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Original Research Paper

Transgenerational changes in *Arabidopsis thaliana* in response to UV-C, heat and cold

Seyed Mohammad Reza Rahavi [1], Igor Kovalchuk *

Department of Biological Sciences, University of Lethbridge, Lethbridge, Canada AB T1K 3M4

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ABSTRACT

It was recently shown that exposure of *Arabidopsis thaliana* to heat, cold or UV-C triggers transgenerational changes in the frequency of homologous recombination and stress tolerance in progeny. We hypothesized that the efficiency of transgenerational changes depends on the time of exposure, the duration of stress or/and stress intensity. To test this, we exposed *A. thaliana* to either heat, cold or UV-C of different severities and durations at 7, 14, 21 and 28 days post-germination (dpg). We found that exposure to stress early in development, namely at 7 dpg, results in beneficial effects on seed size and improved responses to stress in the progeny. Our experiments showed that positive transgenerational changes in response to stress occur when plants are exposed to mild stress early during development, whereas negative transgenerational responses in the form of a lower stress tolerance occur when plants are exposed to severe stress late during development.

1. Introduction

Plants as sedentary organisms are constantly exposed to changes in water availability, temperature and ultraviolet (UV) light irradiation. The sun emits ultraviolet radiation in the long (UV-A), medium (UV-B) and short wave (UV-C) bands; however, only the wavelengths of 290 nm (or longer) can reach the surface of the earth (Rosario et al., 1979). For protecting themselves against UV, plants utilize a variety of mechanisms that either prevent damage or repair the imposed damage (Tuteja et al., 2001). Exposure to UV may also alter chromatin structure and gene expression of a plant. It was shown that UV-B exposure resulted in immediate and heritable epigenetic changes in the control of a silent reporter gene in *Arabidopsis*. This stress-related gene silencing was linked to modifications in histone occupancy and acetylation of histone H3, and it did not depend on changes in DNA methylation (Lang-Mladek et al., 2010).

Temperatures higher than an optimum temperature are considered to be heat stress to plants. Heat stress can disturb cellular homeostasis in plants and cause the inhibition of normal growth and development and even death. Therefore, heat stress can adversely impact all aspects of plant growth and development, including yield and reproduction. Hence, a variety of responses have evolved in plants to deal with heat stress for maintaining cellular homeostasis and minimizing damage. It is assumed that heat shock proteins (HSPs) which are under the control of heat stress transcription factors play a central role in the heat stress response and in acquired thermotolerance (Baniwal et al., 2004). Upon heat exposure, plants invest valuable resources to modify their metabolism and prevent or minimize damage. Disturbances of the steady state of these metabolic pathways may lead to the accumulation of toxic products such as reactive oxygen species (ROS). In fact, there is a relationship between oxidative stress and heat shock response in plants (Zhang et al., 2009; Suzuki et al., 2012). Exposure to heat caused extensive decondensation of 45S rDNA chromatin in rice (Santos et al., 2011). Heat exposure was shown to trigger a release of gene silencing correlated with pronounced alterations in histone occupancy and in histone H3 acetylation with changes in DNA methylation (Lang-Mladek et al., 2010). In Arabidopsis, prolonged heat exposure activated several transcriptionally silent repetitive elements due to loss of nucleosomes and heterochromatin decondensation; the activation of repetitive elements occurred without changes in DNA methylation and with only minor changes in histone modifications (Pecinka et al., 2010).

Low temperature is one of the most important abiotic environmental factors that affects growth, yield and the geographical distribution of plants. In *Arabidopsis thaliana*, initially, cold stress

Abbreviations: DCL, dicer-like; HDD, heat different duration; HDT, heat different temperature; HR, homologous recombination; HRF, homologous recombination frequency; HSPs, heat shock proteins; UV, ultraviolet radiation

* Corresponding author. Tel.: +1 403 329 2579; fax: +1 403 329 2242.
E-mail addresses: seyed.rahavi@gmail.com (S.M. Reza Rahavi), igor.kovalchuk@uleth.ca (I. Kovalchuk).
[1] Tel.: +1 403 329 2628; fax: +1 403 329 2242.

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strongly inhibits photosynthesis but growth recovery occurs after long-term exposure to cold temperatures (Savitch et al., 2001). Physiological responses of plants to cold stress include changes in lipid membrane composition, the accumulation of cryoprotectants and the detoxification of reactive oxygen species (Thomashow, 1999). At the level of chromatin, exposure of maize plants to cold causes an increase in H3K9ac and a reduction in DNA methylation and H3K9me2 at the tandem repetitive sequences, 180-bp and TR-1, thus leading to their transcriptional activation (Hu et al., 2012).

Besides influencing the growth and development of plants, exposure to stresses may also lead to damage to genetic material and DNA, thus causing DNA strand breaks which are in part repaired via homologous recombination (HR). The frequency of somatic HR (HRF) is known to increase upon exposure to a variety of biotic and abiotic stresses, although many of these stresses do not damage DNA directly (Molinier et al., 2005; Boyko et al., 2010; Kathiria et al., 2010; Ries et al., 2000; Boyko et al., 2005, 2010; Kovalchuk et al., 2000; Pecinka et al., 2009; Lucht et al., 2002; Yao and Kovalchuk, 2011). Moreover, it has been shown that a stress-induced increase in somatic HRF can be inherited, leading to the so-called transgenerational changes in HRF (Molinier et al., 2005; Boyko et al., 2010; Kathiria et al., 2010; Boyko et al., 2007).

To date, a variety of stresses have been reported to trigger transgenerational responses, including temperature and water availability, exposure to UV-B, UV-C and salt (Ries et al., 2000; Boyko et al., 2010; Molinier et al., 2006) as well as infection with various pathogens, including viruses (Kathiria et al., 2010) or bacteria (Luna et al., 2012) and even caterpillar herbivory (Rasmann et al., 2012). The documented components of transgenerational response include changes in genome stability, namely an increase in HRF, changes in DNA methylation, histone modifications, gene expression, the metabolites content, a release of gene silencing, and differential stress tolerance (Lang-Mladek et al., 2010; Kathiria et al., 2010; Luna et al., 2012; Rasmann et al., 2012; Bilichak et al., 2012; Slaughter et al., 2012; Mandal et al., 2012). In this respect, transgenerational changes can be positive and negative for plant ability to tolerate stress, although it is often not clear whether changes in HRF, DNA methylation, histone modifications and gene expression are positive or negative in nature, as far as stress tolerance is concerned.

The establishment and strength of transgenerational stress response may be conditioned by the amount of stress applied and possibly by the timing of stress application during plant development. For instance, it has been reported that the most prominent transgenerational increase in genome rearrangements was observed upon exposure to 25–75 mM NaCl, while exposure to 100 mM NaCl showed no effect (Boyko et al., 2010). The progeny of plants exposed to 25 mM NaCl had more pronounced changes in DNA methylation and stress tolerance as compared to those exposed to 100 mM.

In the present work, we assessed whether exposure of *A. thaliana* to different levels of UV-C, heat and cold during different times of development (at 7, 14, 21, or 28 days post germination) would result in different degrees of transgenerational response. We found that, in general, exposure to mild stresses early during development results in the most pronounced positive transgenerational changes in stress tolerance. We suggest that stress applications targeted to agronomically important plants early during development could lead to higher tolerance to stress in the progeny.

2. Results

2.1. The analysis of seed size and yield in the stressed and control plants

Seed yield was significantly higher in 14-day-old plants exposed to UV-C for more than 15 s (Figs. 1A and S1). The treatment of plants at other times during development (except 15 s at 28 dpg) did not result in changes in total seed weight. Seed size in UV-C exposed plants increased if plants were exposed at 7 or 14 dpg and decreased or did not change if plants were exposed at later developmental stages (Figs. 1B and S1). The largest seeds were produced by plants exposed to UV-C at the age of 7 days for duration of 30 s, whereas the smallest—in the group of plants exposed at 21 dpg for 2 min.

In most of the cases, plants exposed to cold had similar seed yields as compared to control plants (Figs. 1C and S1). However, a higher seed yield was obtained from plants exposed to cold for 3 h at 7 or 21 dpg. A lower seed yield was observed in plants exposed to cold at 21 dpg for 24 h (Figs. 1C and S1). Plants exposed to cold for 12 or 24 h at 7 dpg produced larger seeds than control plants (Figs. 1D and S1). Plants exposed to cold at 14 dpg had smaller seeds, whereas plants exposed at 21 and 28 dpg had seeds of a similar size (except those plants exposed to cold for 48 h at 21 dpg) as compared to control plants.

Exposure to different heat treatments for 2 h (heat different temperatures or the HDT group) resulted in a decrease in seed weight if plants were exposed to 37 °C or 50 °C at 7 and 14 dpg (Figs. 1E and S1). Curiously, exposure to 28 °C or 50 °C at 21 and 28 dpg resulted in an increase in seed yield. An increase in seed size upon exposure of 7-day-old plants to 37 °C or 50 °C and a decrease upon exposure to both temperatures at 14, 21 and 28 dpg (Figs. 1F and S1) were observed.

Exposure to 50 °C (heat different durations or the HDD group) resulted in an increase in seed yield if plants were treated at 21 or 28 dpg (Fig. S1). Exposure to heat at 7 dpg did not cause changes in seed yield, whereas exposure at 14 dpg caused a decrease in it. Plants exposed to 50 °C at 7 dpg produced larger seeds, whereas plants exposed at 14 or 21 dpg produced smaller seeds. Seeds of plants exposed at 28 dpg were mostly similar to those of plants from the control group (Fig. S1).

2.2. Changes in stress tolerance in the progeny of exposed plants

Previous research showed that the progeny of stressed plants was altered in response to stress. To analyze progeny performance, plants were exposed either to mild stress at early stages of plant growth or to harsh stress, during rosette formation. Mild stress was defined as a stress that did not cause any visible physiological damage to exposed plants. In contrast, harsh stress caused immediate (visible already in 12 h after exposure) damage to plants, noticeable as extensive leaf bleaching and curling.

2.2.1. Exposure to mild stress

The analysis showed that exposure to mild stress resulted in a larger leaf size in the progeny of plants stressed with UV-C at 21 and 28 dpg as compared to the progeny of control plants (Figs. 2A and S2). In contrast, the progeny of plants exposed to UV-C at 14 dpg had smaller leaves if exposed to mild stress, whereas plants exposed to UV-C at 7 dpg had leaves similar in size to that in control plants (Figs. 2A and S2). The leaves of the progeny of cold-stressed plants exposed to mild stress were larger if the progeny was exposed at 7 and 14 dpg as compared to the progeny of control plants (Figs. 2B and S2). In contrast, the progeny of plants exposed at 21 dpg developed smaller leaves if exposed to mild stress (Fig. 2B). In HDT group, exposure to mild stress of the progeny of plants exposed to 37 °C and 50 °C at 7 dpg resulted in larger leaves than the progeny of control plants (Figs. 2C and S2). Other experimental groups were similar to the control one. As to the HDD group, if plants were exposed to 50 °C for 30 min or 2 h at 7 or 14 dpg, their progeny if exposed to mild stress developed larger leaves (Fig. S2). Other HDD groups were largely similar to the control group.