UNIVERSITY OF LEEDS

LAZY4D

Deeper and steeper root architecture

A dominant, genome editable approach to crop enhancement

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

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Tech ID: 19.657

Patent Literature

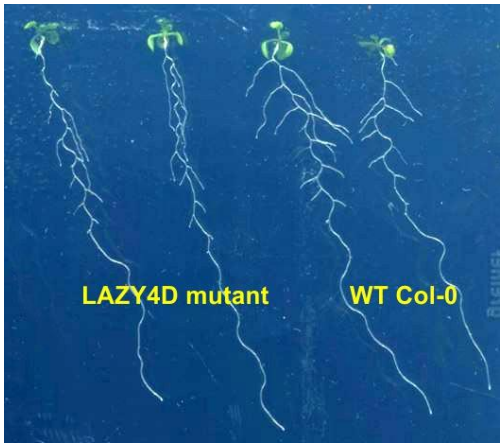
Publications: WO/2021/064402; EP 4038093A1; AR 120136 A1; US 2023-0323384

The angle at which the roots of crop plants grow can have a major effect on the crop's ability to take up nutrients and water from the soil. Deeper root systems enable the crop to make better acquisition of soil nitrogen allowing more sustainable and economic crop production, relying on lower fertilizer inputs. They also enhance access moisture particularly in drier seasons, and may also have a beneficial effect on soil structure. A range of environmental and genetic factors come together to affect the development of the root system in a field crop setting. In a holistic sense, the combination of root length and root angle defines the overall root system architecture and that determines the volume of soil that is available to support the plant's needs. Root angle is therefore a key determinant of crop success and of great agronomic significance but, being difficult to study, it is poorly understood and under-exploited as a route to crop improvement.

Using a forward genetic approach, the group of Professor Stefan Kepinski at the University of Leeds has discovered that steeper root growth angles can be achieved with the mutation of a single nucleotide change in the Arabidopsis *LAZY4* gene, conferring a single amino acid change in the LAZY4 protein. The mutation is completely dominant and is named as "lazy4D".

The *LAZY* gene family are known regulators of gravitropic responses in species as diverse as Arabidopsis, rice and peach. LAZY proteins are conjectured to control plant architecture by coupling gravity sensing to the formation of auxin gradients that override a LAZY-independent mechanism that creates an opposing gravity-induced auxin gradient (Yoshihara and Spalding 2017 *Plant Phys* 175:959-969). Different *LAZY* gene family members are associated with this effect in different parts of the plant. For example, in the roots of rice, the *LAZY* gene *OsDRO1* is associated with root growth angle (Uga *et al* 2013 *Nat Gen* 45:1097-1102). Although the rice *DRO1* is a *LAZY* gene it is not the direct orthologue of the Arabidopsis *LAZY4* gene. *LAZY4* is conserved throughout higher plants and the Leeds group is now implementing approaches to modify *LAZY4* in crops.

The lazy4D mutation lies in a previously undescribed motif of LAZY4. A single base change that results in the loss of an arginine residue (it can be replaced by any of a number of chemically diverse amino acids) causes the phenotype of a steeper root angle of the lateral root system (Fig 1, over). Significantly the mutant alleles are **completely dominant**, thus making them far more tractable for breeding and deployment, and particularly in **hybrid varieties** and **polyploid crop species**. Moreover the lazy4D motif, including the key arginine residue, is very highly conserved in important crops such **maize, wheat, soybean, cotton and oil-seed rape/canola**. The dominant, gain-of-function phenotype seen in the *lazy4D* mutants is consistent with the findings in rice and peach in which homologues of *LAZY4* have been transgenically over-expressed to induce more vertical root angles. Clearly, the Leeds group's approach is far more practical, being achievable by mutant breeding or gene-editing. Equally, it opens up a route to using specific molecular genetic tools for identifying and selecting natural genetic variation for this trait.



In Arabidopsis, *LAZY4* has a paralogue, *LAZY2*, in which the lazy4D motif is also conserved. Introducing the *lazy4D* mutation into *LAZY2* has the same effect as the mutation in *LAZY4*. The Leeds group is currently studying which additional residues in the motif can be modified to achieve the same or similar phenotypes. This is the first report of a gain-of-function mutation in a *LAZY* family gene. Previous reports have described complete knock-outs, transgenic overexpression or gene truncations.

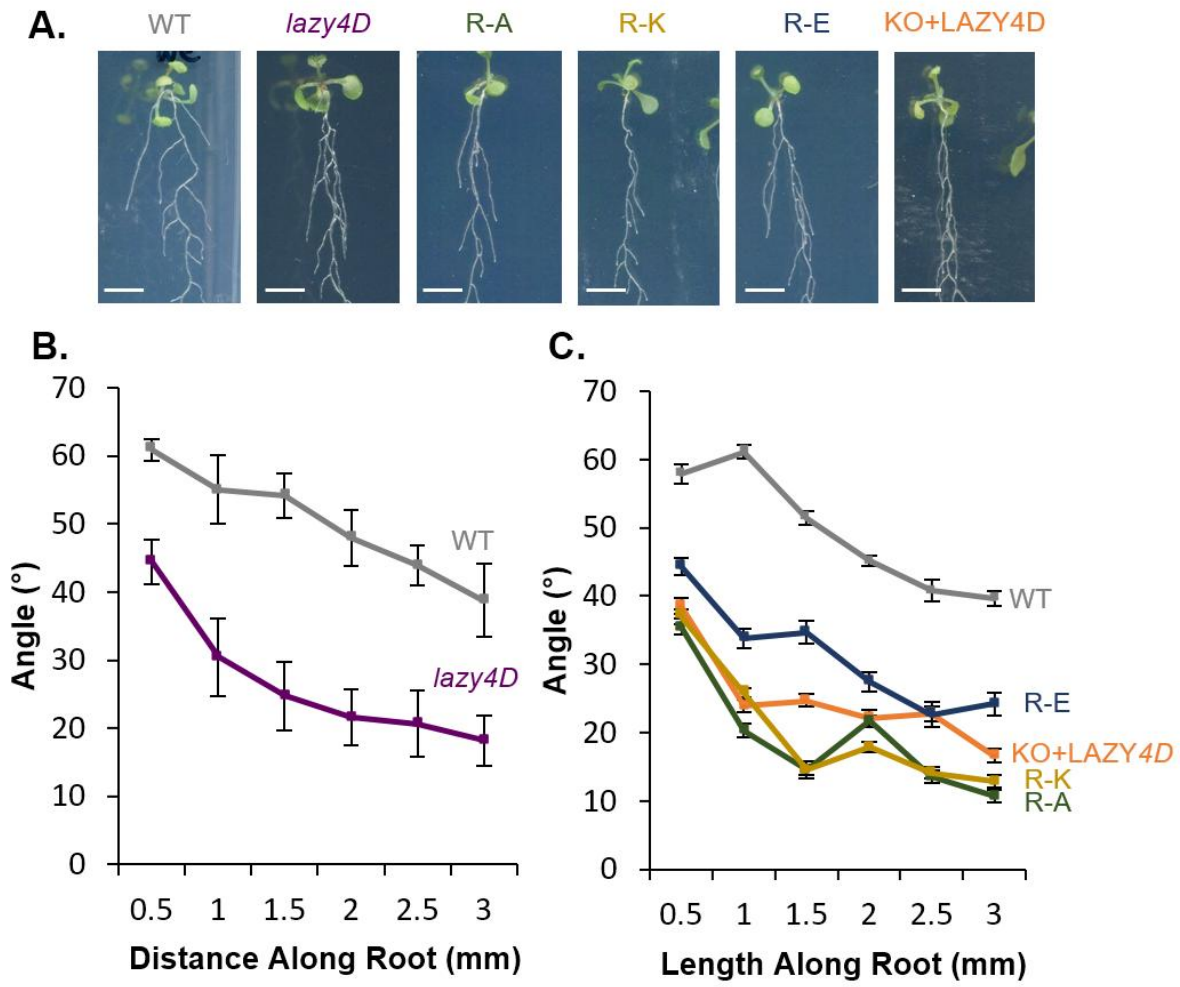


Figure 1: Root angle phenotype of lazy4D and substituted amino acids at the same position.
lazy4D has a significantly more vertical lateral root angle than WTCol-0 (A and B). This is also true for other amino acid substitutions at the lazy4D position (A and C), including for the *lazy4* knockout line trangenically expressing the LAZY4D allele (KO+LAZY4D, orange labels. P<0.05 for all points); scale bars represent 5 mm; error bars represent SEM.

In summary:

- **Simple, single residue change in LAZY4, or paralogues, confers fully dominant steep/deep root phenotype**
- **Highly conserved across plants, including key agricultural crop species**
- **Achievable by mutation breeding and genome editing**
- Allows optimization of root architecture in existing, otherwise elite varieties
- **Provides approaches to screening/selecting existing genetic variation**

The lazy4D technology is patented on behalf of the University of Leeds and is exclusively licensed to PBL. **For more information or licensing interest, please contact PBL.**