Exam 2018
Week 2 - Module Genomes and Phenotypes M1

PART 1 – COURSE QUESTIONS (5 points)

A)The most commonly studied mutations based on genome sequences are SNPs. What are the other categories of genetic aberrations?

B) A gene drive construct is composed of several genetic elements. What are they? What is the role of each element?

C) Describe two possible applications of gene drive technology and give molecular details on the respective gene drive constructs.

PART 2 – ARTICLE ANALYSIS (15 points, each question = 1 point unless indicated, there are 20 questions)

Leaf shape is highly variable in angiosperms. Brassicaceae species within the genus *Capsella* show considerable variation in leaf shape. *Capsella grandiflora* has less dissected leaves than *Capsella rubella*.

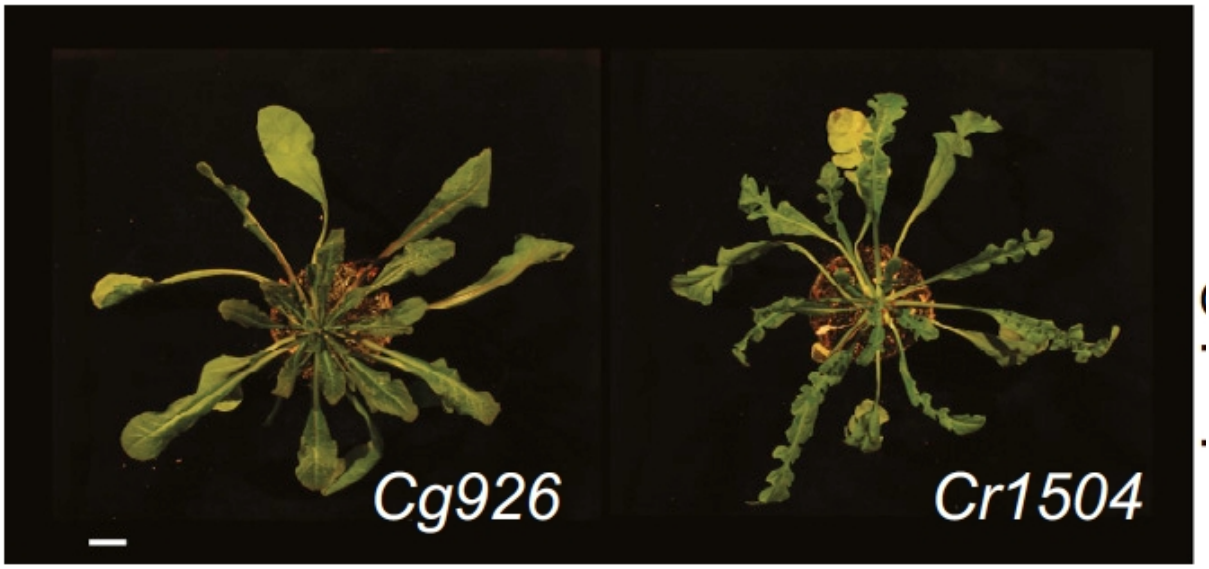

Figure 1. Accession *Cg926* of *Capsella grandiflora* and accession *Cr1504* of *Capsella rubella*.

C. grandiflora and *C. rubella* are closely related and can be crossed to generate recombinant inbred lines (RILs).

1) What are RILs and how are they generated?

A panel of RILs from a cross between *C. grandiflora* accession *Cg926* and *C. rubella* accession *Cr1504* was used to map quantitative trait loci (QTL) underlying the variation in leaf margin dissection.

2) What is QTL mapping and which data are required to perform it?

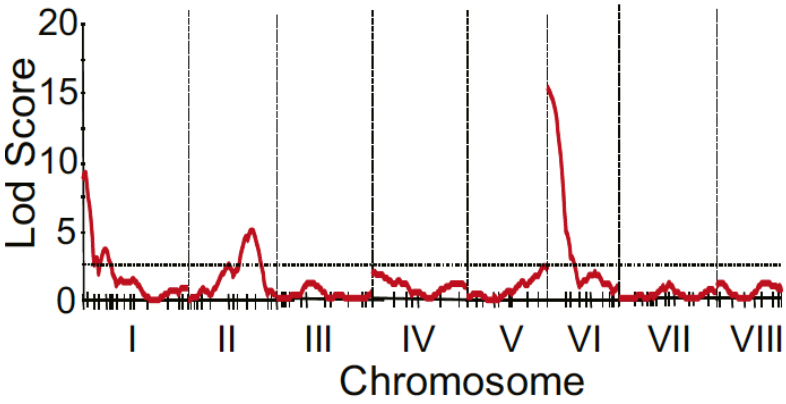

Figure 2. Result of the QTL mapping for leaf dissection.

3) How many QTLs are detected and on which chromosomes are they located?

The introgression of the *C. grandiflora* allele at the strongest QTL in the *C. rubella* genetic background allowed to generate a near isogenic line, *NILgg*. More specifically, *NILgg* was produced by introgressing the *C. grandiflora* allele into a *Cr1504* background by three rounds of backcrossing, followed by selfing this BC3 for two generations, while keeping the QTL region heterozygous.

4) What is an introgression and how is it performed?
5) How can we make sure that our region of interest is introgressed? (0.5 pts)

Leaf shape of the *NILgg* line was compared to that of the *NILrr* line (with the r*ubella* alleles in the *C. rubella* background).

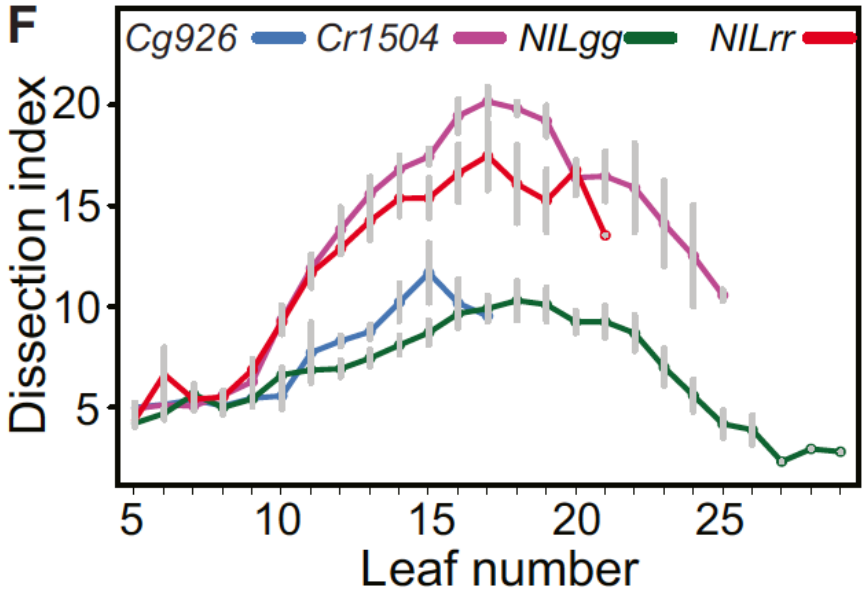

Figure 3. Comparison of leaf dissection in the *Cg926* accession of *C. grandiflora*, the *Cr1504* accession of *C. rubella* and the *NILgg* and *NILrr* lines. The dissection index (perimeter^2/4π x area) is used to quantify leaf dissection. Leaf numbers represent the position of the leaf along the stem (the level of dissection of the leaves varies along the stem).

6) What can you conclude from this graph?

The phenotyping of 1500 plants homozygous for recombinant chromosomes in the major QTL interval between *NILgg* and *NILrr* delimits a region of 110Kb.

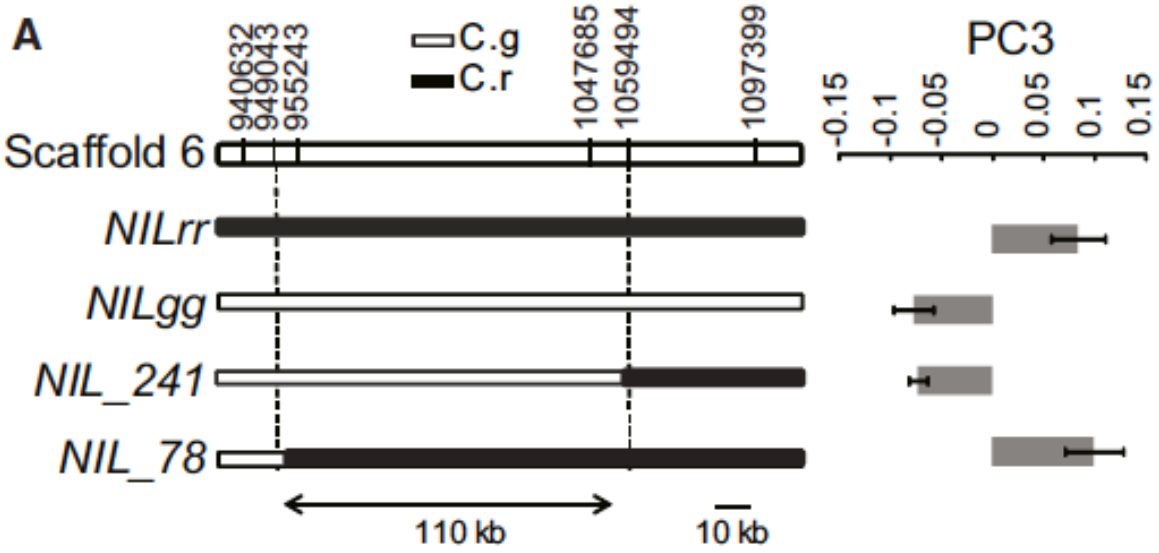

Figure 4. Phenotypic effect of selected recombinant chromosomes. PC3 is a measure of the dissection. Positive values correspond to *C. rubella*-like leaves whereas negative PC3 values correspond to *C. grandiflora*-like leaves.

7) What represent the two dotted lines? (0.5 pts)
8) In NIL_241 and NIL_78, why is the switch from white region to black region not exactly at the position of the dotted line? (0.5 pts)

The 110Kb interval contains three genes: *CrLMI1, CrRCO-A* and *CrRCO-B*. The expression of these genes was measured in *NILgg* and *NILrr* by quantitative RT-PCR (qRT-PCR).

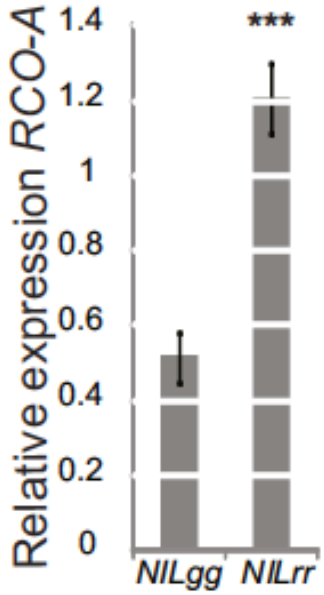

Figure 5. Results of the qRT-PCR expression analysis of *RCO-A* expression. Normalization was done with *CrTUB6* (a *tubulin* gene).

9) What is a RT-qPCR? (0.5 pts)
10) What can you conclude from Figure 5? (0.5 pts)

In addition, expression of RCO-A was analysed by in situ hybridization.

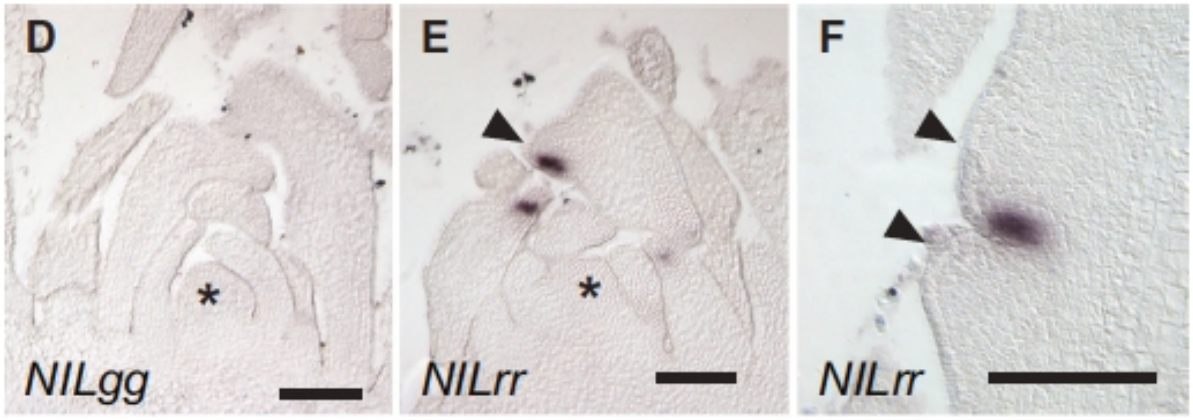

Figure 6. RNA in situ hybridization against RCO-A mRNA in NILgg and NILrr. F: higher magnification. Asterisks indicate shoot apical meristem. Arrowheads mark outgrowing lobes.

11) What is an in situ hybridization on mRNA? (0.5 pts)

The *C. grandiflora* and *C. rubella* alleles differ in 2 single nucleotide changes in the coding region of *RCO-A*, leading to two nonconservative amino acid changes.

12) What can you conclude from the expression analyses of RCO-A by qRT-PCR and in situ hybridizations on the nature of the genetic differences between the RCO-A alleles from C. grandiflora and C. rubella?

The *CgRCO-A* and *CrRCO-A* alleles were transformed in *NILgg*.

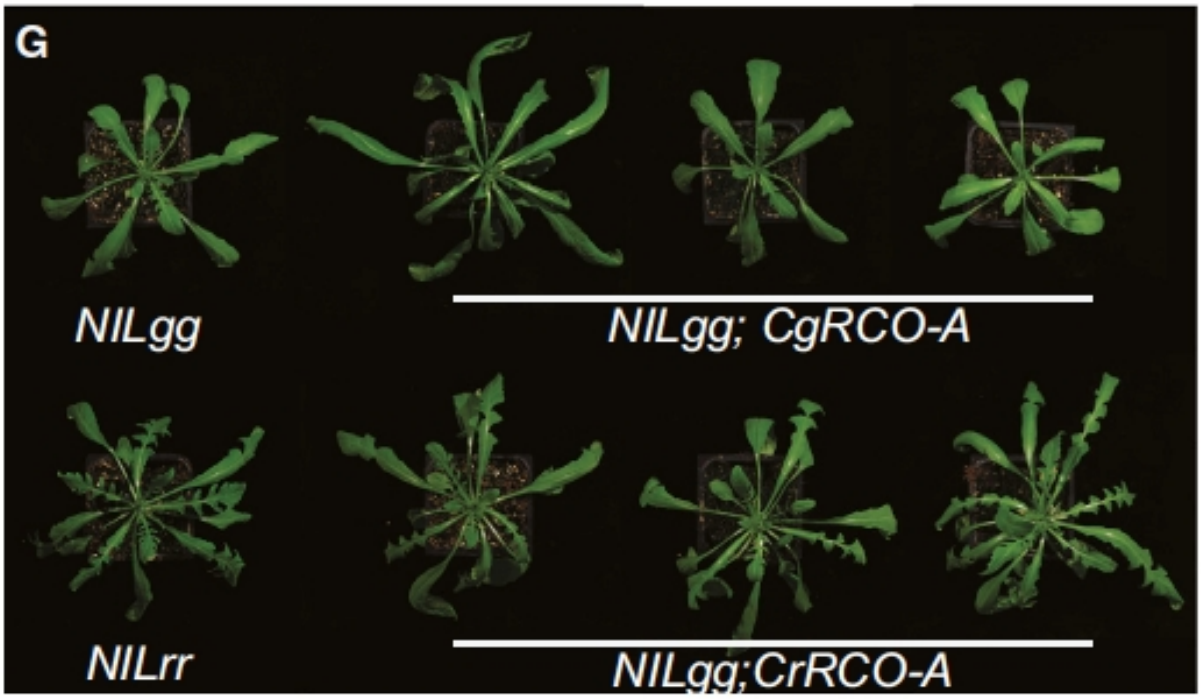

Figure 7. Phenotypes observed when the *CrRCO-A* or *CgRCO-A* alleles are transformed in the *NILgg* background. Independant transformants are shown. A cross between *NILgg* and *NILrr* gives F1 plants which look like *NILrr*.

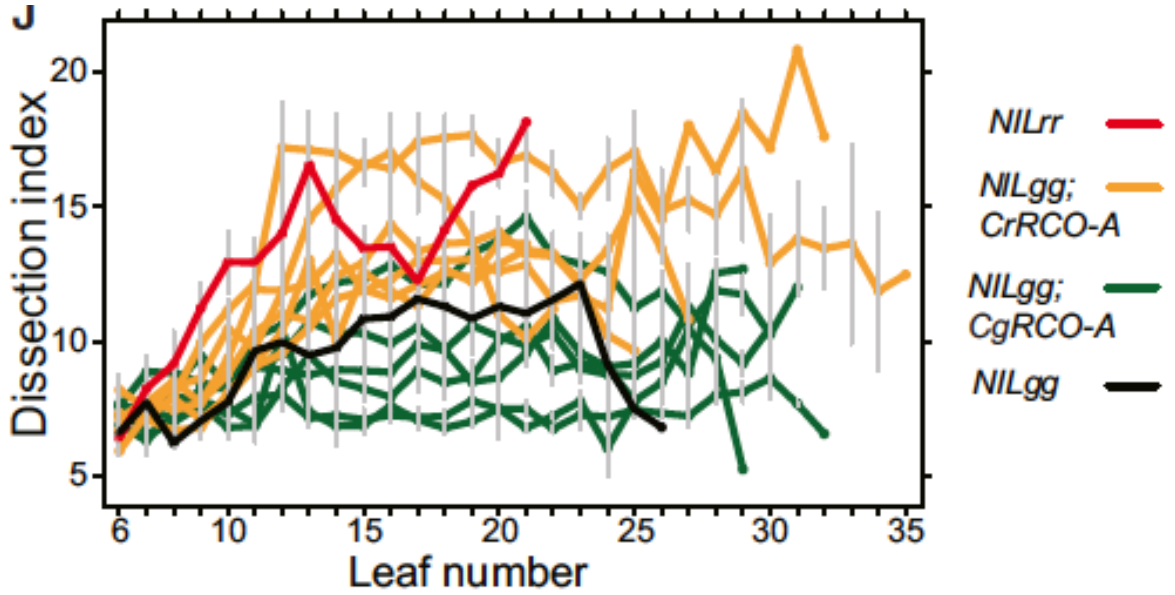

Figure 8. Quantification of leaf dissection in the same plants.

13) What can you conclude about the involvement of the three genes located in the 110kb region on leaf dissection?

Plants were raised at two different temperatures.

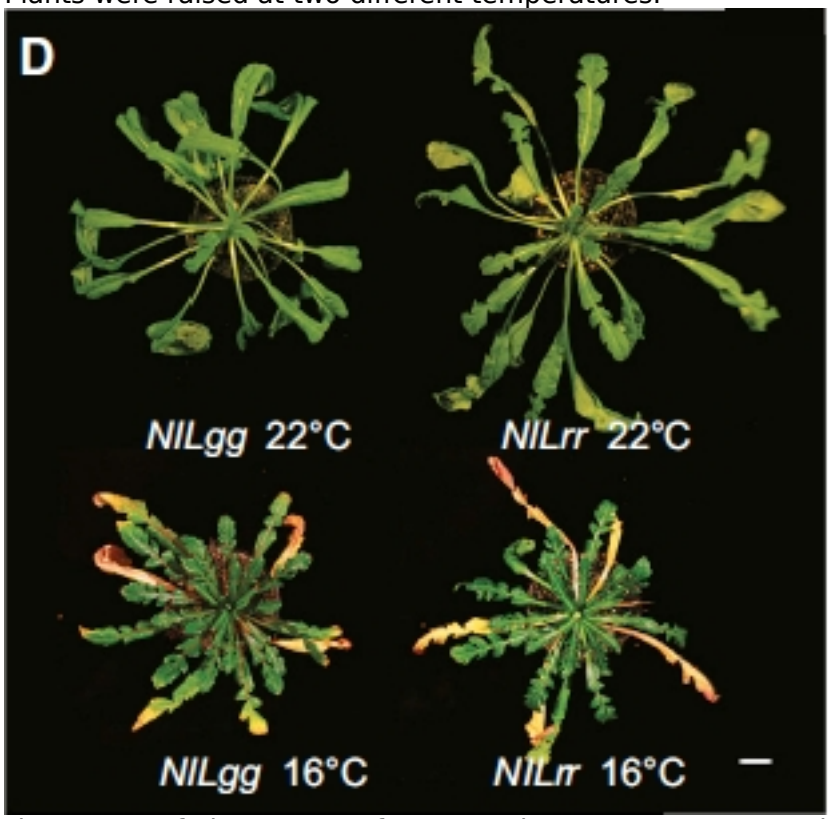

Figure 9. Leaf phenotypes of NILgg and NILrr grown at 22 and 18°C.

14) *What is the effect of temperature on leaf shape? (0.5 pts)*

The expression of *RCO-A* and the other genes in the 110kb interval was measured in NILgg and NILrr grown at two temperatures.

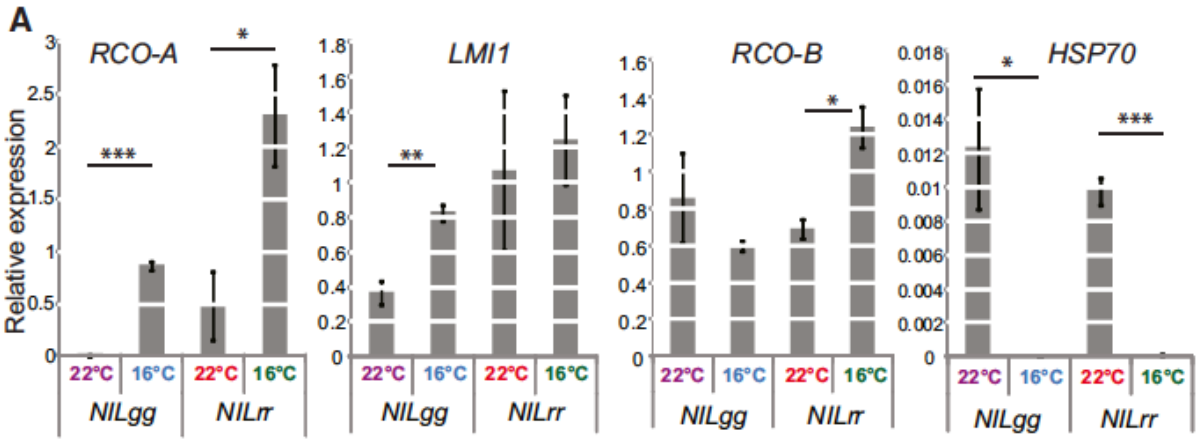

Figure 10: Expression of *RCO-A, LMI1, RCO-B* and *HSP70* in *NILgg* and *NILrr* grown at 22°C and 18°C. *HSP70* is used as a positive control.

15) What can you conclude from this analysis on the mechanisms underlying leaf thermal plasticity in these species?
16) Which experiment would you suggest to test further the role of RCO-A on leaf shape thermal plasticity? (0.5 pts)

Arabidopsis thaliana belongs also to the Brassicae and is characterized by the derived absence of leaf dissection. Interestingly *RCO-A* and *RCO-B* have been lost in *A. thaliana.*

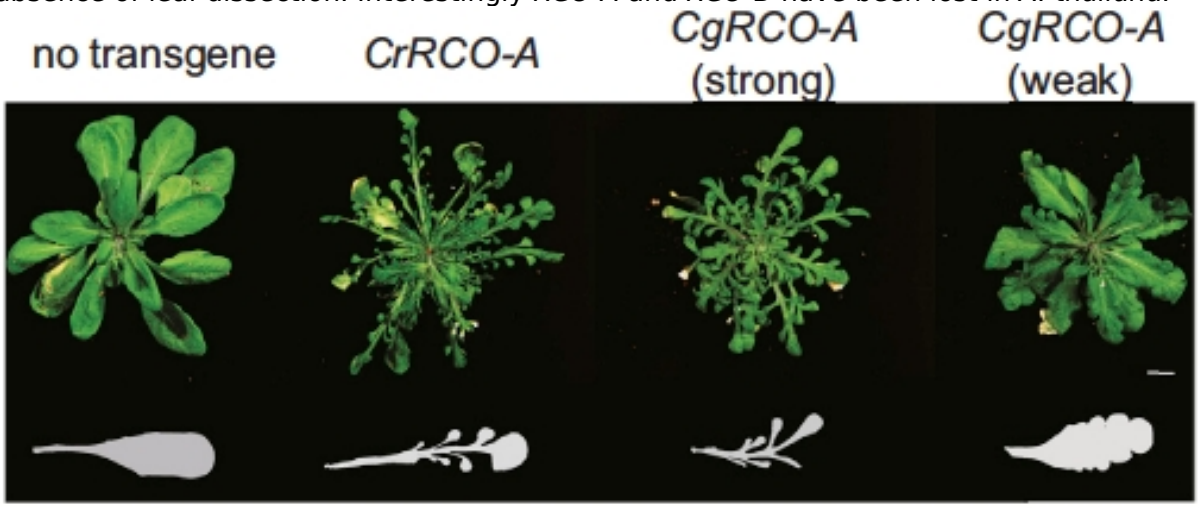

Figure 11. *CrRCO-A* and *CgRCO-A* alleles were transformed in *A. thaliana.* Two different transformant lines are shown for *CgRCO-A*. All plants were raised at the same temperature.

17) How do you explain the difference between the "strong" and the "weak" transformant lines? (0.5 pts)
18) What can you conclude from Fig. 11?

19) How would you qualify RCO-A?

20) How could the involvement of RCO-A in thermal plasticity of leaf shape influence its role in evolution or vice versa? (0.5 pts)