (Fig. 2b,d), with convergence of all root classes on similarly steep angles, suggesting that *EGT1* and *EGT2* act in parallel AGO pathways. Consistent with these architectural changes, clinorotation assays (Supplementary Note 2) revealed graded upward curvature in wild type roots (primary < seminal 1 < seminal 2), which was reduced in *egt1*, intermediate in *egt2*, and largely abolished in the double mutant *egt1 egt2* (Fig. 3a-d). Primary roots showed minimal curvature across genotypes.

We next tested whether loss of AGO disrupts the gravitropic response hierarchy observed using the matched gravistimulus (80–110°) and growth rate normalised framework. Wild type retained a clear response hierarchy (primary > seminal 1 > seminal 2). This hierarchy was weakened in single mutants and abolished in the *egt1 egt2* double mutant (Fig. 3e), where all root classes exhibited similar response kinetics (Fig. 3f and Extended Data Fig. 4). Together, these results demonstrate that EGT1- and EGT2-dependent AGO activity quantitatively determines GSA and is required for root class-specific gravitropic response hierarchy in wheat.

AGO activity establishes root class-specific transcriptional programs

To determine how AGO shapes root class identity, we performed root class-resolved transcriptome profiling of wild type and the AGO mutant *egt1*. Primary, seminal 1, and seminal 2 roots were harvested at matched developmental stage and comparable lengths (Methods; Fig. 4a). In WT, 3,560 genes showed graded expression across root classes (mean + 1 s.d. threshold), following scalar increasing or decreasing trajectories from primary to seminal 2 roots (Fig. 4b,c). These transcriptional gradients mirrored AGO strength inferred from gravitropic and clinorotation assays, with the strongest AGO in seminal 2 roots and the weakest in primary roots (Fig. 1d and Fig. 3d). By contrast, these graded expression patterns were largely lost in *egt1* (Fig. 4b,c), with only 440 genes retaining comparable gradients, indicating a collapse of transcriptional scaling across root classes.

To quantify the extent of this loss, we restricted the analysis to differentially expressed genes (≥ 2 -fold change, FDR-corrected $P < 0.05$; between respective classes in WT versus *egt1*). This reduced the set to 19 genes in *egt1* (out of 174 graded genes in wild type; Fig. 4c) retaining attenuated gradients, indicating widespread loss of transcriptional scaling between root classes. To assess broader transcriptional divergence not restricted to scalar gradients, we performed pairwise differential expression analysis between root classes (Extended Data Fig. 5). In wild type, primary and seminal 1 roots exhibited similar transcriptomes, whereas seminal 2 roots were markedly different. In contrast, *egt1* showed a strong reduction in differentially expressed genes across all pairwise comparisons, indicating convergence of transcriptional states and loss of root class-specific molecular identity.

Genes exhibiting graded expression in WT were enriched for cell wall-associated proteins, peroxidases and redox-related pathways linked to growth regulation and directional cell expansion (Fig. 4d). These

functional gradients were weakened or lost in *egt1*, suggesting that AGO establishes root class identity by modulating cell wall and redox processes in a graded manner.

AGO activity links root architecture to agronomic performance

To assess the physiological consequences of AGO-mediated root architecture, we evaluated wild type, *egt1*, *egt2* and *egt1 egt2* plants under irrigated and non-irrigated field conditions. Under irrigated conditions, grain yield closely reflected differences in root system architecture associated with class-specific GSAs, with progressively steeper genotypes exhibiting progressively reduced performance.

Relative to wild type, *egt1* showed a greater reduction in yield than *egt2*, whereas the *egt1 egt2* double mutant exhibited the strongest reduction, consistent with the near-complete loss of root class-specific angle differentiation and collapse of root system spatial organisation (Fig. 5a). Similar trends were observed for Thousand Grain Weight (Fig. 5c), crop height and hectoliter weight (Extended Fig. 6), indicating broader effects on plant growth and productivity.

Yield penalties were further amplified under non-irrigated conditions, although genotype rankings remained unchanged (Fig. 5b). Thus, excessive root steepening compromised crop performance irrespective of water availability, suggesting that optimal resource acquisition requires coordinated distribution of root growth angles across soil strata rather than uniformly steep rooting. Together, these results establish AGO-mediated patterning of root class-specific GSAs as a key determinant of root system architecture and agronomic performance in wheat.

Discussion

Root growth angle is a major determinant of RSA, yet how multiple stable growth angles are established within a single root system has remained unclear. Here we show that root class-specific GSAs in wheat are generated by a quantitative gradient of antigravitropic offset (AGO) activity acting on a conserved gravitropic response. Primary and seminal root classes differ not in their core gravitropic machinery, but in the extent of AGO activity to which it counteracts gravitropic bending, thereby generating distinct growth angles across the root system.

Our quantitative cereal gravitropism framework resolves an important limitation of conventional reorientation assays, which impose non-equivalent effective gravistimuli across root classes owing to intrinsic differences in GSAs and growth rates. When these factors were controlled, intrinsic gravitropic responses followed a reproducible hierarchy that inversely correlated with AGO strength inferred from clinorotation assays. Genetic disruption of AGO through *EGT1* and *EGT2* abolished this hierarchy, collapsed root class-specific GSAs and produced uniformly steep root systems, demonstrating that AGO is the dominant quantitative regulator of angle determination in wheat. The additive effects observed in the double mutant further indicate that *EGT1* and *EGT2* function through parallel pathways to modulate AGO activity.

At the molecular level, AGO established graded transcriptional programmes across root classes associated with cell wall remodelling, redox regulation and directional growth. Loss of AGO collapsed these transcriptional gradients together with root class-specific molecular identity, suggesting that AGO coordinates developmental patterning of RSA through quantitative regulation of growth-associated pathways. Functionally, disruption of AGO reduced yield under both irrigated and non-irrigated conditions, indicating that optimal crop performance requires coordinated distribution of root growth angles rather than uniformly steep rooting. Together, our findings define a unifying quantitative framework in which conserved gravitropic machinery is tuned by graded AGO activity to generate root system diversity, providing a target for engineering crop architecture.

Methods

Plant materials and growth conditions

Hexaploid wheat (*Triticum aestivum* cv. Cadenza) was used for all experiments and was the parent line used for generation of near-isogenic lines (NILs). Similar sized seeds (width = 3.7 ± 0.1 mm, length = 7 ± 0.5 mm) were germinated overnight in the dark at 18°C on moist germination paper within square Petri dishes. Germinated seedlings were transferred to growth pouches made from folded germination paper (Anchor Paper Co., St. Paul, MN, USA) placed in boxes containing autoclaved water. Plants were grown under controlled conditions at 21°C Day/18°C Night temperatures with a 16-h photoperiod.

Growth-pouch-agar-plate gravitropism assay

For gravitropism assays, 12 cm square petri plates containing 60 ml of solidified 1% water-agar media were prepared. 4 days after germination (DAG), the growth pouches were opened and single seeds, along with germination paper on one side, were transferred to petri plates with agar and sealed with micropore tape (Supplementary Fig. 1). Plates were then rotated by 90° along the vertical axis and imaged every 15 min for 18 h in darkness using an infrared robotic imaging platform²¹.

To generate matched gravistimulus conditions, plates were reoriented at 45°, 90° or 120°, and roots were subsequently grouped according to experienced gravistimulus during analysis. Root tip angles for each root class were quantified using FIJI²² at 0, 1, 2, 4, 6, 12 and 18 h after gravistimulation. Root lengths were measured at corresponding time points using the NeuronJ²³ plugin for FIJI. To account for differences in growth rate, root tip angle at each time point was normalised to root growth at respective timepoint following gravistimulation.

For statistical analyses, roots were first grouped according to experienced gravistimulus. Outliers were removed using the interquartile range (IQR) method, defined as values exceeding $Q3 + 1.5 \times IQR$ or below $Q1 - 1.5 \times IQR$. Root classes and genotypes were compared independently at each time point using one-way ANOVA followed by Tukey's post hoc test in R²⁴.

Simulated microgravity assays

For clinorotation assays, seedlings at 3 DAG mounted on growth-pouch-agar-plate were placed in a 3D clinostat²⁶ operating in random positioning machine (RPM) mode, previously identified as optimal for simulated microgravity experiments (Extended Data Fig. 7, Supplementary Note 2), for 48 h. Plants were subsequently imaged using a DSLR camera. Root angle measurements and statistical analyses were performed in FIJI and R as described above. Outlier values were removed using the IQR method, and root classes were compared using one-way ANOVA followed by Tukey's post hoc test²⁴.

Generation of *egt1*, *egt2* and *egt1egt2* near-isogenic lines

A TILLING population of hexaploid wheat cv. Cadenza²⁶ was screened for deleterious mutations, including premature stop codons and splice acceptor mutations, in all three homoeologues of *egt1* and *egt2*. Details of mutant alleles, genomic locations, KASP marker sequences and Ensembl Plants annotations are provided in Extended Data Table 1.

Loss-of-function triple-mutant NILs for *egt1* and *egt2* were generated using a multi-step crossing and backcrossing strategy (Extended Data Fig. 8). TILLING lines carrying mutations in the A, B and D genomes were intercrossed to generate three-way F₁ plants carrying mutations in all homoeologues. To reduce background mutation load, heterozygous three-way F₁ plants were backcrossed to Cadenza for four successive generations (BC₁-BC₄), with marker-assisted selection performed at each generation using KASP markers. Heterozygous BC₄F₁ plants were self-pollinated to generate segregating BC₄F₂ populations, from which homozygous triple-mutant NILs were selected.

To generate combined *egt1 egt2* mutants, homozygous BC₄F₂ NILs carrying either *egt1* or *egt2* triple mutations were intercrossed. Inter-NIL F₁ plants were selfed, and homozygous sextuple-mutant, single-mutant and wild-type NIL combinations were identified in segregating F₂ and F₃ populations using KASP genotyping.

Assessment of RSA in soil-grown plants using X-ray Computed Tomography

For soil-based assays, 82-mm-diameter plastic cylinders were uniformly filled with sieved (< 2 mm) air-dried Loamy Sand soil at approximately 1.3 g cm⁻³ bulk density. Soil was collected from the University of Nottingham farm site at Bunny, Nottinghamshire (52°51'41"N, 001°07'42"W). Columns were saturated overnight with water before draining for two days to achieve nominal field capacity. Germinated seedlings were transplanted vertically into the column and covered with 1cm of Loamy Sand soil. Three seedlings were planted per column at equal spacing between the centre and edge of the cylinder. Plants were grown for 10 days under the conditions described above.

Columns were scanned at the Hounsfield Facility, University of Nottingham, UK, using a Phoenix v|tome|x M 240 kV X-ray computed tomography scanner (Waygate Technologies). Scans were acquired at 50 μ m spatial resolution using an X-ray tube voltage of 180 kV voltage and 220 μ A current settings. Samples were rotated through 360° in 0.12° increments, generating 3,000 projection images per scan. Each projection was the average of five frames with 333 ms exposure time to reduce image noise. Beam hardening and detector saturation were minimised using a 0.1 mm Cu + 1.0 mm Al filter mounted on the X-ray source. Roots were manually annotated as polylines in VGSTUDIO MAX 2023.1 (Volume Graphics GmbH, Germany), and convex hull volume trait was quantified using an inhouse developed software application for the whole root system.

RNA sequencing and transcriptome analysis

Plants were grown in growth pouches under the conditions described above until individual root classes reached approximately 1 cm in length. For each sample, 0.5 cm root tips were excised and immediately frozen in liquid nitrogen. Twenty individual roots were pooled per biological replicate, and five biological replicates were collected per root class and genotype.

Total RNA was extracted using the Macherey-Nagel™ NucleoSpin™ RNA Plant kit according to the manufacturer's instructions. RNA sequencing libraries for three biological replicates per root class and per genotype were prepared and sequenced by Novogene using Illumina paired-end 150-bp sequencing, generating > 9 GB raw data per sample. Reads were mapped to the *Triticum aestivum* cv. Cadenza reference genome (v2; downloaded from Ensembl Plants), and raw counts were converted to fragments per kilobase of transcript per million mapped reads (FPKM).

To identify genome-wide graded transcriptional programmes across root classes, mean and standard deviation FPKM expression values from three biological replicates were calculated for each gene across primary, seminal 1 and seminal 2 roots. Genes showing increasing or decreasing expression trajectories (primary > seminal 1 > seminal 2 or primary < seminal 1 < seminal 2) exceeding a mean $\pm$ 1 s.d. threshold were classified as graded-expression genes. To assess the effect of AGO disruption on transcriptional scaling, graded-expression profiles identified in wild type were compared with corresponding expression patterns in *egt1* and their FPKM values were used to generate Z-score heatmap in Fig. 4b with the scale bar indicating minimum, intermediate and maximum expression levels.

To restrict analyses to robustly regulated genes, pairwise differential expression analyses were performed between root classes within each genotype (primary versus seminal 1, primary versus seminal 2, and seminal 1 versus seminal 2). Differentially expressed genes (DEGs) were defined using a $\geq$ 2-fold expression threshold and false discovery rate (FDR)-corrected $P < 0.05$. Genes exhibiting both differential expression and graded expression patterns in wild type were subsequently examined in *egt1* to assess loss of transcriptional scaling across root classes. The total number of DEGs in WT and *egt1* were used to generate Fig. 4c. Functional enrichment analyses were performed for genes exhibiting graded differential expression using gene descriptions and UniProt protein identifiers (Supplementray

Table 1). Enriched genes were grouped into functional categories associated with cell wall organisation, redox regulation and growth-related processes (Supplementary Table 2). Some examples showing graded expression and its loss in *egt1* are shown in Fig. 4d.

To understand the broader changes in root class-specific molecular identity in *egt1* compared to WT, the total number of differentially expressed genes across all pairwise root class comparisons was determined for each genotype, irrespective of graded expression behaviour and used to generate Extended Data Fig. 5. GO term analysis was performed using goSeq (*Benjamini–Hochberg FDR-corrected* $P < 0.05$), fold enrichment was calculated using the formula: Fold enrichment = (Number of genes in DEG list associated with GO term / Total number of DEG list) / (Total number of genes in background associated with GO term / Total number of genes in background).

Field trials and plant performance quantification

Field experiments were conducted in 2025 at the John Innes Centre experimental trials site in Bawburgh, Norwich, UK (52.629°N, 1.182°E) using wild-type, *egt1*, *egt2*, and *egt1 egt2* NILs. Genotypes were evaluated under both irrigated and non-irrigated conditions.

Seeds were sown in 6 m × 1.2 m yield plots at a density of 275 plants m⁻² in a randomised complete block design with four replicates under rainfed conditions. An identical block for the irrigated treatment was established adjacent to the rainfed block, separated by a 6 m buffer zone.

Irrigation was applied on a weekly basis at a rate of 15 mm from 1st May to 23rd June, except during the week commencing 26th May, when irrigation was withheld due to adequate rainfall. In total, the irrigated plots received an additional 120 mm of water over the flowering and grain filling periods. In contrast, cumulative rainfall from 2nd March to 25th May, corresponding to the key onset of early growth and biomass accumulation phase, amounted to just 26 mm (Extended Data Fig. 6c). The trial received routine farm-management applications of pesticides and fertilisers to reflect standard agronomic practice.

Plots were assessed for tiller number and crop height prior to harvesting. Tiller number was determined by counting fertile tillers along two 1 m rows per plot and calculating the average. Crop height was calculated as the mean of two measurements per plot, recorded from the soil surface to the canopy height. Plots were harvested with a Wintersteiger Quantum plot combine (WINTERSTEIGER Seedmech GmbH, Neuengeseke, Germany) and final grain yield per plot was recorded. A sample of grain was taken from each plot and the thousand grain weight and grain morphometric data were measured using the MARVIN 2.0 grain analyser (MARViTECH GmbH, Wittenburg, Germany).

Data and materials availability

All data are available in the main text or the supplementary materials.

Declarations

Acknowledgements

We would like to thank Prof. Malcolm Bennett and Dr Gabriel Castrillo for critically reading the manuscript and providing feedback. R.B. acknowledges support from the BBSRC Discovery Fellowship (BB/S011102/1), the BBSRC New Investigator Research Grant (BB/X014843/1), and the BBSRC Partnering Award (BB/X018806/1). A.B., Ad.B., and G.D. acknowledge support from the BBSRC New Investigator Research Grant (BB/X014843/1). A.B. also acknowledges support from the BBSRC Partnering Award (BB/X018806/1).

Author contributions

R.B. conceived the idea. A.B. performed all the experiments. G.D. and Ad.B. supported the experiments. J.S. generated NIL lines and performed field experiments with support from C.U. A.B. and J.S. processed the data. D.W. and J.A. designed the 3D clinostat. A.B., B.A. and C.S. performed X-ray imaging and processing. R.B. and A.B. wrote the manuscript, with input from all co-authors. R.B. supervised the project.

Competing interests

The authors declare no competing interests.

AI Statement

Rephrasing AI features, automated grammar, spelling, punctuation, and basic style suggestions in Grammarly were used to refine and clarify previously written ideas. All core concepts, research, and arguments are the original work of the author.

References

1. DIGBY J, FIRN RD (1995) The gravitropic set-point angle (GSA): the identification of an important developmentally controlled variable governing plant architecture *. *Plant Cell Environ* 18:1434–1440
2. Kirschner GK et al (2024) Genetic regulation of the root angle in cereals. *Trends Plant Sci* 29:814–822
3. Lombardi M et al (2026) Environmental regulation of root growth angle in cereal crops. *Plant Soil*. <https://doi.org/10.1007/s11104-026-08535-2>
4. Waidmann S et al (2019) Cytokinin functions as an asymmetric and anti-gravitropic signal in lateral roots. *Nat Commun* 10:3540
5. Roychoudhry S et al (2023) Antigravitropic PIN polarization maintains non-vertical growth in lateral roots. *Nat Plants* 9:1500–1513

6. Fusi R et al (2022) Root angle is controlled by *EGT1* in cereal crops employing an antigravitropic mechanism. *Proceedings of the National Academy of Sciences* 119
7. Kirschner GK et al (2021) ENHANCED GRAVITROPISM 2 encodes a STERILE ALPHA MOTIF–containing protein that controls root growth angle in barley and wheat. *Proceedings of the National Academy of Sciences* 118
8. Lynch J (1995) Root Architecture and Plant Productivity. *Plant Physiol* 109:7–13
9. York LM, Nord EA, Lynch JP (2013) Integration of root phenes for soil resource acquisition. *Front Plant Sci* 4
10. Lynch JP (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann Bot* 112:347–357
11. Uga Y et al (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nat Genet* 45:1097–1102
12. Smith S, De Smet I (2012) Root system architecture: insights from *Arabidopsis* and cereal crops. *Philosophical Trans Royal Soc B: Biol Sci* 367:1441–1452
13. Alrajhi A, Alharbi S, Beecham S, Alotaibi F (2024) Regulation of root growth and elongation in wheat. *Front Plant Sci* 15
14. Chen J et al (2023) Amyloplast sedimentation repolarizes LAZYs to achieve gravity sensing in plants. *Cell* 186:4788–4802e15
15. Nishimura T et al (2023) Cell polarity linked to gravity sensing is generated by LZY translocation from statoliths to the plasma membrane. *Sci* (1979) 381:1006–1010
16. Han H, Adamowski M, Qi L, Alotaibi SS, Friml J (2021) PIN-mediated polar auxin transport regulations in plant tropic responses. *New Phytol* 232:510–522
17. Swarup R et al (2005) Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nat Cell Biol* 7:1057–1065
18. Ottensschläger I et al (2003) Gravity-regulated differential auxin transport from columella to lateral root cap cells. *Proceedings of the National Academy of Sciences* 100, 2987–2991
19. Band LR et al (2012) Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. *Proceedings of the National Academy of Sciences* 109, 4668–4673
20. Roychoudhry S, Del Bianco M, Kieffer M, Kepinski S (2013) Auxin Controls Gravitropic Setpoint Angle in Higher Plant Lateral Branches. *Curr Biol* 23:1497–1504
21. Wells DM et al (2012) Recovering the dynamics of root growth and development using novel image acquisition and analysis methods. *Philosophical Trans Royal Soc B: Biol Sci* 367:1517–1524
22. Schindelin J et al (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9:676–682
23. Meijering E et al (2004) Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. *Cytometry Part A* 58A:167–176

24. Alboukadel Kassambara (2023) rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.7.2. <https://rpkgs.datanovia.com/rstatix/>
25. Krasileva KV et al (2017) Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences* 114
26. Wahab K, Dan Dragomirescu (2026) wahabk/Clinostat: V0.6.0 - taSPG Update (0.6.0). Zenodo. <https://doi.org/10.5281/zenodo.19509998>

Figures

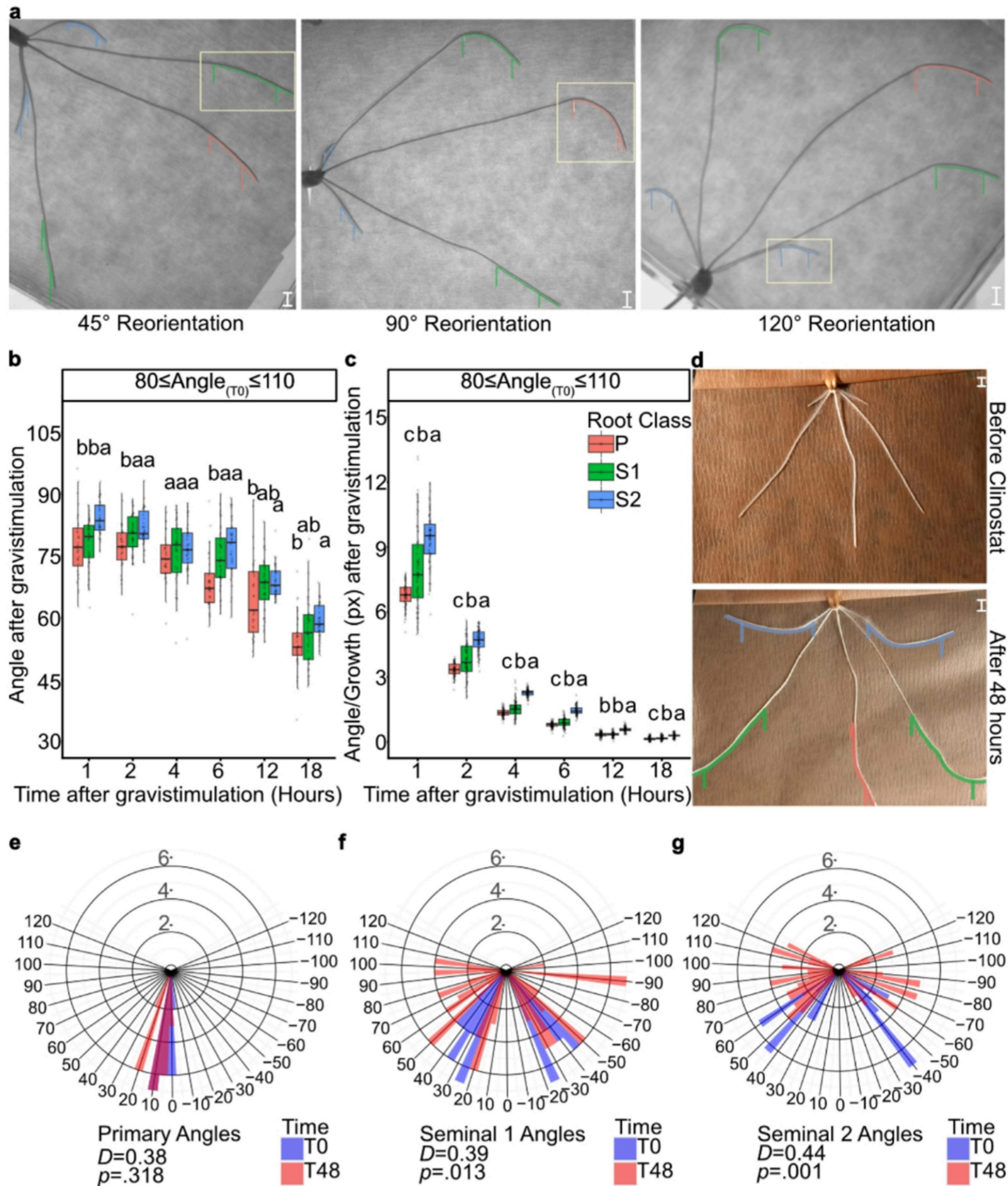


Figure 1

Normalisation of confounding factors reveals root class-specific gravitropic responses.

a, Representative images of gravitropic responses in wild-type wheat (*Triticum aestivum* cv. Cadenza) following reorientation at 45°, 90° or 120° relative to the gravity vector. Scale bar, 5 mm. **b,c**, Time-course analysis of root tip angle (**b**) and tip angle normalised to growth rate (**c**) for primary (P), seminal 1 (S1)

and seminal 2 (S2) roots within the intermediate gravistimulus bin (80-110°) over 18 h. Data are shown as box plots with individual data points overlaid ($n = 35$ seedlings). Different letters indicate statistically significant differences between root classes at each time point (one-way ANOVA, $\alpha = 0.05$). Growth rates for each root class are shown in Extended Fig. 2b. **d**, Representative images of roots before and after 3D clinorotation. Cyan, green and red lines highlight growth trajectories and curvature of P, S1 and S2 roots, respectively. Scale bar, 5 mm. **e-g**, Rose plots showing tip angle distributions of P (**e**), S1 (**f**) and S2 (**g**) roots before clinorotation (T0; blue) and after 48 h of clinorotation (T48; red). Angles from 0° to +120° represent roots on the left side of the vertical axis, and 0° to -120° represent roots on the right. Radial circles indicate frequency from 0 to 10. Data are shown from $n = 20$ seedlings. Kolmogorov-Smirnov (KS) test was used to analyse differences between the two distributions (D statistic shows maximum distance between distributions, α for p-value = 0.05)

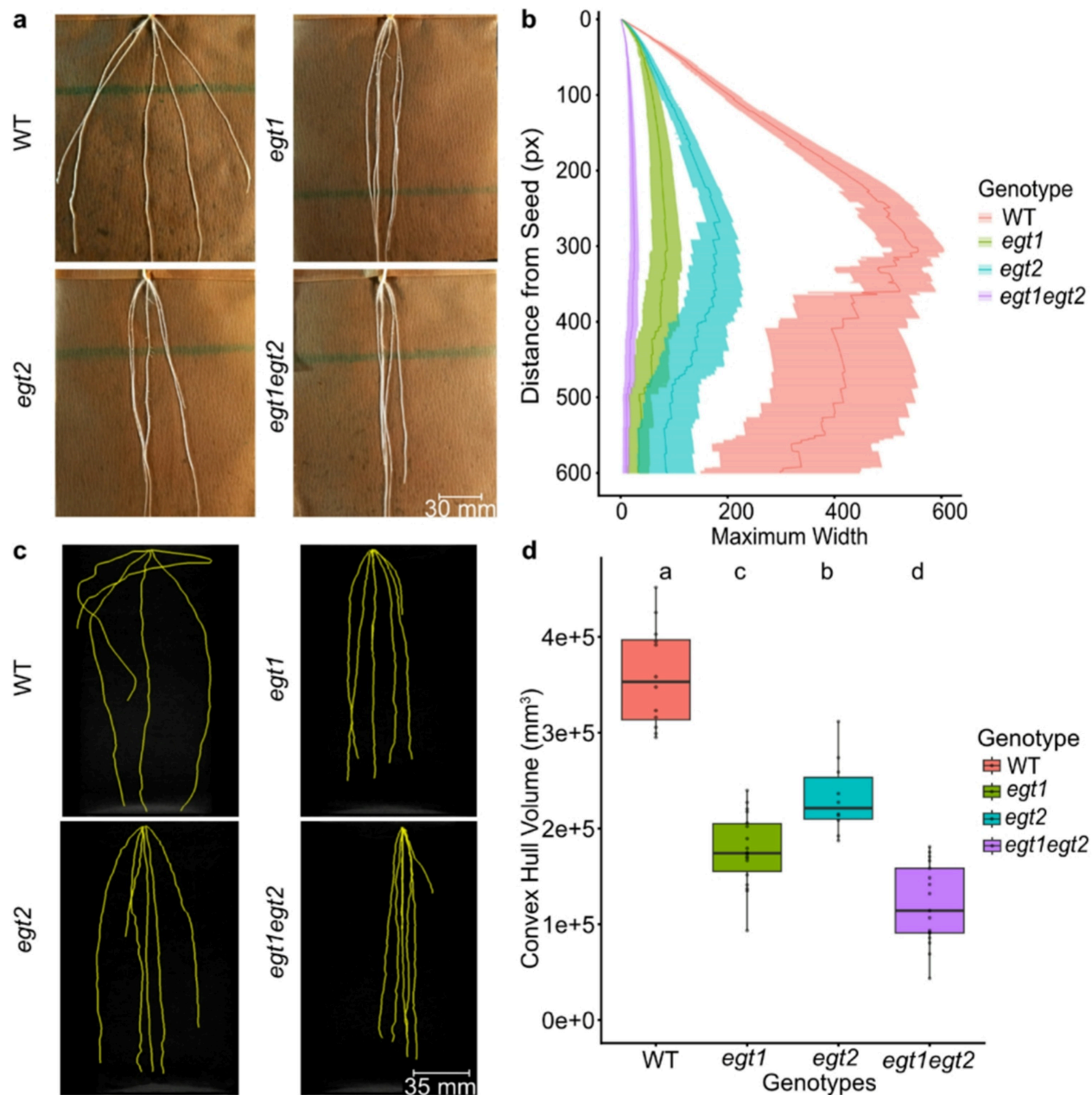


Figure 2

EGT1 and EGT2 act in parallel to control root class specific GSAs in wheat.

a, Representative images of root systems from WT (*Triticum aestivum* cv. Cadenza), *egt1*, *egt2* and *egt1 egt2* double-mutant seedlings grown in growth-pouch assays for 6 days. Scale bar, 30 mm. **b**, Maximum width (lateral spread profile) of RSA for WT *egt1*, *egt2* and *egt1 egt2* double mutants, calculated per pixel from the base of the root system in growth-pouch assays. **c**, Representative 3D rendered X-ray Computed Tomography (CT) images of soil-grown root systems from *egt1*, *egt2* and *egt1 egt2* double

mutants. Scale bar, 35 mm. **d**, Convex hull volume (mm³) of soil-grown root systems from *egt1*, *egt2* and *egt1 egt2* double mutants after 10 days of growth. Data are shown as box plots with individual data points overlaid (n = 30 seedlings for growth pouch assay, n = 15 for soil-based assay). Different letters denote significant differences between genotypes (one-way ANOVA, α = 0.05).

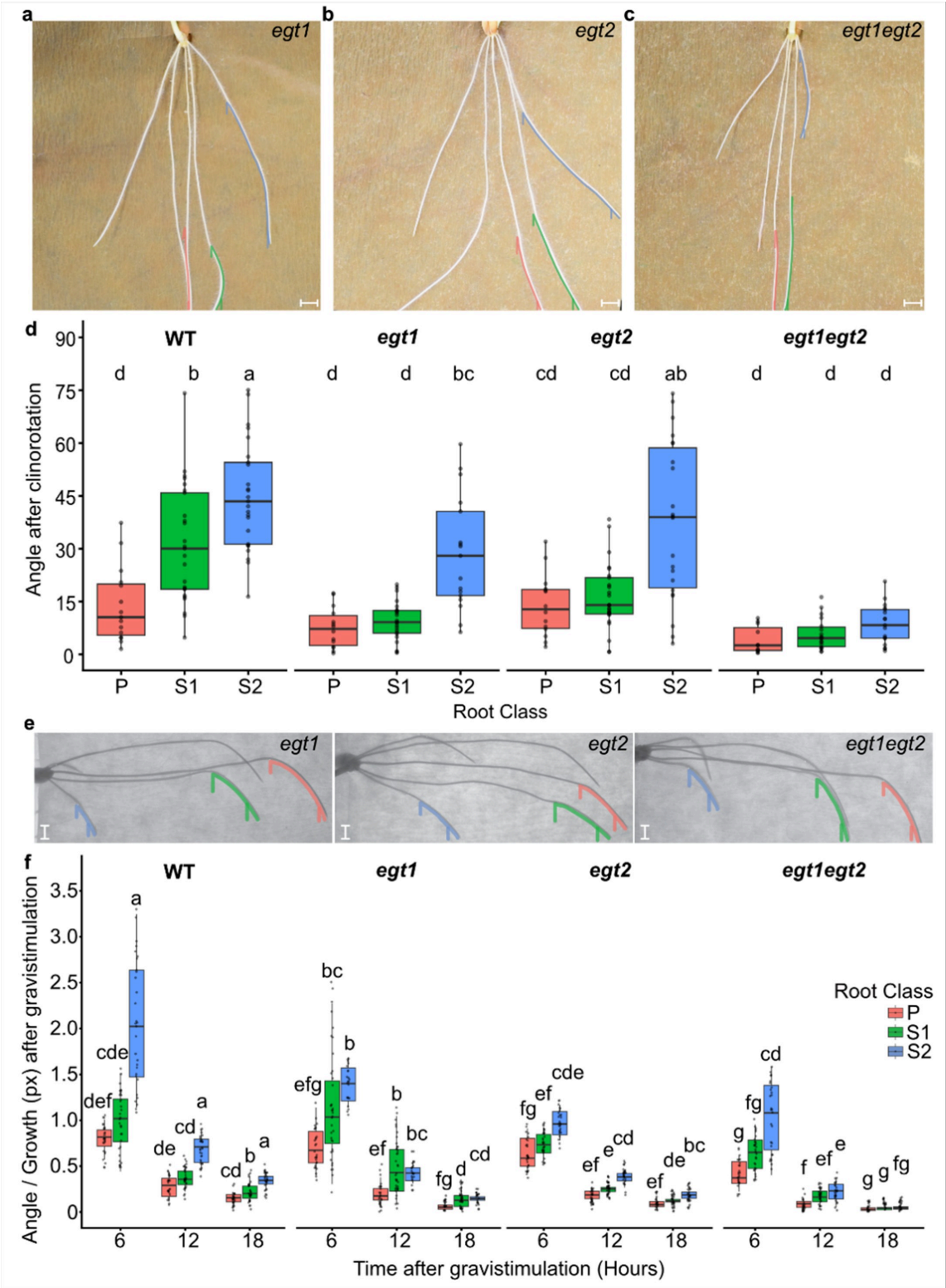


Figure 3

Genetic disruption abolishes AGO activity and gravitropic hierarchy.

a-c, Representative images of root architecture for *egt1* (**a**), *egt2* (**b**) and *egt1egt2* (**c**) before and after 2 days of 3D clinorotation. Cyan, green and red lines indicate growth trajectories and curvature of primary (P), seminal (S1) and seminal (S2) roots, respectively. Scale bar, 5 mm. See Fig. 1d for WT root images.

d, Box plots showing tip angle distributions of P, S1 and S2 roots after 48 h of clinorotation for *egt1* (**d**), *egt2* (**e**) and *egt1egt2* double-mutant. Cyan, green and red lines indicate growth trajectories and curvature of primary (P), seminal (S1) and seminal (S2) roots, respectively. See Figure 1d for WT. Data are shown from $n = 20$ seedlings.

e, Representative images of gravitropic responses in *egt1*, *egt2* and *egt1egt2* following 90° reorientation relative to the gravity vector. Scale bar, 5 mm. As all roots grow between 80-110 GSA, thus 90° rotation alone was used to generate matched gravistimulus magnitude. See Fig. 1a for WT images.

f, Time-course analysis of growth rate normalised root tip angle for P, S1 and S2 roots from WT, *egt1*, *egt2* and *egt1egt2* double-mutant seedlings within the intermediate gravistimulus bin (80-110°) over 18 h. Data are shown as box plots with individual data points overlaid ($n = 30$ seedlings). Different letters denote significant differences between root classes at each time point (one-way ANOVA, $\alpha = 0.05$). Elongation rates for each root class per genotype are shown in Extended Data Fig. 4.

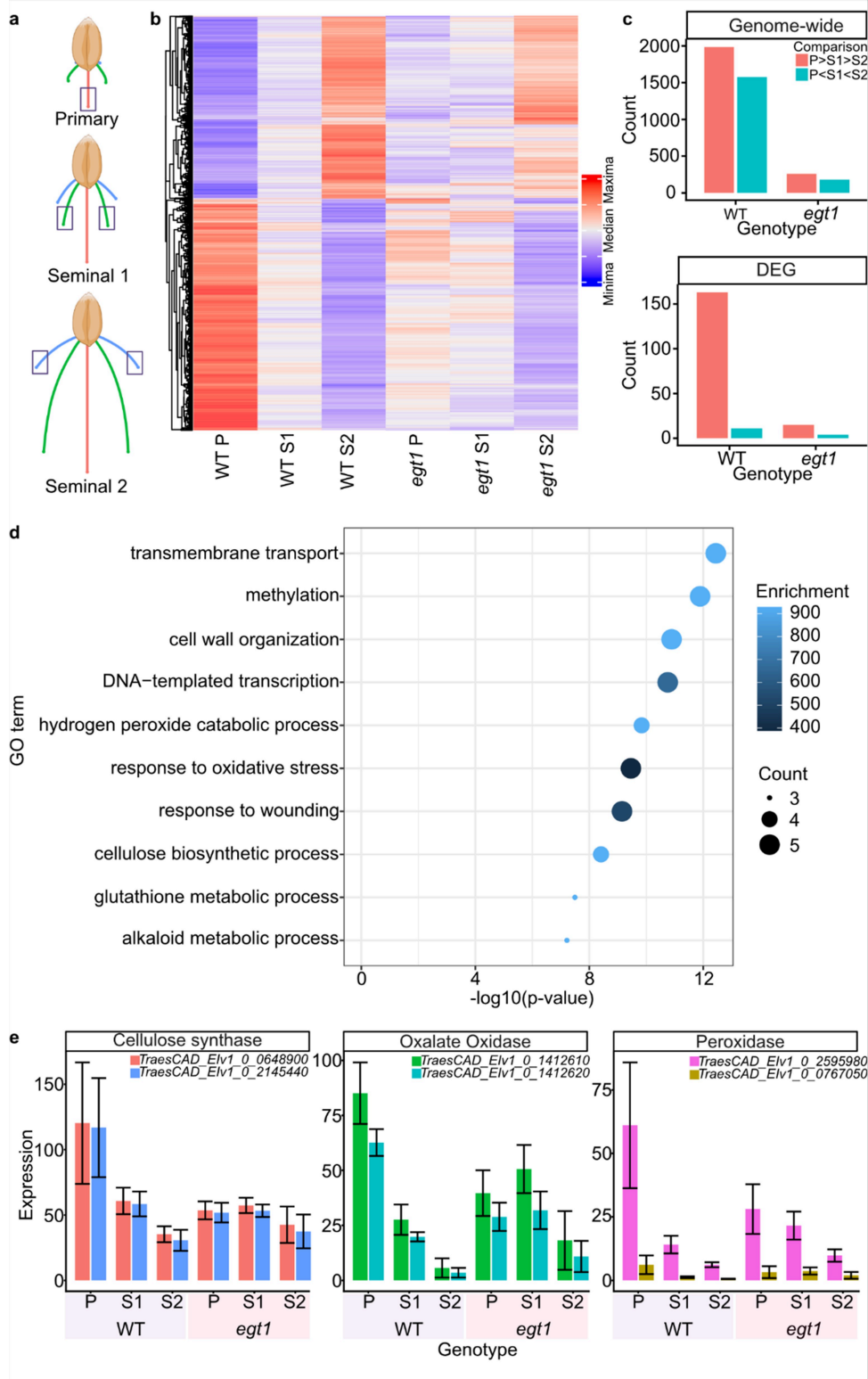


Figure 4

AGO activity establishes root class-specific transcriptional programmes and links root angle regulation to crop performance.

a, Schematic showing root class-resolved transcriptome sampling from primary (P), seminal 1 (S1) and seminal 2 (S2) roots of wild-type wheat (cv. Cadenza) and the partial AGO-loss mutant *egt1*. **b**, Clustered

heat map showing genes with graded expression across root classes in wild type ($P > S1 > S2$ or $P < S1 < S2$), selected using a mean ± 1 s.d. threshold. Expression values are FPKM-normalised and shown as Z-scores, with the scale bar indicating minimum, intermediate and maximum expression levels. Corresponding profiles in *egt1* show loss or attenuation of graded expression. **c**, Number of genes exhibiting graded expression across root classes, identified using a mean ± 1 s.d. FPKM threshold across three biological replicates. Genes were additionally filtered for expression ≥ 1 FPKM across the profile to exclude lowly expressed transcripts. **d**, GO enrichment analysis of genes exhibiting scalar expression across root classes in the “biological processes” category. The x-axis represents $-\log(10)$ of p-value; bubble size represents the number of DEGs assigned to each GO term, and bubble colour reflects fold enrichment value. **e**, Examples of genes encoding cell wall-associated proteins, peroxidases and redox-related components showing graded expression in wild type and loss of this pattern in *egt1*. See Supplementary Table 1 for the full list of differentially expressed genes showing scalar expression.

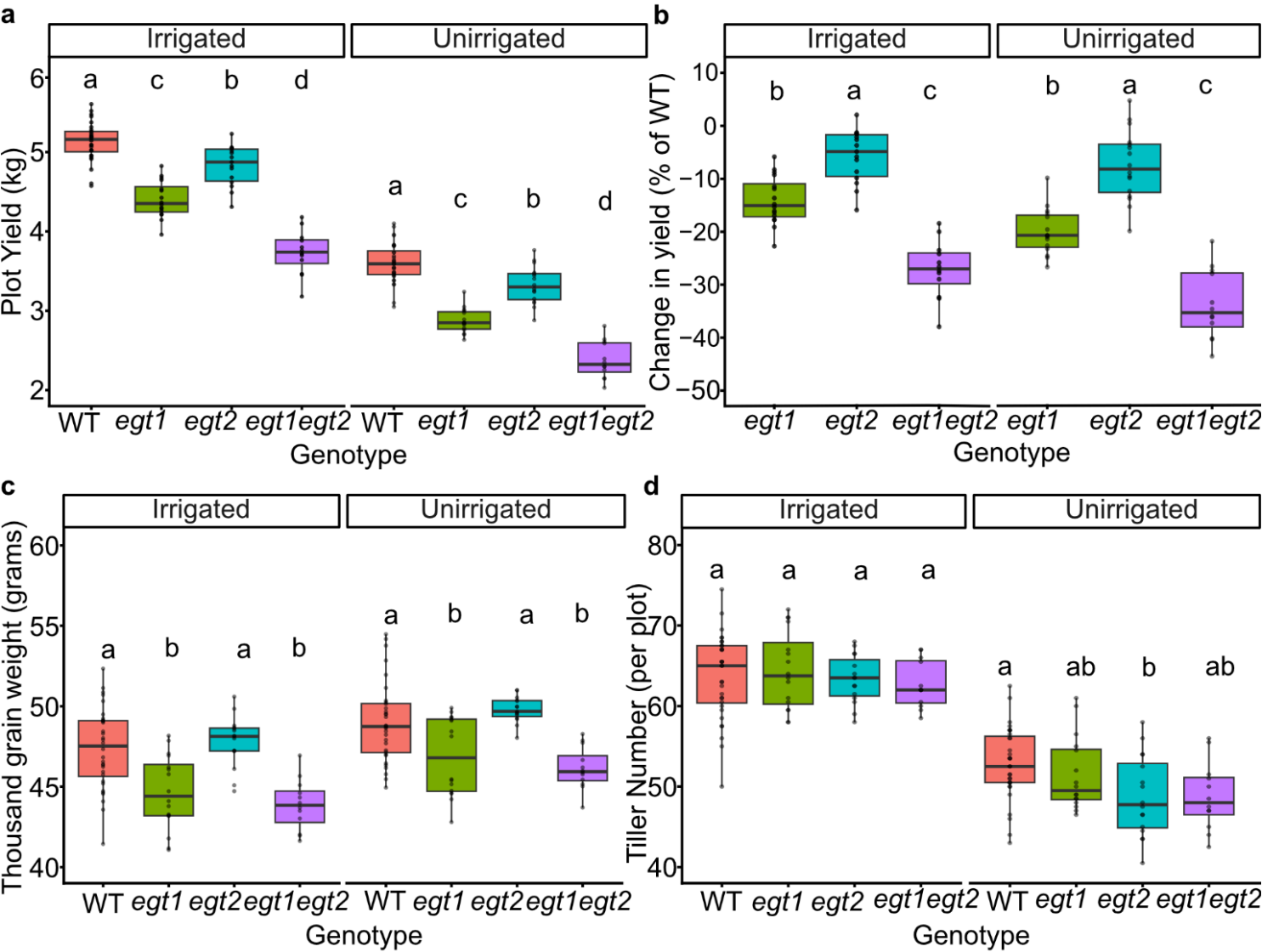


Figure 5

Differences in AGO affects crop performance in irrigated and non-irrigated field experiments.

a, Grain yield of wild-type, *egt1*, *egt2* and *egt1 egt2* plants grown under irrigated and non-irrigated field conditions. Different letters denote significant differences between genotypes within each treatment (one-way ANOVA, $\alpha = 0.05$). **b**, Yield change expressed as a percentage of wild type for *egt1*, *egt2* and *egt1 egt2* plants grown under irrigated and non-irrigated conditions. Different letters denote significant differences between genotypes within each treatment (one-way ANOVA, $\alpha = 0.05$). **c**, Thousand Grain Weight (TGW) of wild type for *egt1*, *egt2* and *egt1 egt2* plants grown under irrigated and non-irrigated conditions. Different letters denote significant differences between genotypes within each treatment (one-way ANOVA, $\alpha = 0.05$). **d**, Tiller number of wild type for *egt1*, *egt2* and *egt1 egt2* plants grown under irrigated and non-irrigated conditions. Different letters denote significant differences between genotypes within each treatment (one-way ANOVA, $\alpha = 0.05$). Whisker plots show distributions with individual data points overlaid ($n = 24$ plots).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalInformation.docx](#)
- [ExtendedDataFigureslegends.docx](#)
- [ExtendedFigures.docx](#)