Expression of flowering genes in *Arabidopsis thaliana* under acute and chronic irradiation

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ABSTRACT

Purpose: The chronic and acute irradiations have different effects on plant ontogenesis, especially during the sensitive flowering phase, which plays an important role in the further seed development and could determine seed yield. The expression of key flowering genes, *Ap1, Co, Gi, FT, FLC,* and *Leafy*, sensitive to irradiation repair gene *RAD51* and proliferation gene *PCNA2* were studied in the wild type *Arabidopsis thaliana* (Columbia ecotype) under the chronic and acute irradiations.

Materials and methods: The chronic irradiation was performed using the radioactive isotope ^{137}CsCl in two total doses of 3 cGy and 17 cGy, with dose-specific rate of 10^{-7} cGy/sec and 6.8×10^{-6} cGy/sec, respectively. The plants were grown under the chronic irradiation during 6 weeks from seeds until the 6.3 stage of flowering. For the acute exposure, the plants were X-ray irradiated one time at the 5.0 development stage (28 day old) by a total dose of 15 Gy with a dose rate of 89 cGy/sec.

Results: After the chronic irradiation with the 3 cGy dose the irradiated plants demonstrated 8 ± 2,8 days earlier flowering than in the control group. However, at the 17 cGy chronic and at the 15 Gy acute doses plants showed 14 ± 3.7 and 2 ± 1.4 day later flowering, respectively. The 3 cGy chronic exposure significantly increased expression of the *Co* gene by a factor of 1.152 (1.087-1.217 95% C.I.) and decreased expression of the *FT* gene by a factor of 0.128 (0.021-0.396 95% C.I.). The 17 cGy chronic exposure decreased expression of the *Ap1* gene by a factor of 0.872 (0.803-0.940 95% C.I.) and the *Lfy* gene by a factor of 0.471 (0.306-0.687 95% C.I.). The 15 Gy acute exposure decreased expression of the *Ap1* gene by a factor of 0.104 (0.074-0.144 95% C.I.) and the *PCNA2* gene by a factor of 0.346 (0.238-0.488 95% C.I.).

Conclusions: Increased expression of the *Co* gene seems stimulated earlier flowering, and decreased expression of the *Ap1* and *Co* genes delayed blooming. The acute exposure increased expression of the *PCNA2* gene and decreased expression of the flowering genes except *Ap1*. In this case, the flowering was less delayed than under chronic dose of 17 cGy. Presumably, it was related to activation of DNA reparation processes under the 3 cGy chronic exposure and the 15 Gy acute irradiation. The *Ap1*, *Co* and *FT* genes play an important role in flowering process under irradiation treatment.

KEYWORDS

ionizing irradiation; flowering genes; gene expression; *Arabidopsis*

Introduction

Ionizing irradiation is a strong environmental stress factor, and biological effects of chronic and acute irradiation on living organisms still need to be more studied. The effects of irradiation exposure on a cell depend on the total dose and dose rate (Yamaguchi et al. 2008). Acute and chronic irradiations can differently affect plant ontogenesis (Kovalchuk et al. 2007). A short exposure of an acute dose can cause less damage in cells, than the same chronic dose (Kellie and Rzucidlo 2011). The acute exposure usually has a targeted effect and directly causes damages (mostly breaks) in DNA molecules by both transferring energy and generating free radicals (Kovacs and Keresztes 2002). The chronic ionizing irradiation has rather stochastic and non-targeted effects (Kovalchuk et al. 2007). It destabilizes genome, activates mobile genetic elements and promotes epigenetic changes in some key genes (Ilnytskyy and Kovalchuk 2011). The impact of high doses of irradiation on the genes activity has been widely studied in plants (Hwang et al. 2016). The effects of acute exposure are more understandable than the effects of chronic irradiation. However, the chronic radiation is very important factor in some environments, such as those contaminated after the nuclear power station catastrophes in Chernobyl and Fukushima (Grodzinsky 1999, Rashydov et al. 2012), where it can remain for a long time aftermath. It is very important to study chronic irradiation effects on sensitive phases of the plant ontogenesis.

Flowering is one of the most important and complex processes in plant development. It is highly sensitive to stress factors (Georges and Périlleux 2005) and strongly depends on several stimuli environmental signals (Castillejo and Pelaz 2008). The yield of many agricultural species depends on optimum time of flowering, flower development and seed fullness, which are under control of complex gene networks. There is a balance between flowering transmissible promoters and transmissible and non-transmissible inhibitors in the vegetative phase in plants. Excess of flowering transmissible promoters induces the generative phase of the ontogenesis in plants (Parenicova 2003).

Some environmental cues such as a light (illumination) day length, temperature, amount of nutrients and endogenous developmental signals are key factors in floral initiation (Fernando and Coupland 2012). Some metabolic pathways are activated by endogenous signals such as hormones and an abundance of nutrients and minerals (Castillejo and Pelaz 2008).

The correct optimum time of flowering is very important in the plant's lifespan. Plants need to accumulate nutritious for an effective reproduction during the vegetative phase (Parenicova 2003). If flowering begins too early, plants might not have enough time to accumulate a necessary amount of nutrition needed for flower development and seed maturation. Plant's production strongly depends on optimal flowering time that can be delayed under stress conditions affecting pollination and seed development. Seeds that are developing under abnormal and uncomfortable environmental conditions often have insufficient time for seed maturation before cold weather or dry periods would begin.

Arabidopsis thaliana is the most widely used plant-model species to study flowering affected by irradiation in detail (The Arabidopsis Genome Initiative 2000). In general, *Arabidopsis* is a very popular plant-model species in molecular plant biology and genetics studies due to its small genome size (1n = 5; 135 Mbp), a short generation cycle and convenient cultivating in the laboratory conditions. The entire *Arabidopsis* genome has been sequenced and is well studied. About 26,000 genes and many molecular pathways were identified in *Arabidopsis* (Pastore et al. 2011) including ~80 genes involved in flowering regulation.

To study effects of irradiation on flowering, we measured expression of the six key flowering related genes, such as *Apetala 1* (*Ap1*), *Constants (Co), Flowering locus C (FLC), Flowering locus T (FT), Gigantia (Gi)* and *Leafy (LFY)* under irradiation and control condition. The genes *Co* and *Gi* are regulated by circadian clock and are key genes in the photoperiod flowering time pathway. The *FT* gene encodes the florigen, a "flowering hormone" or hormone-like protein responsible for controlling and/or triggering flowering in plants (Smaczniak et al. 2012). The *FT* gene is activated in the vascular tissue of leaves too.

The *FT* protein activates the *MADS-box* genes, important regulators of flower development (Jeong and Clark 2005; Kaufmann et al. 2010).

The *FLC* is an important age-sensitive gene that suppresses flowering expression in the vegetative phase. The *Ap1* and *LFY* genes promote floral meristem identity (Siriwardana and Lamb 2012).

The main objective of this study was to find out how chronic irradiation affects expression of the plant flowering related genes. We believe that data obtained in this study will help us better understand mechanisms of stress effect on flowering time. This study may have not only fundamental biological importance, but also important practical applications, e.g. for radionuclide contaminated sites of Chernobyl and Fukushima areas.

We hypothesized that the chronic ionizing irradiation affects the plants reproduction many times more strongly than the X-rays acute irradiation by similar doses. In addition, the factors controlling flowering genes may affect reparation and proliferation under ionizing irradiation. Therefore, we studied also how ionizing irradiation affected expression of reparation and proliferation genes *RAD51* and *PCNA2*, respectively (Corinne et al. 2015).

Materials and methods

Plant cultivation

We used *Arabidopsis thaliana* (Brassicaceae) Columbia ecotype (wild-type) in this study. The seedlings were cultivated in soil under long day illumination conditions (18 hours light and 6 hours dark) (Czechowski et al. 2004) at room temperature. The soil was disinfected by 3% Sodium permanganate solution during 24 hours. The same number of 25 plants was used in the experimental and the control groups. Seeds were disinfected by 12.5 % sodium hypochlorite solution and with 70% ethanol.

Radiation exposure mode

As a stress factor we used acute and chronic irradiation exposures. The chronic irradiation was performed using the ^{137}CsCl irradiation with two total doses of 3 cGy and 17 cGy, and dose rates 10^{-7} cGy/sec and 6.8×10^{-6} cGy/sec, respectively. The plants in the experimental group were grown under chronic irradiation during 6 weeks from seeds until the 6.3 stage of flowering (Boyes et al. 2001). The plants in the control group were grown in the same conditions, but without irradiation. For the acute exposure, the seedlings in 5.0 development stage (28 days old) (Boyes et al. 2001) were irradiated by X-rays one time with an acute dose rate of 89 cGy/sec and a total dose of 15 Gy.

RNA isolation, cDNA synthesis, and real time quantitative PCR

RNA was extracted from the leaves of the 6 week-old plants. The same number of leaves and amount of tissue (~300 mg) were collected per each plant in both experimental and control groups. The RNA extraction was performed using the GeneJET Plant RNA Purification Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). Extracted RNA was used for the *in vitro* reverse transcription with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). To determine the expression activity of the selected genes we used real-time quantitative PCR (RT-qPCR) method (Heid et al. 1996). To perform the RT-qPCR, we either used published or designed specific PCR primers for the six flowering related genes *Ap1, Co, FLC, FT, Gi* and *LFY*, and two sensitive to irradiation genes – the proliferation gene *PCNA2* and the reparation gene *RAD51* using the NCBI BLAST and primer design tools (Table 1). The SYBR Green master mix was used for the RT-qPCR. The *UBQ10* gene was used as an internal reference standard (Nicot et al. 2005). The obtained RT-qPCR data were analysed with the REST 2009 software (Pfaffl et al. 2002).

[Table 1]

Results

The 6.3 phase of flowering (Boyes et al. 2001) appear 8±2.8 days earlier of the *A. thaliana* plants which irradiated at the 3 cGy chronic dose in compare of the control group. However, at the 17 cGy

chronic and at the 15 Gy acute doses the irradiated plants showed flowering 14±3,7 or 2±1,4 days later, respectively, than in the control group (Table 2).

[Table 2]

At the low chronic dose level of 3 cGy expression of the *Co* gene was increased by a factor of 1.152 (1.087-1.217 95% C.I.) in comparison to the non-irradiated control group plants (Table 3). However, expression of the gene *FT* was significantly lower by a factor of 0.128 (0.021-0.396 95% C.I.). The expression of the other genes did not change significantly compared to the control group.

At the high chronic level dose of 17cGy expression was lower for all genes compared to the control group, but statistically significantly only for the *Ap1* and *Lfy* genes, by a factor of 0.471 (0.941-1.342 95% C.I.) and 0.872 (0.803-0.940 95% C.I.), respectively. Expression of the other genes were not significantly different from the control group plants.

[Table 3]

At the acute dose of 15 Gy the *Ap1* and *PCNA2* genes were down-regulated by a factor of 0.104 (0.074-0.144 95% C.I.) and 0.346 (0.238-0.488 95% C.I.), respectively, compared to the control group. The expression of the *Co*, *Gi*, *FLC*, *FT*, *Lfy* and *Rad51* genes was not differed from the control group (Figure 1).

[Figure 1]

Figure 2 summarized expression for all eight genes under all three different ionizing radiation exposure modes.

[Figure 2]

Discussion

Our results revealed that the chronic, as well as acute irradiations affected activity of the flowering, reparation and proliferation genes. The literature data demonstrated that high doses of acute irradiation (100 and 200 Gy) delayed flowering in plants and changed the expression of some genes (Hwang et al. 2016). The recent studies showed that the irradiation sensitive genes were associated with photosystem, phenols, ribo-nucleoside-diphosphate reductase, and with the C2H2 zinc finger family functions in plants treated by 100 Gy at the reproductive stage. The flowering genes were down-regulated under the high acute irradiation doses of 800 Gy (Hwang et al. 2016). The expression levels of the *Co*, *Ap1* and *LFY* transcription factors also responded to the low doses of the chronic and acute irradiation in our research.

The chronic doses affected flowering genes expression more than the acute doses in our study. Previous studies also showed that chronic irradiation affected genes expression and had the different effect on flowering time. Flowering was observed earlier under chronic irradiation than under acute treatment in Kovalchuk et al. (2007). However, we showed that chronic irradiation with 5 times higher dose (3 vs. 17 cGy) affected differently the flowering time (Table 2) and expression of the flowering genes (Figure 1). The chronic irradiation with the 17 cGy total dose had more pronounced effect than with the 15 Gy acute irradiation dose (Figure 1).

Our data showed that different doses of irradiation caused different effects on expression of several flowering genes. Some genes may be more sensitive to environmental factors than others. Our results showed that the *Co*, *Ap1* and *PCNA2* genes were the most sensitive to ionizing irradiation. The *Co* and *FT* genes are involved in the photoperiodic pathway, and they function as circadian signals (Figure 2). The sensitivity of the *Co* and *Ap1* genes to the ionizing irradiation probably provided evidence for their participation in stress-regulation of flowering time.

We demonstrated in our study that radiation exposure is a strong stress factor that affects both flowering time and expression of some important flowering, repair and proliferative genes. The recent

188 studies had also shown that stress factors could cause early or late flowering (Takeno 2012). Stress
189 induced earlier flowering was caused also by low temperature, nitrate stress and low nutrients (Marín et
190 al. 2011).

191 We found that different doses of exposure caused different effect on flowering term. The low chronic
192 dose of 3 cGy stimulated 8 days earlier flowering, but the high chronic irradiation at 17 cGy and the acute
193 irradiation at 15 Gy doses delayed flowering for 14 and 2 days, respectively (Figure 1).

194 Under the low chronic irradiation (3 cGy) an increase of the reparation process leads to the cell fission
195 being not activated, and with an increase of the dose up to 17 cGy the reparation is reduced. Therefore,
196 the dwarfs, plants with shorter heights, increased second peak of flowering and underdeveloped habitus
197 could be observed in an environment contaminated with radionuclides, such as plants growing in the
198 Chernobyl zone (Rashydov et al. 2012). At the acute irradiation of 15 Gy, even with the unchanged level
199 of the reparation process, the activation of the *PCNA2* gene was observed, which indicates restoration of
200 damaged plant via repopulation recovery from unaffected intact cells. The obtained data revealed that the
201 chronic irradiation significantly differed from the acute irradiation by affecting both flowering genes as
202 well as cell proliferation genes.

203 We observed that under the low chronic dose of 3 cGy the up-regulated expression of the flowering
204 gene *Co* accompanied by the 8 days earlier flowering. Opposite, under the high chronic dose of 17 cGy
205 several genes were down-regulated and accompanied by significantly later flowering. The 15 Gy acute
206 exposure dose decreased expression of the *PCNA2*. In this case, the flowering delay was less than at the
207 17 cGy chronic irradiation dose. Presumably, it can be explained by activation of the DNA reparation
208 processes under both the 3 cGy chronic and the 15 Gy acute irradiation exposures. We guess that the *Ap1*
209 and *Co* genes play even more important role in flowering under stress conditions.

210 The effects of irradiation exposure are similar to other abiotic stress factors, such as UV-B/C
211 irradiation, drought, and heat (Llorens et al. 2015). The stress-induced flowering pathway is as important
212 for plant adaptation as photoperiodic and vernalization pathways (Takeno 2012).

The early flowering of *A. thaliana* in response to drought stress, UV-C and pathogens (for example, *Fusarium oxysporum* infection) was demonstrated recently (Takeno 2016). We observed that the low dose of 3 cGy irradiation exposure also promotes early flowering in the *A. thaliana* plants. Published studies have shown also that the gene *FT* could be involved in stress-induced flowering (Takeno 2012). However, we did not observe the increasing expression of the *FT* gene in earlier blooming (King at al. 2008). The photoperiodic *Co* gene was up-regulated and followed by flowering acceleration. Our study showed that effects of the 17 cGy chronic and 15 Gy acute irradiations were similar to the UV-B effect.

The 17 cGy chronic and 15 Gy acute exposure doses delayed flowering. The flowering genes except the *Ap1* gene were down-regulated in chronic irradiation and the 15 Gy acute dose exposure. However, flowering was delayed only for 2 days after the 15 Gy acute irradiation dose.

Some abiotic stress factors, such as cold, osmotic stress and salinity can also delay flowering (Srinivasan et al. 1999; Kotula et al. 2015). Cold temperatures induced degradation of the *CO* protein *via* an ubiquitin/proteasome pathway (Jung et al. 2012). The pathogen infection together with heat, drought and salinity stresses can change normal flowering time in *Arabidopsis* plant by interfering with the photoperiodic pathway (Kazan and Lyons 2016). Salt affected expression of the *FT* gene at the transcriptional and post-transcriptional levels (Li et al. 2007). Salt stress also promoted degradation of the *GI* protein through an unknown ubiquitin/proteasome pathway and negatively affected the *Co* gene expression (Melgar et al. 2012). Drought stress also effectively delayed flowering up to four weeks in some plant species, such as orange (Riboni et al. 2014). This phenomenon negatively affects harvesting and causes harvest productivity losses.

Our study showed that photoperiodic pathway was affected by the ionizing irradiation. Increased expression of the *Co* and *Gi* genes stimulated earlier flowering, and decreased expression of the *Ap1* gene caused delay of blooming at the 17 cGy chronic and 15 Gy acute doses.

However, mechanism of irradiation effects on blooming is still not clearly understood. High level doses more than 1 kGy caused destructive processes in plant cells (Kovacs and Keresztes 2002).

However, medium intensity 0.1-0.4 kGy gamma exposure can also delay germination process and decrease lifespan of plants (Marcu et al. 2013). It is necessary to mention that based on our data the low chronic and acute doses affected also signal transduction genes.

Blooming is sensitive to elevated UV-B, which affects both flowering phenology and flower production. Increasing of UV-B exposure delayed blooming and reduced flower production (Sampson and Cane 1999).

It is known that the ionizing irradiation leads to producing free radicals and ions in biological tissues (Riley 1994). Free radicals can damage or modify key cell components, proteins or ferments and hormones, which are included in important physiological and biochemical processes in a plant ontogenesis. However, morphological, structural, and functional changes depend on the strength and duration of gamma exposure doses (Marcu et al. 2013). Our data showed that the chronic exposure with a high dose of 17 cGy and the acute irradiation dose of 15 Gy had similar effects (Figure 3). The low chronic dose of 3 cCy had opposite effect than the high-level chronic irradiation (17cGy) one. After analysis of low and high chronic doses and acute irradiation effects on flowering and reparations genes we have to notice that there was no correlation between expression levels of flowering genes and the repair gene *RAD51*.

[Figure 3]

Conclusions

We studied effects of chronic and acute irradiation doses on expression of six key flowering genes in *A. thaliana* and revealed that trends in the changes of the flowering genes expression under the stress were closely associated with the transduction signal system through the blooming metabolic pathways.

Our results showed that the chronic irradiation at the high level of dose (17 cGy) delayed blooming by an order of magnitude less than the acute irradiation dose at 15 Gy. Meanwhile the low chronic dose of 3

cCy had the opposite effect, and acceleration of flowering was observed in this case. The low and high chronic irradiation doses significantly affected the flowering genes *Ap1*, *Co* and *LFY*, but changes in their expression level did not correlate with expression of the reparation gene *RAD51*. The data revealed that effectiveness of a chronic irradiation severe differed from acute irradiation by affecting flowering genes activity as well as expression of the cell proliferation gene *PCNA2* in the *A. thaliana* plants.

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Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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Figure legend

Figure 1. Gene expression of the six flowering genes and the *RAD51*, *PCNA2* genes in *A. thaliana* under all three different ionizing irradiation exposure modes.

Figure 2. The scheme of the relations between studied genes and their role in determining of generative phase.

Figure 3. Relationships between the flowering time and the flowering gene expression activity in *A. thaliana* under three different ionizing irradiation exposure modes.