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Analysis of the canola lip mutant

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ANALYSIS OF THE CANOLA LIP MUTANT

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I. Purpose

Canola (*Brassica napus*) is a broadleaf agricultural plant grown mainly in regions of the United States and Canada. Its seeds are used to produce edible oil (canola oil) as well as biofuel. The study of mutagenized wild-type *Brassica napus* seeds in the CAN-TILL project, (http://www.botany.ubc.ca/can-till/), led to the discovery of a phenotype called *Lamina epiphylla* (*LIP*). The *lip* mutant is characterized by changes in leaf shape that cause the leaves to look trumpeted, among other variations. This research project compared wild-type *Brassica napus* leaf development with the *lamina epiphylla* mutant to assess leaf development and to determine the precise gene that causes the mutation. By analyzing the anatomy, histology, and genetics of the *lip* leaf mutant, this investigation will contribute to a deeper understanding of the overall development of this very important oil seed crop. Specifically, understanding the development of leaves, the main photosynthetic organ of the plant, may improve the overall growth of the crop plant. Ultimately this will potentially aid in the production of a larger quantity of seeds which will provide increased oil outputs.

II. Introduction

Canola (*Brassica napus*) is one of the roughly 3,200 species of plants belonging to the mustard (*Brassicaceae*) family. Wild-type canola plants typically have broad, flat leaves. Leaves require juxtaposition of expressing genes on the upper (adaxial) and lower (abaxial) surfaces of the lamina in order to produce a flattened leaf (Chitwood *et al.,* 2007). With each surface composed of distinct cell types, differentiation of the internal leaf is a result of patterning events that occur

when the leaf is developing (Chitwood *et al.,* 2007). While the conventional bifacial leaf has a distinct upper and lower surface, there are many variations of leaf symmetry. One variation is the unifacial leaf where cell types on both adaxial and abaxial surfaces are symmetrical (Chitwood *et al.,* 2007).

In the CAN-TILL project, wild-type canola plants were treated with the chemical EMS (ethylmethane sulphonate), where a mutated plant that produced furrowed, trumpet-shaped leaves and/or filamentous outgrowths was identified (http://www.botany.ubc.ca/can-till/). Preliminary histological results on the mutant leaves showed that adaxial cell types were located on the outside of the trumpet leaves while cell types on the abaxial side were either on the inside of the trumpet or missing (J. Nowak, pers. communication). This data also showed that segregation of the *lip* mutant arises from heterozygous plants in a typical Mendelian ratio of 3:1 (heterozygous to homozygous plants). Morphologically, heterozygous plants look similar to wild-type plants while homozygous plants (*lip* mutants) have trumpet-shaped leaves with many filamentous processes and ectopic epi-laminar leaf outgrowths (J. Nowak, pers. communication).

While the gene that causes the *lip* mutation is unknown, leaf polarity in the model plant *Arabidopsis*, a species closely related to canola, has been studied extensively (Byrne, 2006). Most significantly, the *Arabidopsis PHABULOSA* (*PHB*) mutant bears a striking similarity to the *lip* mutant (McConnell & Barton, 1998). It has been established that development of leaf polarity depends upon "the precise spatial and temporal expression of regulatory genes which pattern tissues and control cell fate" (Williams *et al.,* 2005, p. 3657). The *PHB* mutation was mapped to a

miR165/166 complementary site and that disruption of the microRNA site caused the mutations observed in the *phb-1d* mutants (Mallory *et al.,* 2004). In *Arabidopsis*, the "developmental regulators targeted by miRNAs are five members of the class III homeodomain-leucine zipper (HD-ZIP) family of transcription factors (Sessa *et al*., 1998) that include the genes *REVOLUTA (REV), PHABULOSA (PHB), PHAVOLUTA (PHV), CORONA (CNA)* and *ATHB8*" (Williams *et al.,* 2005, p. 3658). *PHV, PHB and REV* microRNAs (mir165/166) are all involved in regulating the polarity of leaves by defining adaxial cell fate (Kim *et al.*, 2005; Emery *et al.,* 2003; Juarez *et al.*, 2004; Kidner & Martienssen, 2004; McConnell & Barton, 1998; McConnell *et al.,* 2001; Mallory *et al.,* 2004; Zhong and Ye, 2004). When the mir155/166-directed regulation is altered, *phb, phv,* and *rev* mutants show tissue radialization (McConnell & Barton, 1998; Williams *et al.,* 2005; Prigge *et al.,* 2005). A similar mutant to the *phb* and *phv* mutants was identified in the plant *Antirrhinum* (snap dragon). Observations of the *PHANTASTICA (PHAN)* mutant was found to cause a similar phenotype to the *phb* and *phv* mutants where leaves were radialized and adaxial tissues were lost (Waites & Hudson, 1995; Waites *et al.,* 1998). Thus, regulation of leaf polarity is well established in many other species, including *Arabidopsis* and *Antirrhinum.*

III. Research Questions

The research project that forms the basis of this thesis compared the canola *lip* mutant with wild-type *Brassica napus* plants in an effort to determine the gene that is responsible for causing the *lip* mutation. Based on the published results of

prior research conducted on *Arabidopsis*, this study focused on a possible homologous candidate gene, *REVOLUTA-like* gene, from the HD-ZIP III family of genes.

IV. Methodology

This research consisted of growing wild-type, heterozygous and homozygous (*lip* mutant) *Brassica napus* plants to observe the leaf anatomy, both morphologically and histologically, as well as beginning genetic analysis of the plants. All of these procedures provided a greater understanding of the phenotypic and genotypic differences between wild-type and the *lip* mutant *Brassica napus* plants.

Plant Growth

Seeds were sown directly onto soil with ~24 wild-type and 24 heterozygous seeds sown out at the same time. These seeds were placed in a growth chamber (24^0C) for one week and then transferred to a greenhouse where they were observed and tissue samples collected, as necessary. Out of the 24 heterozygous seeds planted, on average, 6 plants expressed the *lip* phenotype.

Morphology

Photographs were taken of the wild-type, heterozygous and *lip Brassica napus* plants to document their overall morphology. Photographs at high and low magnification were taken to document whole leaves as well as specific traits (margins, petiole/blade region) on the leaves.

Anatomy and Histology

Petioles, lip margins, lateral margins and tip margins were sectioned from wild-type, heterozygous and *lip* mutant plants and placed directly into 70% formalin acetic acid alcohol (FAA) for fixation. These plant tissues were then dehydrated through an ethanol series, placed into paraffin and embedded, sectioned at a thickness of 7 micrometers at an angle of 7 degrees, mounted onto slides and stained outlined inSinha lab protocol (1997).

Genetics

DNA and RNA were extracted from the leaves of wild-type, heterozygous and *lip* mutant plants using the protocol as outlined by the manufacturer (QIAGEN).PCR was performed using 45 cycles, a temperature gradient from 55°C to 75°C and primers designed from the coding region. The primers used were: *Arabidopsis AS1* [NM_129319.3], F: aggatggtgagatgggaaga, R: gctgaggtaaggaacccaaa, *Antirrhinum* MYB-related transcription factor [AJ005586.1], F: tttggggcaatgggcaaagg, R: ctagccaaggttgattcaagacc, *Cardamine AS1* [DQ512733.1], F: gcgtcatggaagttgctctcc, R: tcaggggcggtctaatctgcaa, *Nicotiana phantastica* [AY559043.1], F: gggtcgaggcaagaactgggct, R: cttaggcggccagggacacac, *Pisum* MYB-related transcription factor *PHAN1* [AF299140.2], F: caagttttgagaaaccggctgtggg, R: caatgcattgcatcaacccc, *Solanum phantastica* [NM_001247347.1], F: gaggagtttgggaggatggaaactg, R: gaaccgtctaaaggagcaagg and *Zea mays rs2* [AF143447.1], F: gctactgctcgccaggtcccctc, R: agcgacggtgtggtgagcggc.

V. Results

Morphology

Wild-type

B*rassica napus* wild-type plants have bifacial leaves that consist of a blade and petiole (Figures 1A, 2A). Wild-type leaves exhibit polarity, having a distinct adaxial and abaxial surface. The adaxial surface is the top portion of the leaf (orientated toward the sun) and the abaxial surface is the bottom portion of the leaf (side facing away from the sun) (Figure 2B). Leaves have lobate margins, which means that they are indented at the margin but the indentation does not run to the midline. Venation in wild-type *Brassica napus* plants shows a reticulate pattern where the smaller diameter veins form a reticulate (netted) pattern. Petioles are horseshoe-shaped with flaps of adaxial tissue running along the sides (Figure 3 A-E).

Heterozygous

Morphologically, heterozygous *Brassica napus* leaves are similar to their wild-type counterparts in that there is a distinct blade and petiole. Like wild-type, heterozygous leaves are also bifacial and show polarity on the adaxial (Figure 1B Leaves 4,5) and abaxial (Figure 1B Leaves 1-3) surfaces . Despite these similarities, there are some heterozygous leaves that show a distinct morphological difference from wild-type leaves. For instance, some leaves lack petiolar flaps (Figure 1B Leaf 1) while others have a thickened petiole and lack a distinct blade/petiole region (Figure 1B Leaf 5). Also, rather than having rounded leaves that are typical of wild-type leaves, some heterozygous leaves are more ovate (Figure 1B Leaf 5). However, similar to wild-type leaves, heterozygous *Brassica napus* leaves have lobate margins,

a reticulate vein pattern and petioles that are horseshoe-shaped with adaxial tissue running along the sides (Figure 3F-I).

Lip Mutant

While wild-type and heterozygous *Brassica napus* plants are morphologically similar, the leaves of the *lip* mutant plants show a severe change in leaf morphology. There is a large degree of phenotypic variation between leaves as well as variation within a plant (Figure 1C).. One of the leaf phenotypes that was observed on *a lip* plant was trumpet-shaped leaves (Figure 1C Leaves 1,2,4). Trumpeted leaves have no filamentous processes along their petioles and blades (Figure 1C Leaf 2). Additionally, these leaves do not have any ectopic epi-laminar ("lip") blade outgrowths. The margins of these leaves are also lobate and showed a reticulate vein pattern (Figure 4C,D).

A second leaf phenotype observed in the *Brassica napus lip* mutant were trumpeted-shaped leaves with one or more filamentous processes extending from either the petiole or leaf blade or both (Figure 4G,H). The lateral margins of these leaves were also lobate and showed a reticulate vein pattern (Figure 4E,F).

A third leaf phenotype observed in the *Brassica napus lip* mutant has secondary outgrowths with smaller trumpeted-shaped leaves growing along the petiole of the main leaf (Figure 4I,J). In this type of leaf phenotype, filamentous processes may also be seen on the blade and/or petiole (Figure 4K). The lateral margins of both the main leaves and the smaller leaf outgrowths are lobate and both the main leaf and the smaller secondary leaf outgrowth also show a reticulate vein pattern (Figure 4J,L).

The fourth phenotype observed in the *Brassica napus lip* mutant is the phenotype from which the *lip* mutant gets its name. Ectopic epi-laminar outgrowths develop on the abaxial surface of the leaf (Figure 5A). These outgrowths resemble lips, hence the name of the mutant. It was found that the cells of these outgrowths resemble adaxial-type of cells even though the outgrowths develop on the abaxial surface of the leaf (Figure 5D,E). They also can have one or several filamentous processes coming from the petiole and/or the leaf blade (Figure 5F).

The *lip* mutant has a flattened blade with non-trumpeted leaves. The leaf margins are also lobate; however, the shape of the blade is not completely round or ovate like wild-type and heterozygous leaves (Figure 5A). Also, unlike wild-type and heterozygous leaves, which have one primary vein running down the middle of the leaf and secondary veins emerging from the primary vein, the major veins (primary and secondary veins) of this *lip* leaf show a palmate pattern (several major veins diverging from a central point) (Figure 5C).

Leaves that have ectopic epi-laminar outgrowths tend to be non-trumpeted (Figure 5A). In some cases, though, leaves may appear slightly trumpeted at the petiole/blade junction (Figure 5B). It was observed that the epi-laminar outgrowths never appear on leaves that are fully trumpeted (Figure 1C Leaves 1,2,4). The petioles in all phenotypes of the *Brassica napus lip* mutant plants were completely radial. This differs from the wild-type and heterozygous *Brassica napus* plants which have horseshoe-shaped petioles (Figure 5F and 3D,E). Another difference observed in the *lip* mutant petioles is that they lack flaps of adaxial tissue running along them

as is seen in both wild-type and heterozygous plants. However, mutant leaves do have ectopic outgrowths along the petiole (Figure 5F)

Anatomy

To assess changes in the internal anatomy of the leaves and petioles, cross sections were made from wild-type, heterozygous and homozygous plants.

Petiole Anatomy

Petioles of wild-type *Brassica napus* leaves resemble a horseshoe (Figure 6A,B) where several vascular strands are grouped (vascular bundles) along the margin of the petiole on the abaxial side forming the horseshoe shape. These vascular bundles have xylem in the middle surrounded by an area of phloem which is oriented towards the abaxial surface (Figure 6B).

Cross sections of petioles of heterozygous *Brassica napus* leaves showed a similar anatomy to that of wild-type leaves (Figure 6C,D). Heterozygous petioles also were horseshoe-shaped with several vascular strands grouped along the edge of the petiole on the abaxial side forming a U-shape (Figure 6C). Like wild-type petioles, the vascular bundles have xylem in the middle surrounded by an area of phloem on the abaxial side (Figure 6D).

In contrast to the wild-type and heterozygous *Brassica napus* plants, petioles of the *lip* mutant plants from both non-trumpeted and trumpeted leaves were completely radialized (Figure 6E,G). In non-trumpeted leaves, several vascular strands were bundled near the center of the petiole in a radial pattern (Figure 6E). The arrangement of the vascular cells resemble those seen in wild-type and

heterozygous petioles, where xylem tissue is surrounded by phloem (Figure 6F). However, in these petioles, the phloem is on the side of the vascular strand that is closest to the center of the petiole. In trumpeted *lip* mutant leaves, there is only one area of xylem in the center of the petiole. Interspersed among the xylem are several circular bundles of phloem that are distinct from both one another and the xylem (Figure 6G). The phloem shows no pattern of orientation as is observed in the petioles of wild-type, heterozygous and *lip* mutant non-trumpeted leaves (Figure 6H).

Leaf Anatomy

Cross sections from the lateral and tip margins of wild type *Brassica napus* leaves show that vascular bundles have a collateral arrangement; The xylem is oriented towards the adaxial surface of the leaf and the phloem was oriented towards the abaxial side (Figure 7A,B). Mesophyll cells in wild-type plants were cuboidal in shape (Figure 7C). Heterozygous leaves, like those of the wild-type plants, the vascular bundles have a collateral arrangement and Mesophyll cells were also cuboidal in shape (Figure 7D,E).

Cross sections taken from the *lip* margins of non-trumpeted *Brassica napus* *lip* mutant leaves showed that the vascular bundles were collateral but smaller than the bundles observed in both wild-type and heterozygous plants (Figure 7F). The vascular strands of the non-trumpeted *lip* mutant plants had xylem orientated towards the adaxial side and phloem orientated towards the abaxial side of the leaf. The mesophyll cells of these plants was much less regular than that of the wild-type and heterozygous plants. In addition to this, the mesophyll cells were also observed

to be slightly more rounded than the epidermal cells of the wild-type and heterozygous plants (Figure 7G).

In cross sections of the tip margin, including the mid-vein of *the* trumpeted leaf mutants, the vascular bundle was smaller when compared to the ones seen in wild-type and heterozygous plants (Figure 7H). Tip margin sections, not including the mid-vein, showed very few vascular bundles and observed bundles were very small in diameter (Figure 7I). Unlike the vascular strands of the wild-type, heterozygous and *lip* mutant plants with non-trumpeted leaves where the xylem faced the adaxial side and the phloem faced the abaxial side, the vascular strands of the trumpeted leaves did not have a clear collateral arrangement of xylem and phloem and were not specifically orientated towards the adaxial or abaxial surface of the leaf (Figure 7I).

Molecular Biology

DNA Extraction

DNA from whole leaf tissue of *Brassica napus* wild-type, heterozygous and *lip* mutant plants was extracted for all three plant types. Successful DNA extraction of all three genotypes was confirmed on an agarose gel (Figure 8A)

RNA Extraction

RNA from whole leaf tissue from leaves of *Brassica napus* wild-type, heterozygous and lip mutant plants was extracted. An agarose gel showed successful RNA extraction with two clear bands (18S and 28S ribosomal bands) was observed for all genotypes (Figure 9C)

RNA extracted from marginal and internal leaf tissue of *Brassica napus* wild-type, heterozygous and *lip* mutant plants was also successful for all three genotypes (Figure 9D,E).

RNA extracted from both stem and petiole tissue failed to produce any measurable RNA for wild-type, heterozygous and *lip* mutant *Brassica napus* plants. RNA bands were extremely faint on the agarose gels, indicating very low RNA concentration (Figure 9A,B).

PCR

A PCR using all primers was conducted and no PCR product was present (data not shown). A subsequent PCR was conducted using the same PCR conditions as the first PCR run, but with the addition of magnesium chloride to increase specificity of the primer binding to the DNA. This PCR run used only the *Arabidopsis AS1*, *Cardamine AS1* and *Solanum phantastica* primers. After running an agarose gel, bands were observed for all three primers (Figure 8B). However, none of the bands matched the expected size of the gene fragment (Figure 8B).

VI. Discussion

This study analyzed changes in the adaxial-abaxial polarity in leaves of the *Brassica napus lip* mutant. Wild-type *Brassica napus* plants have broad, flat, bifacial leaves that have an upper (adaxial) and lower (abaxial) surface. However, there are many variations from the conventional bifacial leaf. In the *Brassica napus lip* mutant, unifacial leaves are seen in which the leaves show one type of surface (Chitwood *et al.,* 2007). This study sought to determine the morphological, histological and

genetic components that cause the *lip* mutation.

The leaf anatomy of the *Brassica napus lip* mutant suggests that it could be either an adaxial or abaxial mutant. The orientation of its vascular tissue with xylem surrounding the phloem or in other cases the complete absence of phloem, seems to indicate that it is an abaxial mutation as is seen in the *Arabidopsis phabulosa-1d* (*phb-1d*) mutant. Like the *Brassica napus lip* mutant, trumpet-shaped leaves also occur in the *phb-1d* mutant (Figure 10A,B). Along with this, the *phb*-1d mutant has vascular bundles where xylem surrounds the phloem (McConnel & Barton, 1998). Similarily, the more radialized leaves of the *phb-1d* mutant "either entirely lack a vascular strand or possess single xylem elements in which phloem is not seen" as was seen in the *lip* mutant (McConnel & Barton, 1998, p. 2937).

Even though in many ways the *lip* mutant resembles the *phb-1d* mutant in *Arabidopsis,* differences in leaf phenotype were observed. For instance, in the *phb-1d* mutant, lip-like structures are not observed. These ectopic structures, however, do occur in *PHAN* mutants (Figure 10C,D) (Waites & Hudson, 1995). Waites and Hudson (1995) found that the PHAN protein produces a dorsalizing factor and when the amount of dorsalizing factor is reduced, ectopic ridges appear on the upper surface of the leaves. Their findings would suggest that the *lip* mutation may be caused by an adaxial gene.

In order to definitively conclude whether the *lip* mutation is caused by an adaxial or abaxial factor, molecular analysis of the *lip* mutant must be conducted. PCR was performed in this study in an attempt to isolate product from an array of gene sequences that may potentially be the gene causing the mutation. However, the

PCR's were unsuccessful. The lack of product may have been due to sequence unspecificity. Alternatively, it may have been because the PCR was not performed on the *lip* mutant plants that actually have the defect in the gene. The next step will be to test these genes via PCR in the heterozygous and homozygous lines.

Based on the preliminary work of J. Nowak (pers. communication), her phylogenetic analysis suggests that the *lip* mutation may be a *REV-like* gene. While her findings provide some indirect evidence that the *lip* mutation may be a *REV-like* gene, it is impossible to be certain until genetic sequencing is performed on the *lip* mutant. Sequencing of the gene will be conducted in future experiments.

Future research should conduct PCR tests on the heterozygous and homozygous *Brassica napus* lines. Eventually, plants there are homozygous for the *lip* mutant should be sequenced to isolate the gene responsible for the mutation.

Canola is an important crop for a wide variety of reasons. Canola plants are grown to produce oil that is used in many foods, canola meal which is used as a supplement for livestock as well as biofuel. The research that this thesis is based on will contribute to a better understanding of leaf development in Canola plants and in time, allow farmers to produce more from this important crop.

VII. Acknowledgements

I would like to thank Dr. Julie Kang, Department of Biology, for her inspiration and guidance. This project would not have been possible without her help.

I would also like to thank Julia Nowak, University of British Columbia, for

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Finally, I would like to thank Jessica Moon, Ph. D., Director of the University of Northern Iowa Honors Program, for her help throughout the thesis process.

VIII. Figures

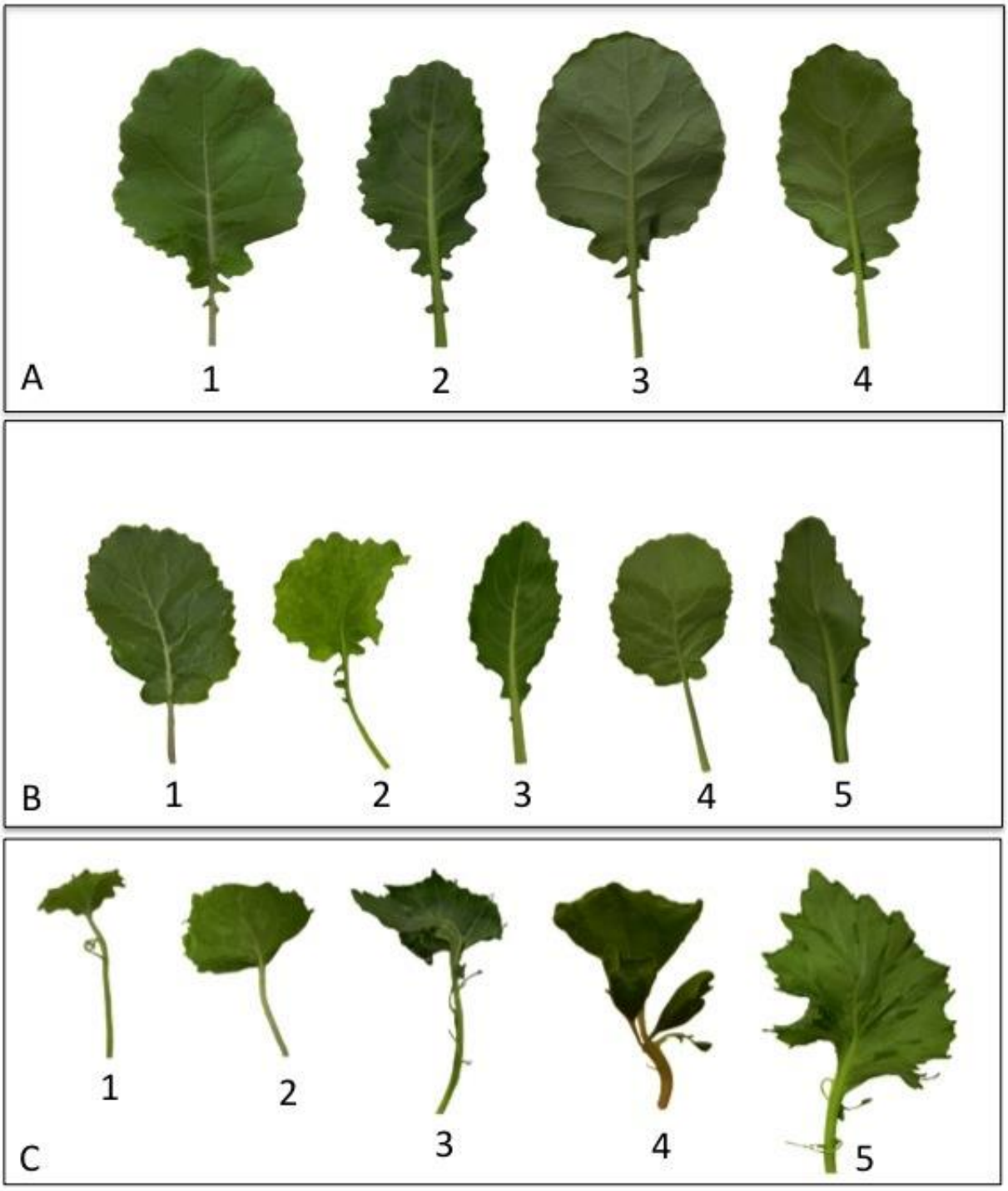

Figure 1: Leaf morphology. **A:** Wild-type comparative leaf morphology. **B:** Heterozygous comparative leaf morphology. **C:** *Lip* mutant comparative leaf morphology.

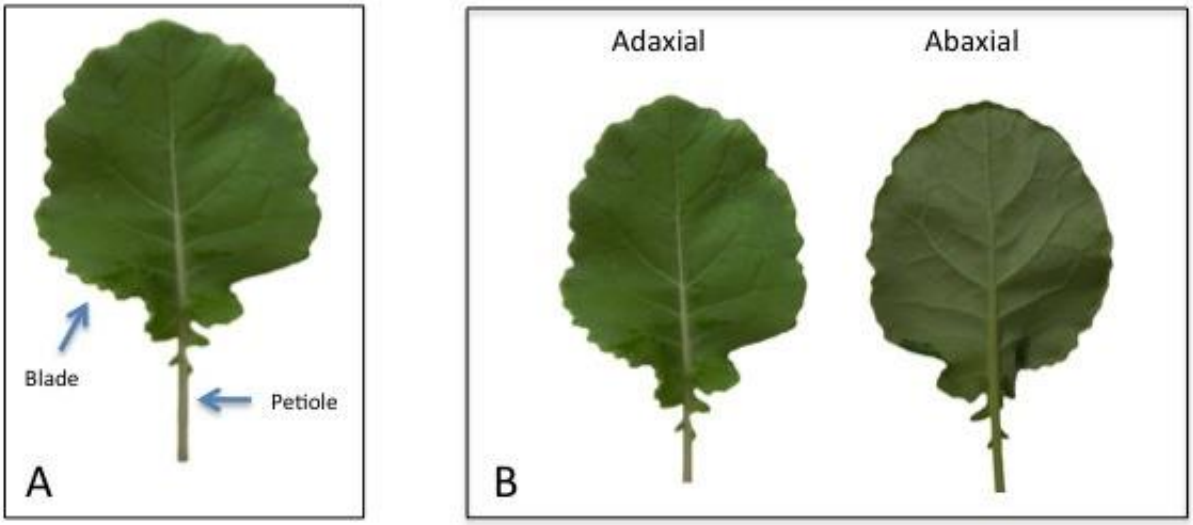

Figure 2: Parts of a *Brassica napus* leaf. **A:** *Brassica napus* leaf showing blade and petiole. **B:** Wild-type leaves showing adaxial and abaxial sides.

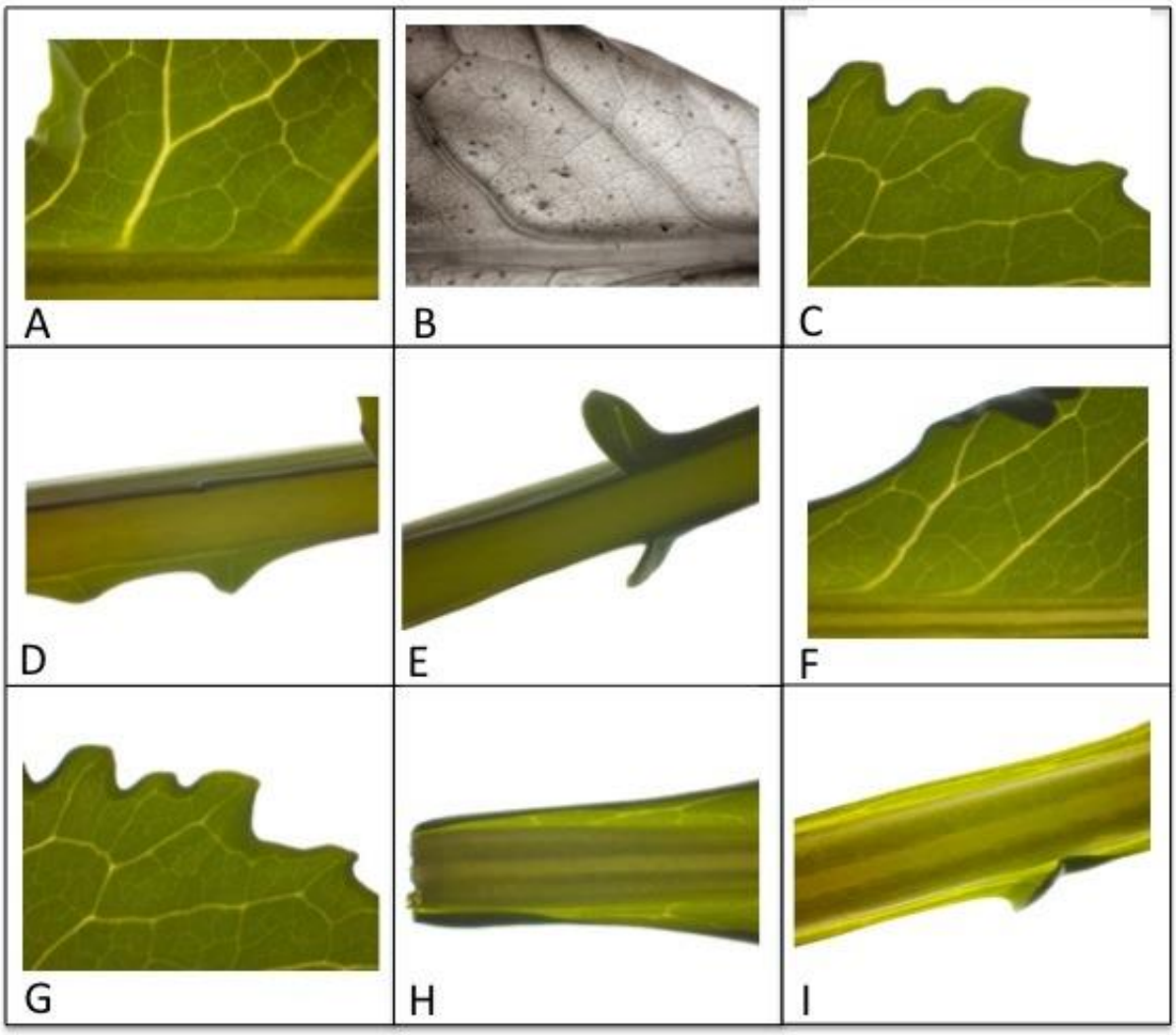

Figure 3: Wild-type and heterozygous leaf morphology. **A, B:** Vein pattern in wild-type leaves. **C:** Leaf margin of wild-type leaf. **D, E:** Wild-type petioles. **F:** Vein pattern in heterozygous leaf. **G:** Leaf margin of heterozygous leaf. **H, I:**

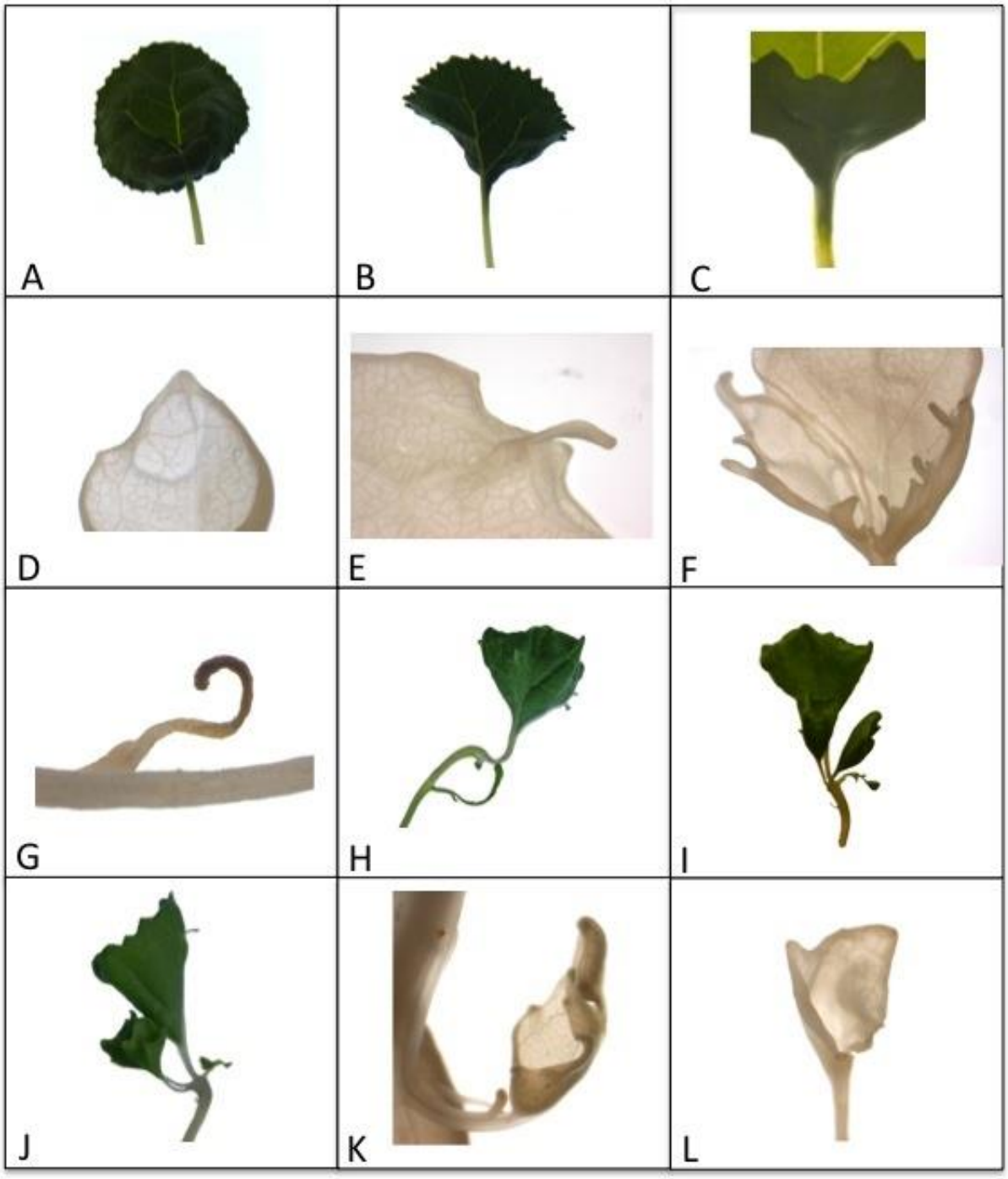

Figure 4: *Lip* mutant leaf morphology. **A, B:** Trumpeted leaves of the *lip* mutant. **C:** Leaf margin of a trumpeted *lip* mutant leaf. **D:** Vein pattern of a trumpeted *lip* mutant leaf. **E, F:** Filamentous processes extending from *lip* mutant leaves. **G, H:** Filamentous processes extending from the petiole of a *lip* mutant leaf. **I-K:** Secondary leaf outgrowths and filamentous processes occurring trumpeted leaves of *lip* mutant plants. **L:** Vein pattern of a secondary leaf outgrowth.

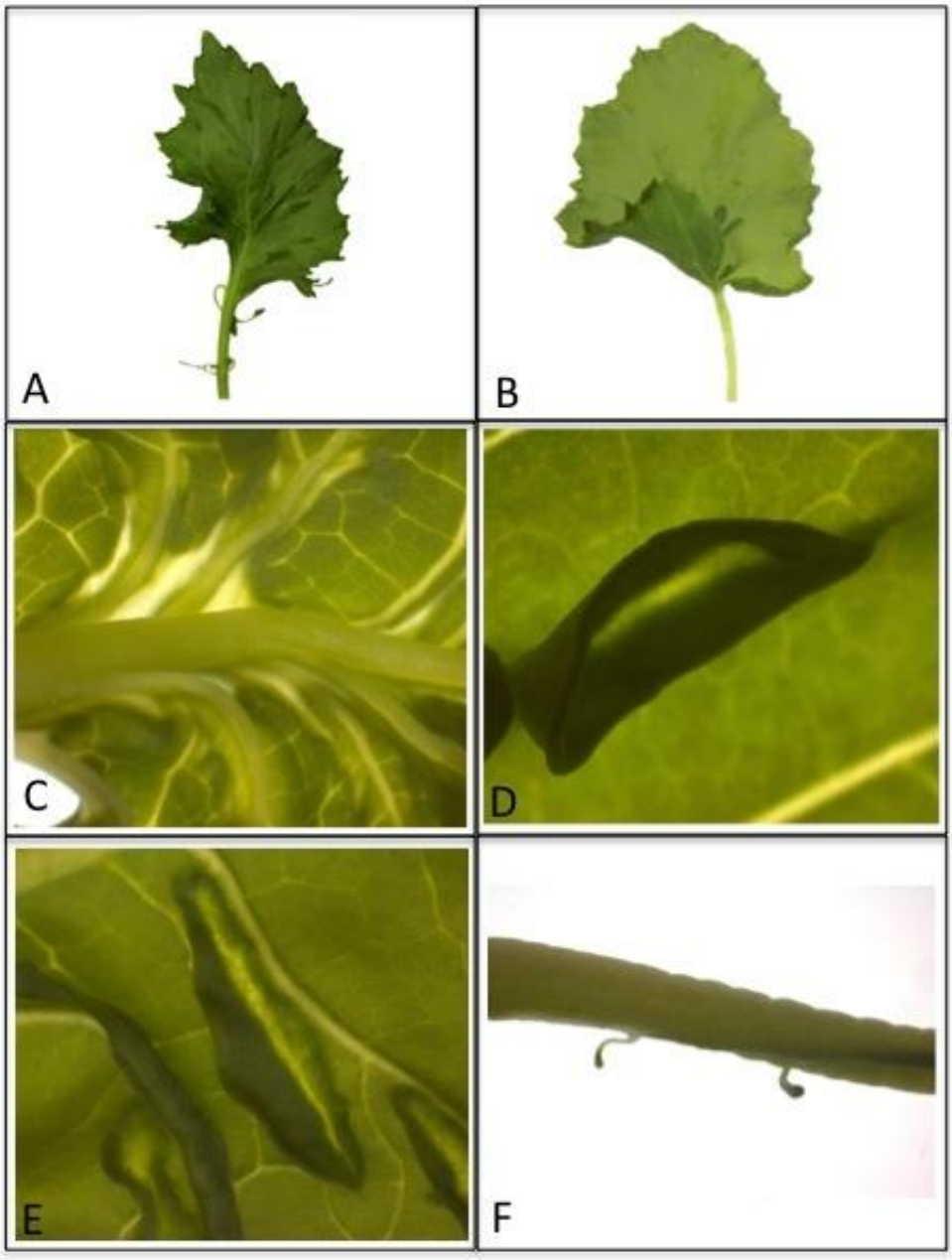

Figure 5: Ectopic epi-laminar growths occurring on *lip* mutant leaves. **A:** Abaxial side of a non-trumpeted *lip* mutant leaf showing ectopic epi-laminar growths. **B:** *Lip* mutant leaf with ectopic epi-laminar growths that is slightly trumpeted at the petiole/blade junction. **C:** Vein pattern in non-trumpeted *lip* mutant leaves. **D, E:** Ectopic epi-laminar growths on *lip* mutant leaves. **F:** *Lip* mutant petiole showing filamentous processes.

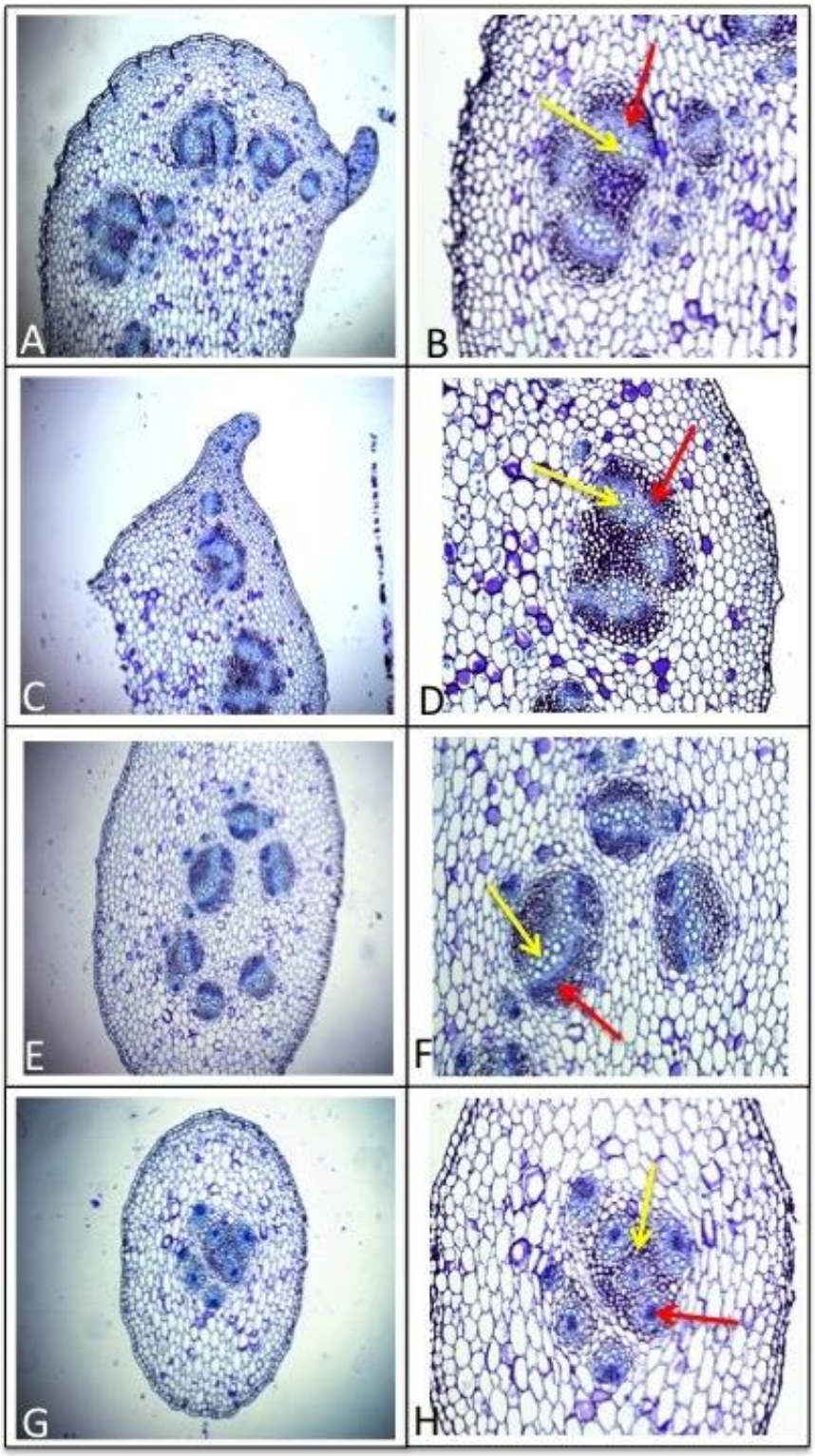

Figure 6: Petiole cross sections. Yellow arrows denote xylem, red arrows denote phloem. **A, B:** Wild-type petiole cross sections. **C, D:** Heterozygous petiole cross sections. **E, F:** Non-trumpeted *lip* mutant petiole cross sections. **G, H:** Trumpeted *lip* mutant petiole cross sections.

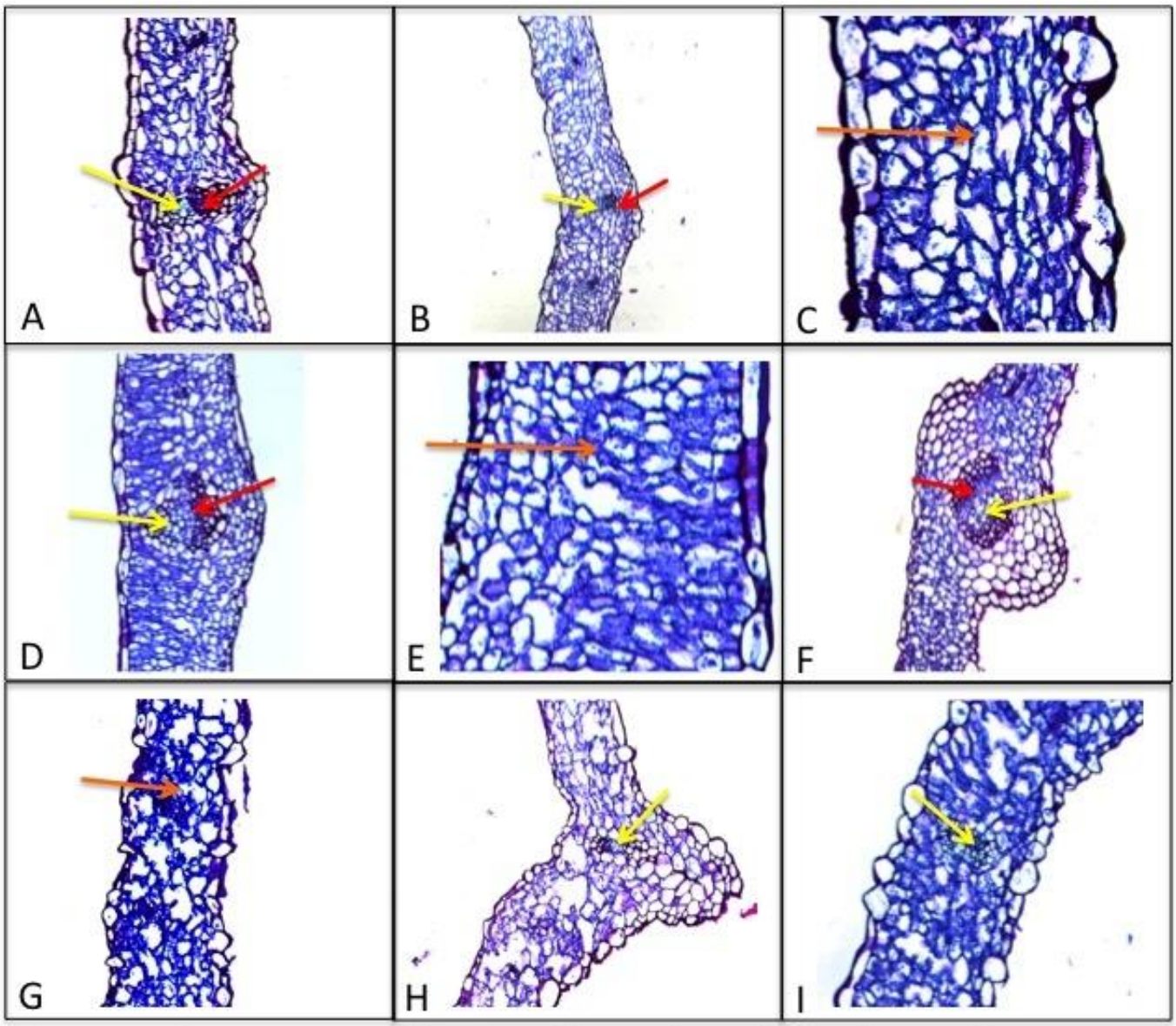

Figure 7: Leaf cross sections. Yellow arrows denote xylem, red arrows denote phloem, orange arrows denote mesophyll cells. **A:** Cross section of wild-type leaf lateral margin showing vascular bundle. **B:** Cross section of wild-type leaf tip margin showing vascular bundle. **C:** Cross section of wild-type leaf showing mesophyll cells. **D:** Cross section of heterozygous internal leaf tissue showing vascular bundle. **E:** Cross section of heterozygous internal leaf tissue showing mesophyll cells. **F:** Cross section of *lip* mutant non-trumpeted *lip* margin leaf tissue showing vascular bundle. **G:** Cross section of *lip* mutant leaf tissue showing mesophyll cells. **H:** Cross section of *lip* mutant trumpeted leaf tip margin including the mid-vein showing the vascular bundle. **I:** Cross section of *lip* mutant trumpeted leaf tip margin not including the mid-vein showing vascular bundle.

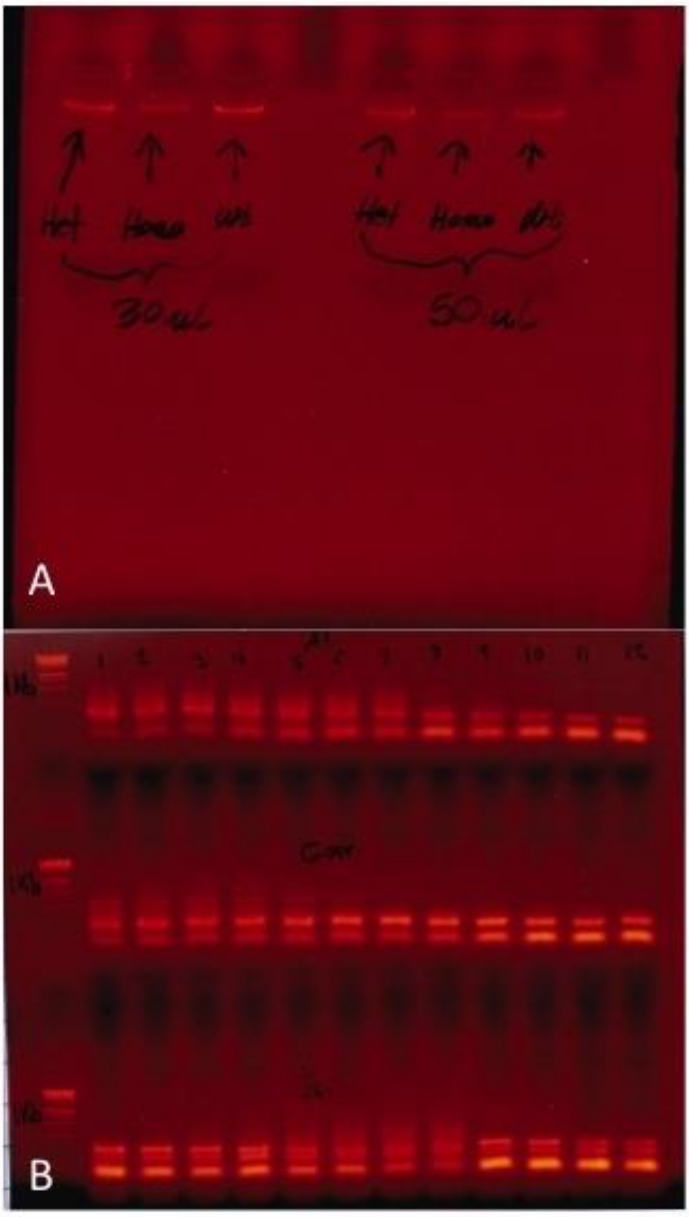

Figure 8: DNA results. Wt denotes wild-type, Het denotes heterozygous and Homo denotes *lip* mutant plants. **A:** Agrose gel containing RNA extracted from whole leaf tissue of wild type, heterozygous and *lip* mutant plants. **B:** Agrose gel showing the results of a PCR test run using *Arabidopsis AS1 (*denoted by At), *Cardamine AS1 (*denoted by Car) *and Solanum phantastica* (denoted by Sol) primers.

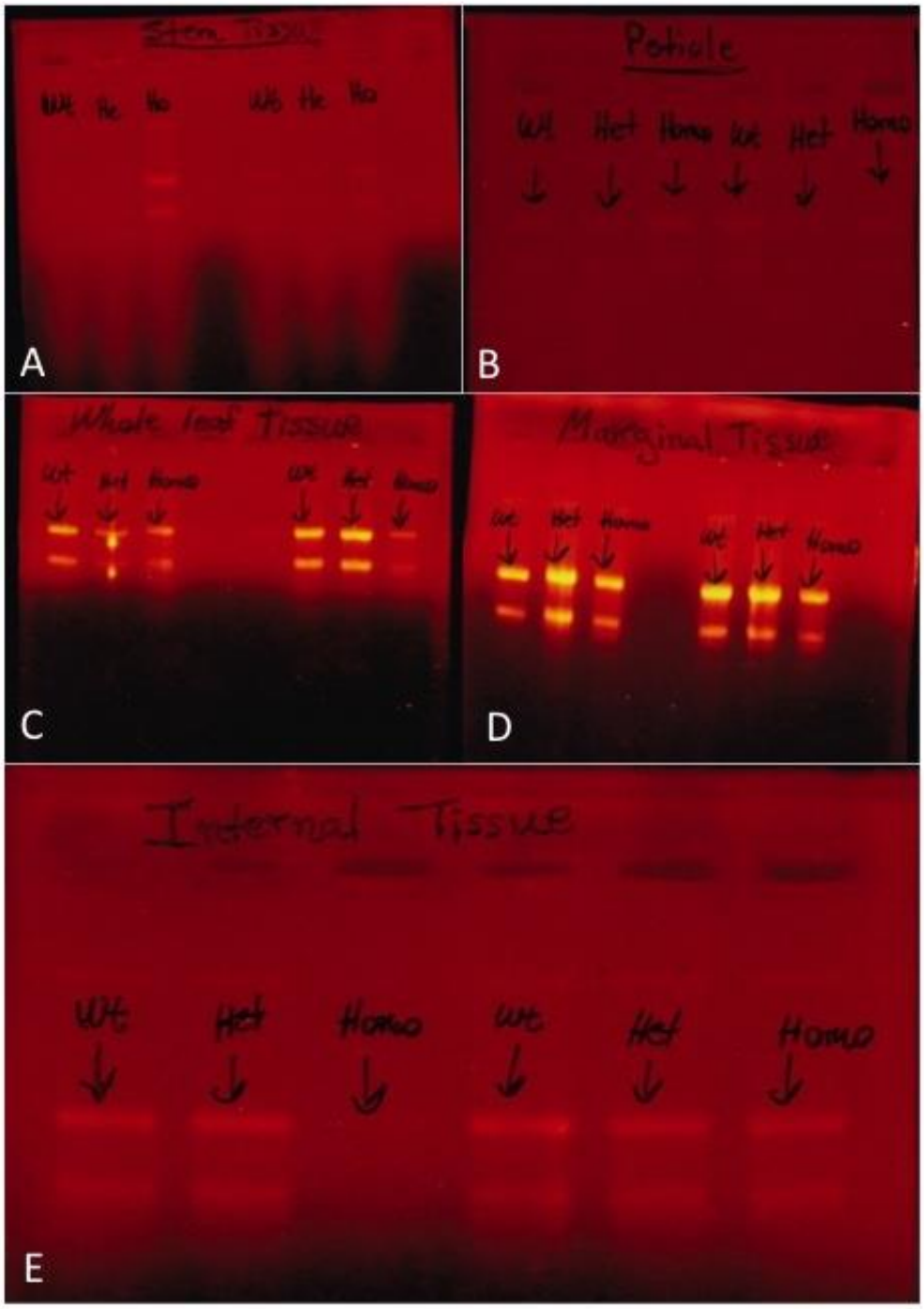

Figure 9: RNA results from extractions performed on wild-type, heterozygous and *lip* mutant plants. Wt denotes wild-type, Het denotes heterozygous and Homo denotes *lip* mutant plants. **A:** Agrose gel showing RNA extracted from stem tissue. **B:** Agrose gel showing RNA extracted from petioles. **C:** Agrose gel showing RNA extracted from whole leaf tissue. **D:** Agrose gel showing RNA extracted from marginal tissue. **E:** Agrose gel showing RNA extracted from internal tissue.

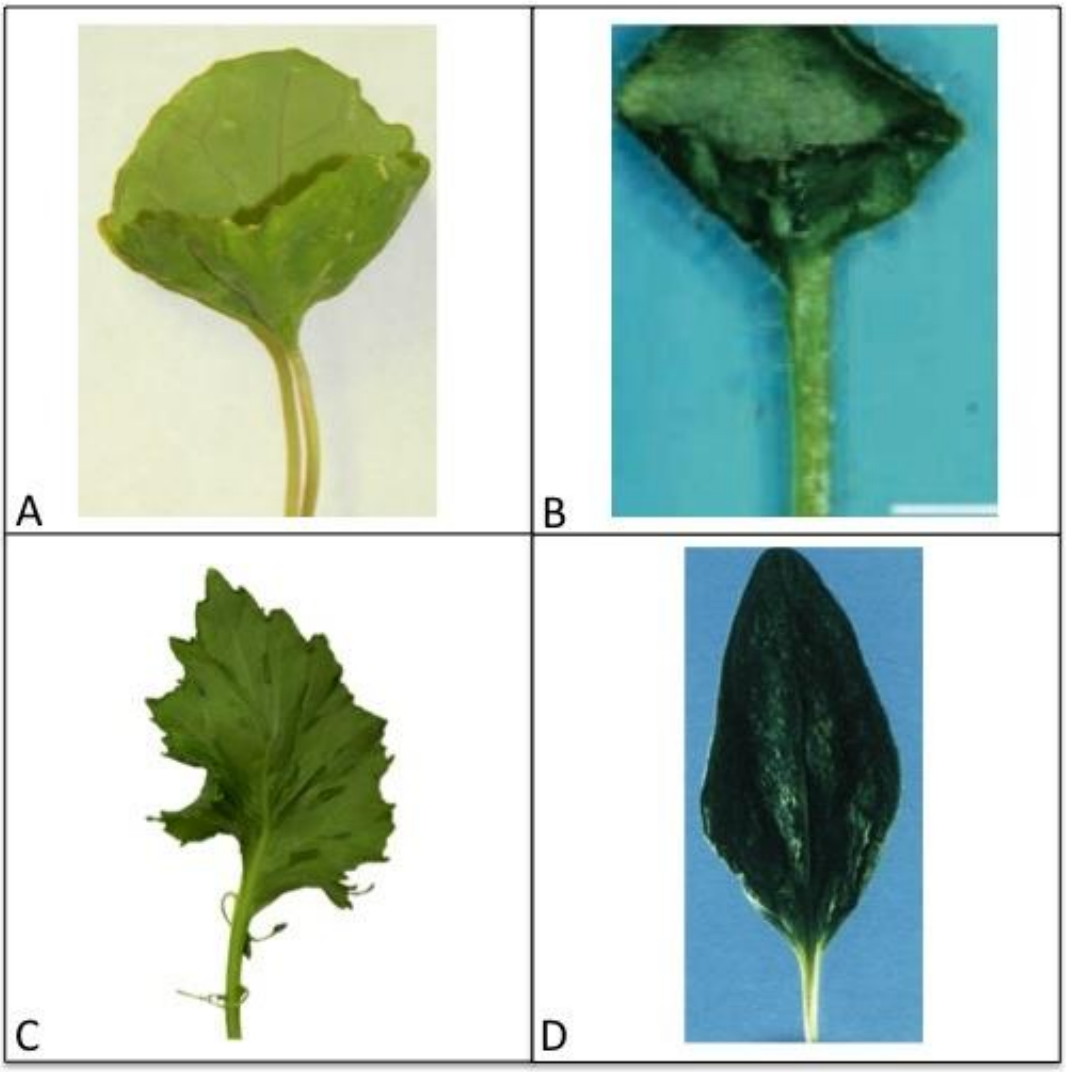

Figure 10: Comparison pictures. **A:** *Lip* mutant trumpeted leaf. **B:** *Phb-1d* mutant trumpeted leaf. **C:** *Lip* mutant non-trumpeted leaf with ectopic outgrowths. **D:** *PHAN* mutant leaf with ectopic outgrowths.

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This Study by: Matt Rafferty

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has been approved as meeting the thesis or project requirement for the Designation University Honors.

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Date Dr. Julie Kang, Honors Thesis Advisor

5/9/14 _____
Date Dr. Jessica Moon, Director, University Honors Program