ВІСНИК
УКРАЇНСЬКОГО ТОВАРИСТВА ГЕНЕТИКІВ І СЕЛЕКЦІОНЕРІВ

UDC 577.39 [58.084.1]: 575.224.22

THE ROLE OF JASMONATE SIGNALING PATHWAY IN PLANT'S FLOWERING GENES RESPONSE TO IONIZING RADIATION

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***Aim**. This study aimed to characterize the role of the jasmonate signaling pathway in flowering genes response to acute and chronic ionizing irradiation in plants. **Methods**. We used the wild-type Arabidopsis thaliana and jasmonic pathway defective jin mutant of Col 0 ecotype in our experiments. The chronic irradiation was provided by ^{137}CsCl with a total dose of 17 cGy and a dose rate of 6.8×10^{-6} cGy/s. The acute irradiation experiment was performed on 21 days old plants at the 5.0 stage (Boyes et al., 2001) by X-rays in a total dose of 5 Gy with a dose rate of 89 cGy/s. The length of stems and leaves was measured in post-irradiation period. The molecular genetic analysis was done using real-time PCR. We determined the relative expression of key flowering genes AP1, GI, FT, CO, ACT2 with UBQ10 used as reference genes. Statistical analysis of phenotypic parameters was done using Student's t-test in GraphPad Prism 8 software. The quantitative PCR data were analyzed in the REST 2009 software, QIAGEN. **Results.** The plant groups differed significantly by the stem length (p>0,05). The study revealed decreased expression of CO, GI and FT genes in jin mutants. The overexpression of AP1 in jin mutants under chronic irradiation may cause cell division errors and impact flower development. In contrast, AP1 expression in WT plants was near to normal = 1 under chronic irradiation. These results suggest the involvement of the jasmonate pathway in the regulation of plants flowering during the irradiation. **Conclusion**. Based on the results of our study, we hypothesize that jasmonic acid has a stabilizing effect on the rate of cell differentiation in plants under chronic irradiation. Despite the uncovered role of jasmonic acid in A. thaliana flowering the exact mechanism of its action remains unclear and requires further investigation.*

***Keywords:** jasmonate signaling, jasmonic acid, JA, flowering, ionizing radiation, real-time PCR, relative expression.*

Introduction. Ionizing radiation (IR) plays an important role in plants evolution as a primordial stressor (Caplin, Willey, 2018). IR is a strong stress genotoxic factor for living plant cells and known to cause genetic alterations in plants. The effects are dose and intensity dependent. Acute and chronic irradiations have different mechanisms of action and cause different effects in the plant organism (Hong et al., 2018).

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Various exposure and intensity combinations were studied on the Arabidopsis model (Boyes et al., 2001). Acute exposure to radiation leads plants to a damage of macromolecules in plant cells. It causes the occurrence of free radicals and DNA molecule breakage. It was established that the median lethal acute radiation dose (LD50) for *A. thaliana* is of 300 Gy. Chronic irradiation has a stochastic and non-targeted effect. Preferably, it leads to the activation of mobile genetic elements and epigenetic changes in the genome (Kovalchuk et al., 2007). Especially, chronic irradiation has the greatest impact on plant ontogenesis (Nesterenko, Rashydov, 2018; Dmitriev at al., 2018). Chronic radiation is predominant in radiation polluted areas after man-made disasters, such as the accidents at the Chernobyl, Ukraine, and Fukushima, Japan nuclear power plants (Rashydov et al., 2012).

Flowering is one of the most stress-sensitive stages of plant growth and development. The correct development of seeds depends on accurate flowering and its delay may lead to a mismatch with appropriate temperature regime and the presence of insect pollinators. The time of seed maturation closely depends on signal system transduction, especially the influence of the jasmonate signal pathway.

The jasmonic acid (JA) is seen as structural and, in some cases, functional, an analog of prostaglandins the animals. A jasmonate signaling pathway is getting activated by cell surface receptor SR160 followed by mechanical damage of the plant (Ruan at al., 2019). The JA dependent physiological processes are seed maturation, the formation of viable pollen, root growth, the launch of an aging program, protective responses to biotic and abiotic stressors (Kolupaev, Karpets, 2010).

Materials and methods

For our study, we used the wild-type (WT) *Arabidopsis thaliana* plants of the ecotype Columbia 0 and *jin* mutant line *(jasmonate insensitive).* Plants were cultivated in sterile soil, long day (18/6) conditions at room temperature. The sterilization was done soil was sterilized by a 3 % solution of sodium permanganate for 24 hours. The seeds were sterilized with 12.5 % sodium hypochlorite solution and then washed with 70 % ethanol. Experimental groups include 20 plants each. The experimental data acquired from three technical and three biological repeats. Plants were exposed to chronic radiation ^{137}CsCl with a total dose of 17 cGy with an irradiation rate of 6.8×10^{-6} cGy/s. The experimental plants were placed into the radiation with a constant rate of radiation for 6 weeks starting from the seeds germination phase to the flowering phase. The control group of plants was grown under identical conditions except for the radiation influence. In the case of the acute mode of irradiation, 21 days old plants at the 5.0 stage (Boyes et al., 2001) were irradiated by X-ray with an 89 cGy/s radiation rate. The total acute dose was 5 Gy.

The measurement of the plant's growth indicators such as the length of the stem and the size of the leaf were taken every 3 days, of the post-irradiation period. The statistical analysis was done by Student t-test using GraphPad Prism 8.

To analyze genes of interest expression real-time PCR was utilized. To do so total RNA was extracted from the leaves of the plants using the set of QIAGEN Rneasy Plant mini kit (Germany). The cDNA was synthesized using ThermoFisher Scientific Maxima First Strand cDNA Synthesis Kit (USA). The reverse transcription reaction was performed according to the kit manufacturer instruction. For each reaction 1 µg of RNA was used.

The expressions of key flowering genes *AP1, GI, FT, CO* were calculated relative to reference genes *Actin (ACT2)* and *UBQ10*. For qPCR analysis cDNA was diluted 1:10, and primers 1: 100. SYBR Green fluorophores (Thermo Scientific Maxima SYBR Green qPCR Master Mix) were used for signal detection. A three-stage amplification program with an annealing temperature of 55°C was used, the detection was carried out via the green fluorescent signal detection channel.

Statistical analysis of the quantitative PCR data was done by a standard curve constructing. The data were normalized to the expression value of the *ACT2* or *UBQ10* reference genes and control samples. The calculations were performed using the REST 2009 QIAGEN software (Pfaffl at al., 2002; REST 2009 Software User Guide, 2009). Relative expression value was calculated for each of the genes. The expression level values were compared with reference gene expression = 1. The confidence intervals and p values were calculated by randomization algorithms of REST. The hypothesis test P(H1) represents the probability of the difference between the sample and control groups (REST 2009 Software User Guide, 2009).

Results and discussion

The phenotypic differences among the studied groups were revealed. The plant groups did not differ significantly in the stem length (fig. 1).

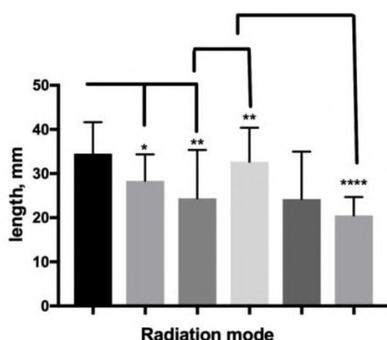

Fig. 1. The stem length in the different experimental groups. The legend is:

Jin 5 Gy; Jin control acute; WT_5 Gy; WT control; Jin 17cGy; Jin control chronic

* p < 0,05; ** p < 0,001; **** p < 0,0001

The stem length of *jin* mutants exposed to radiation (5 Gy) was significantly higher than that of untreated *jin* mutants (two-tailed Student t-test, p-value = 0.0150); the stems of irradiated with 5 Gy WT plants were significantly shorter in comparison to *WT* non-irradiated plants (two-tailed, p-value = 0.0021). Moreover, *jin* mutants had significantly higher stem length than WT plants after the acute irradiation (5 Gy) (two-tailed, p-value = 0.0022).

We did not found any significant impact of chronic irradiation on *jin* mutants morphological parameters. However, non-irradiated mutants had a smaller stem length compared to non-irradiated WT plants. There was no significant change in leaf length in different experimental groups.

The analysis showed the differences in gene expression profiles among different plant groups and differences between the control and experimental groups (table).

Table. The relative gene expression of key flowering genes under ionizing radiation

jin (non-irradiated)					
Gene	Expression level (*P<0,05)	Std. error	95 % C. I.	P(H1)	Trend
CO	0.333*	0.311 - 0.369	0.295 - 0.380	0	↓
GI	0.42*	0.393 - 0.441	0.386 - 0.453	0.021	↓
AP1	0.235*	0.212 - 0.263	0.198 - 0.276	0.021	↓
FT	0.802	0.734 - 0.857	0.719 - 0.920	0.061	↓
ACT2	1				
WT *Col 0* (17 cGy chronic irradiation)					
CO	0.906	0.576 - 1.321	0.531 - 1.462	0.582	↓
GI	0.969	0.854 - 1.100	0.772 - 1.190	0.718	↓
AP1	0.872*	0.830 - 0.929	0.803 - 0.940	0	↓
FT	0.647	0.352 - 1.010	0.273 - 1.541	0.404	↓
UBQ10	1				
*jin (*17 cGy chronic irradiation)					
CO	0.712*	0.620 - 0.855	0.550 - 0.905	0.012	↓
GI	0.629*	0.565 - 0.697	0.537 - 0.712	0.028	↓
AP1	1.768*	1.301 - 2.144	1.231 - 2.246	0.074	↑
FT	1.168	0.971 - 1.413	0.908 - 1.486	0.35	↑
ACT2	1				
jin (5 Gy acute irradiation)					
CO	1.225	1.167 - 1.272	1.143 - 1.294	0.092	↑

GI	1.448*	1.202 - 1.652	1.130 - 1.804	0.041	↑
AP1	0.397*	0.349 - 0.461	0.322 - 0.485	0	↓
FT	0.821	0.676 - 0.913	0.667 - 1.134	0.202	↓
ACT2	1				
WT *Col 0* (3 Gy acute irradiation)					
CO	1.621*	1.398 - 1.919	1.284 - 2.000	0.033	↑
GI	1.735*	1.608 - 1.912	1.563 - 1.984	0.026	↑
AP1	2.148*	1.820 - 2.589	1.659 - 2.744	0	↑
FT	2.659*	2.523 - 2.804	2.514 - 2.809	0	↑
ACT2	1				

* *Statistically significant trend of treatment.* Relative expression values were compared with reference gene expression =1. Interpretation: expression value <1 means the gene is downregulated; expression value >1 means the gene is up-regulated. The confidence intervals and p values were calculated by randomization algorithms of REST. The hypothesis test P(H1) represents the probability of the difference between the sample and control groups (REST 2009 Software User Guide, 2009).

The trends of the key flowering genes activity in WT plants and *jin* mutants under different irradiation modes are presented in figure 2.

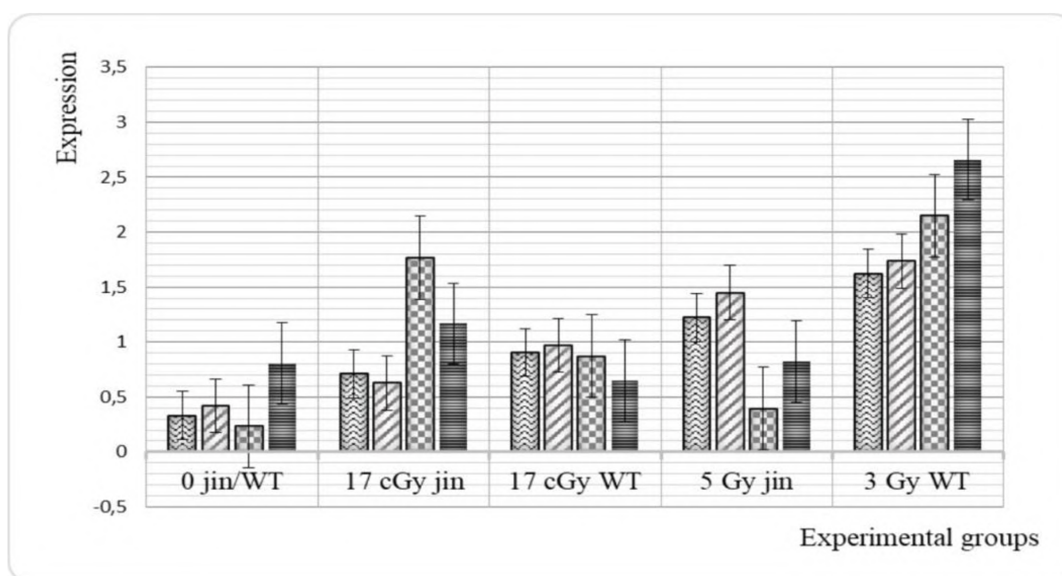

Fig. 2. The trend of key flowering genes expression in WT and *jin* mutants depends on the irradiation mode. The legend is: *CO* *GI* *AP1* *FT* Relative target genes expression values were compared to reference gene expression =1. In the case of the expression value <1 the gene is down-regulated if expression value >1 the gene is up-regulated.

Our data (fig. 2) demonstrate that *AP1* gene expression is dramatically increased during chronic irradiation in *jin* mutants. *AP1* gene regulates cell differentiation and determines the apical meristem to floral way development (Gregis at al., 2006). Cell differentiation is very important for correct flowers and gametes formation. The overexpression of *AP1* may potentially lead to cell division and differentiation errors.

CO, GI, FT expressions decreased in both WT and *jin* mutant groups under chronic irradiation. At the same time, *jin* mutants had lower expression of *CO* and *GI* genes than non-irradiated mutants.

In previous studies, the authors studied the effect of JA on plant response to abiotic stress. Low temperatures, nitrate stress, dehydration or nutrient deficiency cause early flowering (Kolupaev, Karpets, 2010; Lugovaya, 2014). Other authors have

observed the effect of exogenous JA during salt stress in four-week-old WT and *jin1* plants (Yastreb, 2016).

In our study, we also showed JA participation in the response of the flowering genes to radiation. We think that jasmonic acid has a regulatory effect on the flowering genes in Arabidopsis plants under chronic irradiation. According to our previous results, low-dose acute X-ray exposure has the different effects on plants then low-dose chronic exposure (Kryvokhyzha et al., 2018).

Conclusions

The present study uncovers the role of JA in the regulation of flowering in *Arabidopsis thaliana* plants during radiation exposure. The key flowering genes *APETALA 1 (AP1), GIGANTIA (GI), FLOWERING LOCUS T (FT), CONSTANTS (CO)* expression were explored in WT and *jin* mutants. The overexpression of *AP1* under chronic irradiation may cause cell division errors and affect flower development. Based on the results of our study, we hypothesize that jasmonic acid has a stabilizing effect on plant cell differentiation under chronic low-dose radiation exposure.

Acknowledgments

The research was supported by the Seventh Framework Program of the European Union under Marie Curie Action International Research Staff Exchange Scheme (FP7-PEOPLE-2013-IRSES), project ID: 612587 «Plant DNA tolerance».

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Presented by I. O. Andreev
Received 22.05.2019

РОЛЬ ЖАСМОНАТНОГО СИГНАЛЬНОГО ШЛЯХУ У ВІДПОВІДІ ГЕНІВ ЦВІТІННЯ НА ІОНІЗУЮЧЕ ОПРОМІНЕННЯ

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Мета. Метою даного дослідження є вивчення ролі жасмонатного сигналінгу у регуляції генів цвітіння рослин під впливом іонізуючого випромінювання. **Матеріали і методи**. Для проведення експерименту ми використовували рослини *Arabidopsis thaliana* дикого типу та мутанти *jin* з дефектом жасмонатного сигнального шляху. Хронічне опромінення ^{137}CsCl відбувалося у дозі 17 сГр з потужністю 6,8 * 10-6 сГр/сек. Одноразове короткотривале опромінення (3 та 5 Гр, з потужністю 89 сГр/с) рослин відбувалося на стадії розвитку 5,0 на 21 день вегетації (Boyes et al. 2001). В період після опромінення проводили виміри довжини квітконоса та листа. Молекулярно-генетичний аналіз проводили за допомогою ПЛР у реальному часі. У даному експерименті визначали відносну експресію ключових генів цвітіння *AP1, GI, FT, CO*. Як референсні гени використовували *ACT2* та *UBQ10*. Статистичний аналіз проводили за допомогою критерія Стюдента у програмі GraphPad Prism 8. Аналіз кількісних даних ПЛР був проведений за допомогою програми REST 2009, QIAGEN. **Результати**. Групи рослин мали статистично значущі відмінності за висотою стрілки ($p > 0.05$). В дослідженні показано пригнічення експресії генів *CO, GI* і *FT* у мутантів *jin*. Надекспресія AP1 у мутантів *jin* при хронічному випромінюванні може викликати помилки поділу клітин і впливати на розвиток квітки. Експресія *AP1* в рослинах дикого типу була наближеною до нормальної = 1 під час хронічного опромінення. **Висновки**. Виходячи з результатів нашого дослідження, ми припускаємо, що жасмонова кислота має стабілізуючий вплив на активність клітинної диференціації у рослин під впливом хронічного опромінення. Проте, точний механізм впливу жасмонової кислоти на цвітіння рослин *Arabidopsis thaliana* залишається неясним і потребує подальшого дослідження.

Ключові слова: жасмонатний сигналінг, жасмонова кислота, ЖК, цвітіння, іонізуюче випромінювання, ПЛР у реальному часі, відносна експресія.