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Gene expression patterns in wheat coleorhiza under cold- and biological stratification

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ABSTRACT

This study assessed germination of wheat seeds under cold and biological stratification and determined the expression level of gibberellins (GA) and abscisic acid (ABA) genes in coleorhiza. Both cold and biological stratification significantly ($P < 0.05$) enhanced the rate and efficacy of germination. The spatial distance between the fungal endophyte and the seed can be a determining factor of biological stratification as seeds in direct contact with fungal endophyte showed the highest rate and efficacy of germination. Consistently high expression of GA3ox2 gene was found in wheat coleorhiza throughout the tested period of germination. The expression of ABA biosynthesis gene, TaNCED, was substantially higher in cold stratification seeds, reflecting the role of abscisic acid in stress-adaptation. Overall, this study provides molecular evidence of the importance of coleorhiza in germinating wheat seeds, in addition to reporting that the spatial distance between symbiotic partners may be a critical factor driving mycovitality.

1. Introduction

During last three decades, world wheat production declined 5.5% due to climate changes, threatening food security (Lobell et al. 2011). Wheat is one of the most widely used cereal crops in the world. Although Canada produces nearly 5% of world's total wheat, it exports more than 75% of its total production (Canadian Wheat Board, 2010). Climate is a key driver of plant ecophysiology, phenophases, and reproduction efficacy. Hence, consequences of a global warming on each stage along the plant developmental continuum seem likely (Cleland et al. 2007). In a mature seed, the non-vascularized multicellular embryonic tissue that shields the quiescent meristematic tissue of radicle and elongates during imbibition and early germination before radicle emergence in monocot seeds is called coleorhiza (Sargent and Osborne, 1980). Although coleorhiza was initially perceived as a mere protective layer, its diverse morphology, anatomy, functioning, and key role in seed dormancy or germination have recently been elucidated (Barrero et al. 2009). Seed germination is one of the vital phases in plant life cycle; it regulates plant emergence and early adaptation to natural or agricultural ecosystems and as such it is the foundation of crop production (Weitbrecht et al. 2011). The impact of fungal endophytes on seed germination is well-established (Warcup, 1985; Vujanovic et al. 2000). Fungal endophytes help seed break their morphophysiological dormancy and maintain vitality, leading to successful germination; this critical phenomenon is known as 'mycovitality' (Vujanovic and Vujanovic, 2007). The use of hydrothermal modeling demonstrated that rate and energy of germination in wheat can be significantly improved by employing fungal endophytes (Hubbard et al. 2012). Furthermore, mycobionts can enhance heat and drought tolerances of wheat, which in turn aid seeds attain 50% germination in ~2 days (Hubbard et al. 2013). This is particularly important as seeds are the key generative organs in regeneration and dispersal of flowering plants (Baskin and Baskin 2004). The beneficial impact of fungal endophytes on crop seed germination has considerable implications for agronomy and plant biotechnology. Seeds are a fundamental constituent of the world's diet and cereal grains add up to half of the global per capita energy intake (Bewley, 1997). Hence, assessment of conditions that improve symbiotic seed germination is of strategic importance.

Stratification is a long-known process of "activation" that facilitates the release of dormancy and onset of germination (Koller et al. 1962). In wild conditions, seeds endure natural stratification that

Abbreviations: ABA, abscisic acid; GA, gibberellins; PDA, potato dextrose agar; qPCR, quantitative real-time PCR.

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is seed-testa softens up on frozen ground in winter and triggers embryo to rupture the testa. Thus, stratification was traditionally perceived as a cold related signaling mechanism of breaking seed dormancy, without considering biological or fungal symbiotic activators. Cold stratification in which seeds are kept at dark and cold (~4 °C) conditions is a well-acknowledged method of seed germination enhancement. It is often reported as the most effective way of promoting seed germination (Schutz and Rave, 1999). Cold stratification has been studied in many species including weeds (Milberg and Andersson, 1998), grass (Schutz and Rave, 1999), pine (Carpita et al. 1983), tobacco (Wu et al. 2008), and rice (Mukhopadhyay et al. 2004). Recent transcriptomic studies discovered importance of coleorhiza in regulating dormancy in cereals (Barrero et al. 2009). Because of the obvious importance of cold stratification it is imperative to understand the molecular mechanisms and drivers of this process. However, very few studies have examined the effect of cold stratification on seed germination and expression patterns of functional genes, especially in wheat. Since many of the functional genes associated with seed germination in wheat are well-established, wheat can serve as a model plant for disentangling the impact of stratification. For example, the genes GA3-oxidase 2 and *14-3-3* are known as GA biosynthetic gene and negative regulator of GA biosynthesis pathway, respectively (Ji et al. 2011; Zhang et al. 2007). The NCED gene is well known for its role in ABA biosynthesis pathway whereas ABA 8′-hydroxylase gene is involved in ABA catabolic pathway (Ji et al. 2011). These key functional genes can be employed to assess the effect of stratification on seed germination in wheat.

Microorganisms that colonize and cause asymptomatic infections in healthy plant-tissues are called endophytes (Strobel et al. 2004). Considerable number of studies have shown that fungal endophytes are capable of producing volatile compounds that can affect various phenophases in plants (Mitchell et al. 2010; Strobel et al. 2001, 2004). Although early physiological studies confirmed that spore germination and growth of microorganisms have been enhanced by volatile compounds released from germinating seeds (Schenck and Stotzky, 1975), it is still poorly known if volatiles discharged by microbial endophytes or microbe-seed physical contact have the same effect on seed response and germination (Vujanovic et al. 2000). The role of fungal endophytes in enhancing seed germination was recently recognized in *Gramineae* including wheat, as a natural phenomenon with potentially important biotechnological implications (Clay, 1987; Hubbard et al. 2012; Vujanovic, 2007). Although both cold and symbiotic stratification are individually well-acknowledged for last few decades, comparison of these mechanisms with regard to seed germination and functional gene expression patterns remains elusive. This study aims to disentangle the GA-ABA expression patterns in wheat seed during cold and biological stratification to link germination rate and gene expression patterns in this critical phenophase. The overarching goal of this study is to understand the mechanisms by which endophytes affect seed germination and to assess if the volatile compounds produced by endophytes influence plant gene expression that in turn may affect seed dormancy and germination. Specifically, the following research questions were asked: (1) are the rate and efficacy of symbiotic seed germination stimulated by physical contact and/or spatial distance between two symbiotic (photobiont and mycobiont) partners; and (2) is the percentage of seed germination linked to different expression patterns of GA and ABA biosynthesis genes when exposing the seed to biological *vs.* cold stratification?

This study provides evidence for the hypothesis that fungal endophytes improve wheat seed germination by modulating the expression of germination-related genes.

2. Materials and methods

2.1. Wheat seeds and sterilization protocols

Seeds of the durum wheat cultivar AC Avonlea produced by Agriculture and Agri-Food Canada Seed Increase Unit Research Farm (Indian Head, Saskatchewan) were used in this study. The seeds used were produced under greenhouse conditions, and were certified to be free of microbes (Hubbard et al. 2012). To identify the best suitable protocol that efficiently sterilizes seed-surface without affecting seed quality and vitality, we compared four widely acknowledged seed-sterilization methods: sterilization by 95% ethanol (Zhang et al. 2007, 2008), sterilization by 5% sodium hypochlorite (Abdul-Baki, 1974), combined 95% ethanol and 5% sodium hypochlorite (Abdellatif et al. 2009), and sterilization by chlorine gas (Desfeux et al. 2000). Chlorine gas sterilization protocol was the most effective method showing 80% germination and completely free of contaminants and thus, it was selected to sterilize the seeds.

2.2. Cold and biological stratification

For cold stratification, surface sterilized seeds were kept on moist filter paper at 4 °C cold-room for 48 h (Mukhopadhyay et al. 2004; Wu et al. 2008). For biological stratification, sterilized seeds were incubated in presence of SMCD 2206, an endophytic Ascomycota mitosporic fungal isolate deposited in the Saskatchewan Microbial Collection Database (SMCD). SMCD 2206 strain was selected by Dr. Vujanovic from a much larger collection of hundreds of fungi because of it positive impacts on wheat agronomic traits including yield subjected to biotic stress (Hubbard et al. 2013). This fungus differs from other wheat endophytes in terms of macroscopic and microscopic appearance, growth rate as free-living organisms and tolerance for heat or drought stress (Hubbard et al. 2012). Both *in vitro* and *in planta* studies demonstrated the endophytic nature of SMCD 2006 being colonizer of asymptomatic wheat (Hubbard et al. 2012, 2013). Because of its positive impact on seed germination and stress tolerance in wheat, SMCD 2206 was selected in this study. Fungal endophyte was grown on potato dextrose agar (PDA) at room temperature in darkness for at least three days before use. To assess indirect fungal stratification, an agar plug (5 mm^2) of the endophyte dissected from the margins of a parent colony was placed in the center of a 90 cm petri dish with PDA, ensuring an indirect contact *via* exchange of volatiles. Then 10 surface sterilized seeds were place at the periphery of the petri dish encircling the fungal agar plug at approximately 4 cm distance. All petri dishes were sealed with 5 layers of Parafilm® (Pechiny Plastic Packaging, Menasha, WI) to avoid diffusion of volatile/gaseous compounds. The impact of direct-contact of the fungal endophyte was elucidated by placing a 3 mm^2 agar plug between two adjacent surface sterilized wheat seeds and 5 mm^2 plug in the center of the PDA plates (Abdellatif et al. 2009). All treatments were carried out with three replicates of PDA plates with 10 surface sterilized wheat seeds on each plate. Petri dishes were incubated at ~20 °C in darkness.

2.3. Germination percentage

Wheat seed germination was recorded during 3 days after sowing on agar plates. At that time, seeds in direct-contact were colonized by fungus hyphae while seeds in indirect-contact assays were non-colonized meaning that fungus-seed communication was only possible *via* diffusible or volatile chemical signals transferred from the sender to the receiver cells (Bruns and Read, 2000). The germination assessment was applied to distinguish developmental and gene expression changes during the early seed