

**The roots of rye (*Secale cereale* L.) are capable of synthesizing benzoxazinoids**

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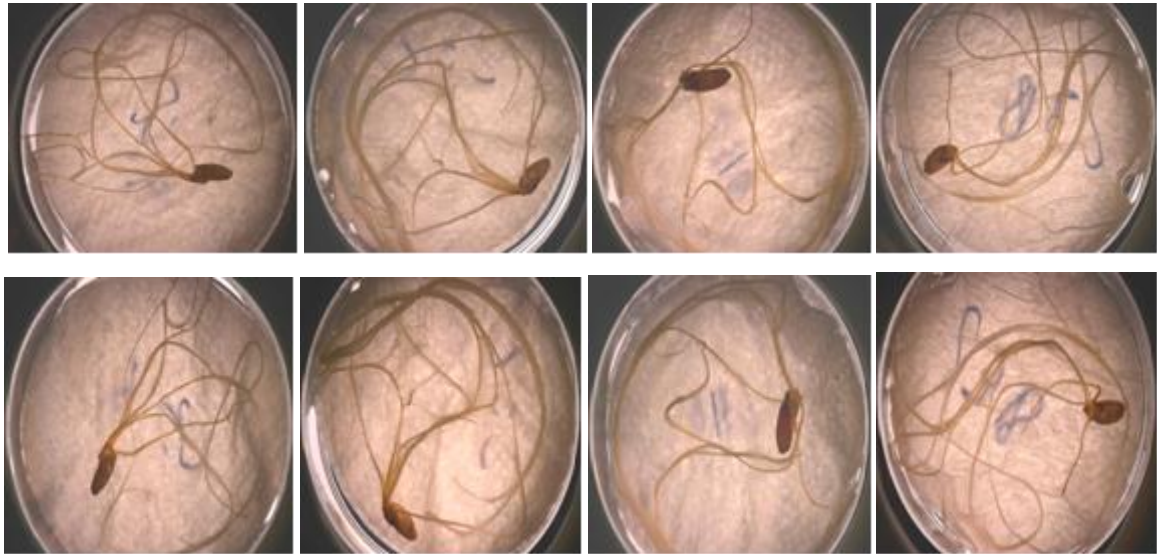


Figure S1. Twelve-day-old roots developed from seeds deprived of coleoptile of rye inbred line L318.

Table S1. Relative transcript levels of *ScBx1* and *ScIgl* in rye RDC, RIV, R2W, and L2W.

Analyzed organ/organoid	Genes	
	<i>ScBx1</i>	<i>ScIgl</i>
RDC	0.0014 (0.0002)	0.0224 (0.0100)
RIV	0,0002 (0.0000)	nd (-)
KI R2W	0.0028 (0.0004)	0.0090 (0.0010)
KII L2W	0.0697 (0.0067)	0.0020 (0.0010)

RDC - roots developed from seeds deprived of the coleoptile at 2 days after sowing

RIV - roots developed *in vitro*

KI R2W - control reference organ: roots from 2-week-old seedlings

KII L2W - control reference organ: leaves from 2-week-old seedlings

nd – not detected

Values in brackets are standard errors.

The same methodology was used to analyse *ScIgl* and *ScBx1* transcript levels (described in the main text). The sequences of primers used in PCR were as follows:

F: AACACCAGCTACACCATCAGAG

R: GTGGGTTTACAGTCGCCCTA

Table S2. Contents of six benzoxazinoids in RDC, RIV, R2W, and L2W of rye inbred line L318.

Analyzed organ/organoid	BX [ $\mu\text{g}/\text{mg d.m.}$ ]					
	HBOA	GDIBOA	DIBOA	GDIMBOA	DIMBOA	MBOA
RDC	0.028 (0.001)	0.070 (0.000)	0.248 (0.003)	0.772 (0.001)	1.194 (0.008)	0.410 (0.021)
RIV	0.004 (0.000)	0.010 (0.001)	0.003 (0.000)	nd -	0.010 (0.001)	0.001 (0.000)
KI R2W	0.018 (0.000)	0.318 (0.013)	0.114 (0.006)	8.073 (0.000)	0.536 (0.013)	0.427 (0.013)
KII L2W	0.202 (0.090)	2.507 (0.726)	4.475 (1.405)	0.230 (0.128)	0.013 (0.010)	0.044 (0.015)

RDC - roots developed from seeds deprived of the coleoptile at 2 days after sowing

RIV - roots developed *in vitro*

KI R2W - control reference organ: roots from 2-week-old seedlings

KII L2W - control reference organ: leaves from 2-week-old seedlings

nd – not detected

Values in brackets are standard errors.

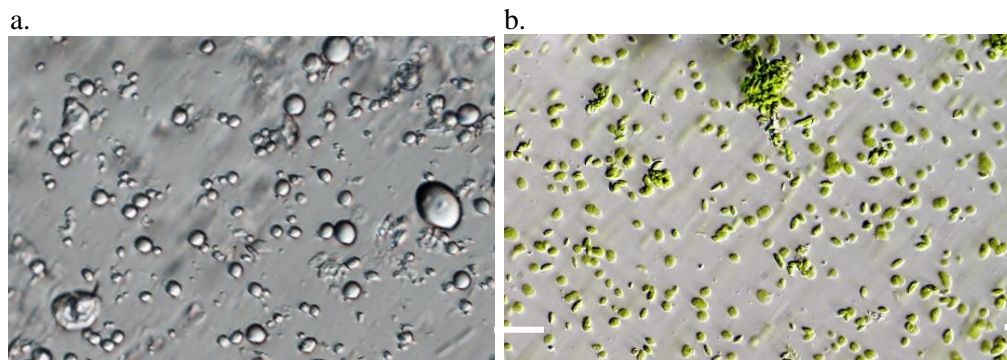


Figure S2. Plastids isolated from roots developed from (a) seeds deprived of coleoptile at 2 days after sowing and (b) leaves of 2-week-old seedlings of rye inbred line L318. Scale bars = 50 µm.

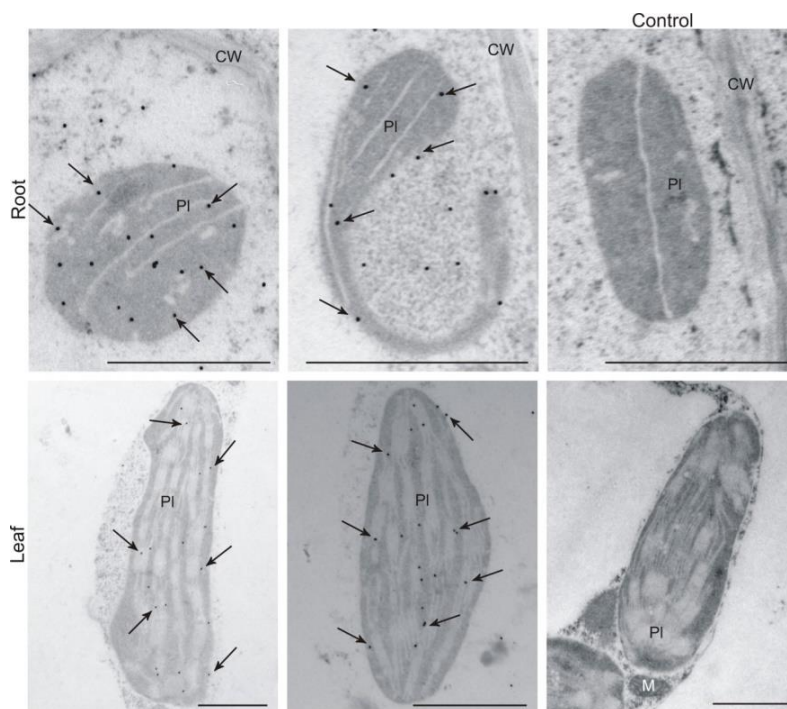


Figure S3. Localisation of indole-3-glycerol phosphate lyase by immunogold labelling and transmission microscopy in roots (R2W) and leaves (L2W) of 2-week-old seedlings of rye inbred line L318. Abbreviations: CW- cell wall, M- mitochondrion, PI- plastid. Scale bars = 1 µm.