Characterisation of the maize leaf patterning mutants *Wavy auricle in blade1-R* and *milkweed pod1-R*

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Abstract

The maize leaf has three main axes of growth, with an asymmetric distribution of tissue types along each axis. This study focuses on three mutants, *Wavy auricle in blade1-R* (*Wab1-R*), *liguleless1-R* (*lg1-R*) and *milkweed pod1-R* (*mwp1-R*) that disrupt axial patterning of maize leaves. Dominant *Wab1* mutations disrupt both medial-lateral and proximal-distal patterning. *Wab1* leaf blades are narrow and ectopic auricle and sheath-like tissues extend into the leaf blade. Previous analyses have shown that *Lg1* acts cell-autonomously to specify ligule and auricle tissues. The current study reveals additional roles in defining leaf shape. The recessive *lg1-R* mutation exacerbates the *Wab1-R* phenotype; in the double mutants, most of the proximal blade is deleted and sheath tissue extends along the residual blade.

A mosaic analysis of *Wab1-R* was conducted in *Lg1* and *lg1-R* backgrounds to determine if *Wab1-R* affects leaf development in a cell-autonomous manner. Normal tissue identity was restored in all *wab1/-* sectors in a *lg1-R* mutant background, and in three quarters of sectors in a *Lg1* background. These results suggest that *Lg1* can influence the autonomy of *Wab1-R*. In both genotypes, leaf-halves with *wab1/-* sectors were significantly wider than non-sectored leaf-halves, suggesting that *Wab1-R* acts cell-autonomously to affect lateral growth.

mwp1-R is a recessive mutation that specifically affects patterning of sheath tissue. Characterisation of the *mwp1-R* phenotype revealed that *mwp1-R* husk leaves and the sheaths of vegetative leaves develop pairs of outgrowths on the abaxial surface associated with regions of adaxialised tissue. *In situ* hybridisation confirmed that disruptions to adaxial-abaxial patterning are correlated with misexpression of leaf polarity genes. Leaf margins and fused organs such as the prophyll are most severely affected by *mwp1-R*. The first two husk leaves normally fuse along adjacent margins to form the bi-keeled prophyll. In the most severe cases the *mwp1-R* prophyll is reduced to an unfused, two-pronged structure and keel outgrowth is significantly reduced. We speculate that the adaxial-abaxial patterning system has been co-opted during evolution to promote outgrowth of the keels in normal prophyll development.

The results of this study place *Mwp1*, *wab1* and *Lg1* in a network of genes that regulate leaf polarity and axial patterning.

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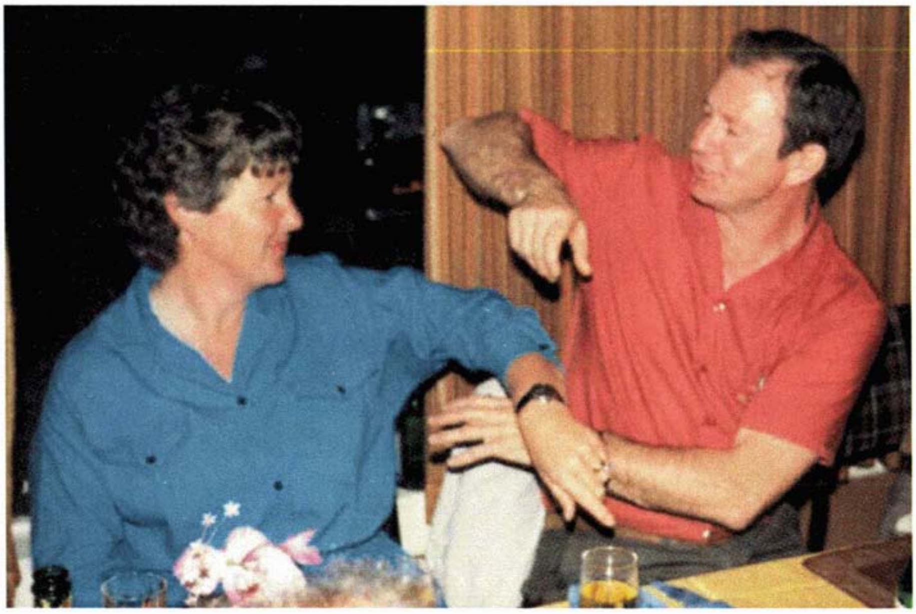

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List of abbreviations

BSA	bovine serum albumin
°C	degrees Celsius
d	day
DEPC	diethylpyrocarbonate
dicot	dicotyledon
DIG	digoxigenin
DNA	deoxyribonucleic acid
DPX	dibutylphthalate polystyrene xylene
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
FAA	formaldehyde, acetic acid, ethanol
GFP	green fluorescent protein
h	hour
kV	kilovolt
L1, L2, L3	cell layers in the shoot apical meristem and lateral organs
LM	lateral meristem
M	molar
mg	milligram
min	minute
miRNA	microRNA
ml	millilitre
mm	millimetre
mM	millimolar
monocot	monocotyledon
mRNA	messenger ribonucleic acid
NBT/BCIP	5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt/nitro-blue tetrazolium chloride
nm	nanometre
NTP	nucleotide triphosphates
P0, P1, P2	plastochron number
PBS	phosphate buffered saline
RNA	ribonucleic acid
s	second
SAM	shoot apical meristem

SEM	scanning electron microscopy
SSC	sodium chloride, sodium citrate
SSPE	sodium chloride, sodium phosphate, EDTA
TBS	tris buffered saline
TE	tris, EDTA
tRNA	transfer ribonucleic acid
µg	microgram
µl	microlitre
µm	micron
w/v	weight by volume ratio
v/v	volume to volume ratio