Unraveling the cold response in Draba

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Summary

Draba is a genus with species occurring worldwide in various geographical regions. The genus, thus, provides the opportunity to investigate how species have adapted to different environments. This thesis compares the cold response of *Draba* species originating from tropical-alpine regions with species from arctic-alpine and temperate regions. Tropical-alpine regions are characterised by a year-round growing season, which differs considerably from the seasonal arctic-alpine and temperate regions. In equatorial high altitudinal (i.e., tropical-alpine) mountains low temperatures occur on a daily basis; within 24hours a warm 'summer' day can be followed by a cold night during which freezing is not exceptional. In contrast, arctic-alpine regions are generally characterized by long, cold winters where even during summer months the possibility exists that temperatures drop below zero. Plants occurring in these regions have to be prepared for frequent periods of freezing. Those growing in temperate regions, have to cope with periods of frost only during winter and early spring. We hypothesise that the cold response of temperate plants differs from that of their arctic- and tropical-alpine relatives.

Cold is a limiting factor to the distribution of plants worldwide. Since plants are sessile organisms they have to adapt to the local circumstances present in their habitat in order to survive. Plants have developed a wide array of responses to cope with low temperatures. This can include morphological adaptations such as leaves covered with trichomes (i.e., hairs), a compact growth form, or small leaves arranged in rosettes. Plants also adapt to cold at a physiological level by producing extra sugars or metabolites, which function as antifreeze compounds and protect the cell from freezing damage. In addition, some plant species have the option of 'cold acclimation'. This is a process by which plants can enhance their tolerance to sub-zero temperatures after a pre-exposure to low but non-freezing temperatures.

When a plant is exposed to cold, a signaling cascade is triggered which results in an increased ability of plants to withstand frost. This process is generally referred to as the cold response pathway of plants and several genes crucial to this process have been characterized. The focus of this thesis is twofold: first we have investigated the expression of genes involved in the *cold response pathway* in different *Draba* species. Second, we have investigated the juvenile survival and electrolyte leakage (i.e., degree of cellular damage) of the same species in response to *freezing*.

To investigate the cold response of different *Draba* species a unique experiment was designed to simulate the natural variation in light and temperature occurring in equatorial mountains. Juvenile plants were grown under a 21/15°C (day/night) temperature regime with a 12h photoperiod. The subsequent cold treatment involved a shift to a 21/4°C (day/night) temperature regime during which the 12h photoperiod was maintained. These experimental conditions were chosen in order to simulate the natural circumstances present in tropical high altitudinal mountains of Ecuador. Similar temperature and light conditions are encountered in spring and fall in temperate regions and occasionally in arctic-alpine regions. The main focus of this thesis is the comparison of the cold response pathway between two temperate *Draba* species (*D. verna* and *D. muralis*), one arctic-alpine (*D. nivalis*) and one tropical-alpine *Draba* species (*D. hookeri*). Throughout the controlled experiments, *Arabidopsis thaliana* was included as a frame of reference.

Research in the model plant system *Arabidopsis thaliana* has shown that *CBF* genes (coding for transcription factors) play a crucial role in the cold response of plants. In response to cold *CBF* genes are induced triggering the expression of *COR* genes, which in turn results in an enhanced cold tolerance. This transcriptional response is commonly referred to as the '*CBF* regulon' and has been shown to be functional in many plant species. To investigate if and how the *CBF* regulon is involved in the cold response of *Draba* species, we cloned homologous genes from *Draba* by means of a PCR strategy. This thesis reports the identification of three classes of *CBF* and two classes of *COR* genes (one of which proved to have an unusual structure) in *Draba*. Our results demonstrate that the investigated genes are induced in response to cold treatment and that the kinetics of *CBF* and *COR15* genes differ between *Draba* species. The most abundant *CBFc* gene, present in the two temperate species, is expressed earlier in response to cold than *CBFa* present in the arctic- and tropical-alpine species. This difference in induction of both *CBF* genes did not translate into a different timing of *COR15* induction in the different *Draba* species. In other words, the sequence of induction was always the same, but the timing between *CBF* and *COR15* expression varied. Remarkably, *CBF* and *COR15* expression levels were 10 to 20-fold lower in *Draba* than in *Arabidopsis*. We, therefore, conclude that the identified *CBF* genes in *Draba* do not fulfill the same function as in *Arabidopsis*. Additional research by means of, for example, overexpression or knockout lines (transgenic plants) is needed to gain a better insight into the functional role of the *CBF* regulon in *Draba.*

In order to investigate whether the obtained differences at expressional level resulted in differences at metabolite level, the proline and sugar content of *Draba* plants was subsequently analyzed (by E. Zuther of the MPI in Golm). Proline levels, which increase in response to cold in *Arabidopsis*, did not change in response to cold treatment in *Draba*. Temperate *D. muralis* had a constitutive high proline level, thereby calling for additional research. Raffinose, a sugar known to accumulate during cold acclimation in *Arabidopsis* does not appear to accumulate in two of the three investigated *Draba* species in response to cold. No clear-cut relationship between induction of gene expression and accumulation of proline and sugars appears to exist in *Draba*.

After having investigated the molecular aspects of the cold response we investigated the freezing tolerance of different *Draba* species next. This was done by determining the juvenile survival of seedlings at temperatures ranging from –5°C to –15°C. Additionally, the amount of tissue damage that occurred during freezing was investigated. During both experiments we determined whether cold acclimation resulted in a higher freezing tolerance. When all species were grown under the same conditions prior to their exposure to sub-zero temperatures, some species proved to be more freezing tolerant than others. Surprisingly, *Arabidopsis thaliana* ecotype Col-wt proved to be the most freezing tolerant species. Of all investigated *Draba* species, the two temperate species turned out to be the most freezing tolerant, while tropical-alpine *D. hookeri* was most freezing sensitive. With the exception of *D. hookeri,* cold acclimation resulted in an enhanced freezing tolerance in all species. The observed differences in freezing tolerance of acclimated tropical-alpine, arctic-alpine and

temperate *Draba* species suggest adaptations to the temperature variation of their natural habitats at a genetic level.

Tropical-alpine *D. hookeri* proved to be the most freezing sensitive *Draba* species that displayed a poor ability to enhance its freezing tolerance by cold acclimation. In an environment where large shifts in temperature occur within a 24h period, we do not expect a gradual increase in freezing tolerance to be useful to the survival of the plant. This in contrast to species occurring in arctic-alpine or temperate regions that can enhance their freezing tolerance gradually due to the shifts in season. Therefore, a release of selective pressure may have resulted in a loss of ability for *D. hookeri* to cold acclimate. Nonetheless, this species survives well in its natural habitat. This suggests that this habitat may be less extreme compared to other climates or other *Draba* species.

Finally, to investigate whether the cold response pathway is also induced under natural circumstances the *CBF* expression of five *Draba* species growing in their natural habitat in Ecuador was analyzed. Despite different weather conditions in the various research sites, all investigated species showed an induction of *CBF* expression. A comparison between the average expression levels measured in the field and the laboratory demonstrated that adult plants in the field have higher expression levels (>10-fold) than juvenile plants in the laboratory. In addition, expression patterns as found in the laboratory do not match those found in the field. Contrary to the results from the laboratory experiments, the field results also revealed *CBF* induction at dawn. This suggests a possible role for *CBF* at daybreak when temperatures are still low, but plants already start to photosynthesize. Detected differences between field and laboratory experiments highlight the importance of more field experiments, despite the challenges one faces in the field – especially 3900m above sea level – and the amount of factors one cannot control.

Although *Draba* is not yet a model system like *Arabidopsis*, it is a genus worth investigating. Especially the amount of variation present at genetic, morphological, and physiological level is plenty. This genus, further, offers the possibility to compare species from different geographical regions with one another, but also plants with differing growth forms, annual and perennial plants, as well as diploid and polyploidy species. Due to its difference in freezing tolerance with *Arabidopsis*, *Draba* could provide novel information in relation to genes or transcriptional pathways not yet discovered in other (model) taxa.