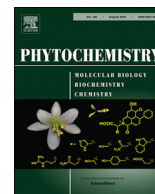




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Effect of growth temperature on glucosinolate profiles in *Arabidopsis thaliana* accessions

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ABSTRACT

Glucosinolates are plant secondary metabolites with important roles in plant defence against pathogens and pests and are also known for their health benefits. Understanding how environmental factors affect the level and composition of glucosinolates is therefore of importance in the perspective of climate change. In this study we analysed glucosinolates in *Arabidopsis thaliana* accessions when grown at constant standard (21 °C), moderate (15 °C) and low (9 °C) temperatures during three generations. In most of the tested accessions moderate and pronounced chilling temperatures led to higher levels of glucosinolates, especially aliphatic glucosinolates. Which temperature yielded the highest glucosinolate levels was accession-dependent. Transcriptional profiling revealed also accession-specific gene responses, but only a limited correlation between changes in glucosinolate-related gene expression and glucosinolate levels. Different growth temperatures in one generation did not consistently affect glucosinolate composition in subsequent generations, hence a clear transgenerational effect of temperature on glucosinolates was not observed.

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1. Introduction

The Earth's climate change is a regular issue in politics and society. But also natural scientists, among them plant biologists, are dealing with the potential consequences of climate change. With a predicted overall temperature increase, but with high inter-regional variability, and an increased frequency of weather extremes, plants all over the world risk to experience a change of their abiotic environment. While crop production in current key producing regions will be negatively impacted, some high-latitude regions currently too cold to grow crops might become more suitable in the future (Porter et al., 2014). Those changes will affect plant growth and phenology as well as the chemical composition of plant tissues, and thereby impact biological processes such as

plant-insect and plant-pathogen interactions (Ahuja et al., 2010a). Understanding how environmental factors affect the level and composition of secondary metabolites in plants is therefore of importance and has been actively studied (Hannah et al., 2010; Nakabayashi and Saito, 2015; Ramakrishna and Ravishankar, 2011). One important group of plant secondary metabolites with a well-documented role in plant defence against pathogens and pests but which are also known for their health benefits are glucosinolates, produced by plants of the Brassicales (Ahuja et al., 2010b; Björkman et al., 2011).

Glucosinolate biosynthesis is known to be influenced by developmental parameters (Brown et al., 2003), genetic variation (Bellostas et al., 2007; Kliebenstein et al., 2001a), biotic stressors (Hopkins et al., 2009; Mewis et al., 2006) as well as numerous exogenous abiotic factors. Those factors include nutrition (Falk et al., 2007; Gerendas et al., 2009), photoperiod and light quality (Engelen-Eigles et al., 2006), air composition (Himanen et al., 2008; Schonhof et al., 2007b), water (Mewis et al., 2012) and temperature. The effect of temperature on glucosinolates has been studied under field or controlled growth conditions in some Brassicaceae species

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such as cabbage (Rosa, 1997; Rosa et al., 1994; Rosa and Rodrigues, 1998), kale (Steindal et al., 2015; Velasco et al., 2007), broccoli (Guo et al., 2014; Mølmann et al., 2015; Steindal et al., 2013), turnip (Francisco et al., 2012) and watercress (Engelen-Eigles et al., 2006). Accessions of the model plant *Arabidopsis thaliana* (L.) Heynh. present a natural variation in their response to growth temperature (Hasdai et al., 2006; Lefebvre et al., 2009; Zhen and Ungerer, 2008) and in their glucosinolate composition (Kliebenstein et al., 2001a). Therefore different *A. thaliana* accessions were used in our study to investigate the effect of the growth temperature on glucosinolate levels.

Another important aspect in the context of plant adaptation to changing conditions that has gained attention lately is the question whether plants possess a transgenerational stress memory, what the underlying mechanisms might be and how such a memory might affect plant traits (Hauser et al., 2011; Heard and Martienssen, 2014; Holeski et al., 2012). Little is known about the transgenerational effect of abiotic (stress) conditions on the levels of secondary metabolites in plants. Recently, the effect of drought stress in the parent generation on the levels of glucosinolates in the offspring was reported for *Boechera stricta* (A. Gray) A. Löve & D. Löve (Alsdurf et al., 2013). Similarly, in our experiments we did not

only assess the effect of different growth temperatures on glucosinolates in *A. thaliana* for one generation, but also for subsequent generations (Fig. 1) experiencing a different temperature than the parents.

2. Results

2.1. Effects of growth temperature on seed germination of *Arabidopsis thaliana* accessions

The germination of *A. thaliana* F1 plants was assessed after seven days (Fig. 1 and Supplementary Fig. 1). The accessions C24, Col-0, Cvi and *Ler* had similar germination rates, ranging from 75 to 91%, at 21 °C, while Ru-0 showed much lower germination at this temperature than the other accessions. At 15 °C, the germination rate of all five accessions was reduced compared to 21 °C, but to different extents. While the germination rate of Col-0 was only slightly affected, Cvi showed a germination that was reduced to 22%. Ru-0 was most affected in germination by the temperature, with less than 4% of Ru-0 seeds having germinated after 4 day at 15 °C. None of the seeds had germinated at 9 °C after seven days. Eventually, germination rates at 9 °C and 15 °C reached levels

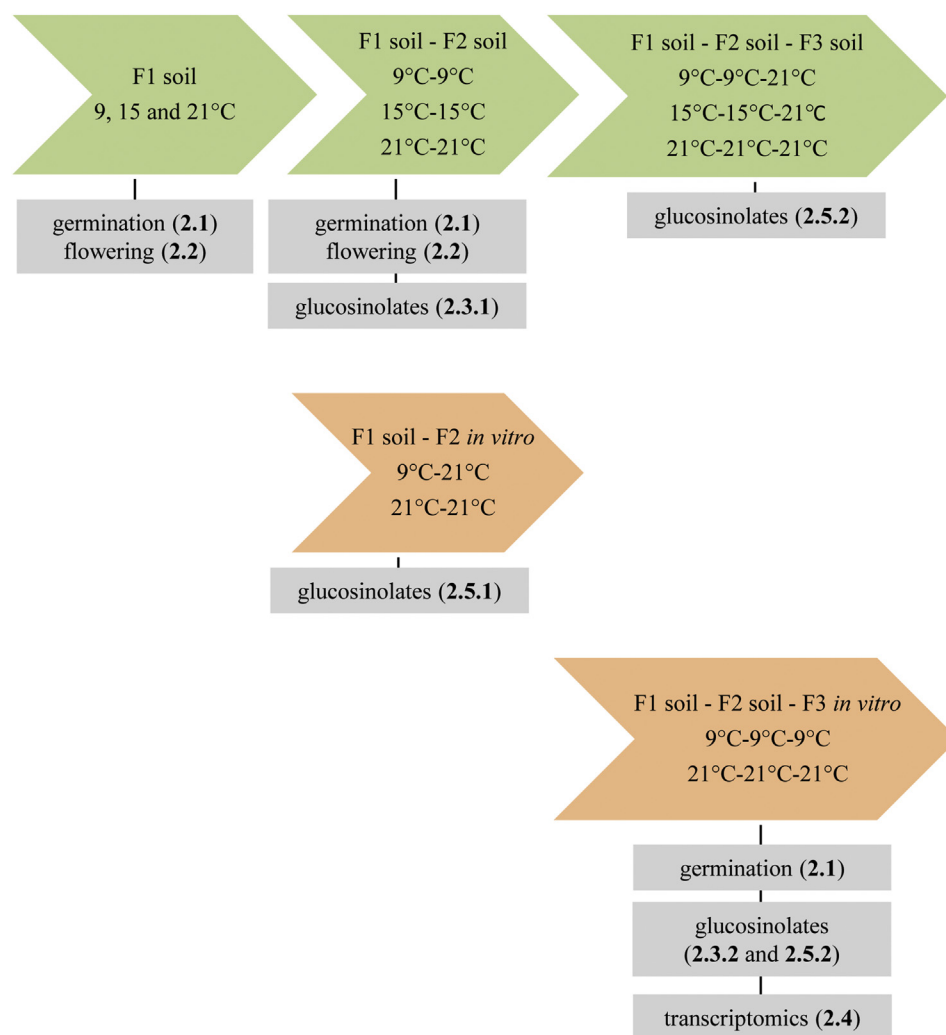


Fig. 1. Schematic overview of the setup for growth experiments of the five *A. thaliana* accessions at three growth temperatures described in this study, indicating at what generation plants were monitored for seed germination and flowering or tissue was harvested for glucosinolate analysis or transcriptional profiling. Numbers in brackets refer to sections of the Results part.

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