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IN SILICO CHARACTERIZATION OF B-1,3-GLUCANASE PROMOTER FROM *DROSERA BINATA*

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ABSTRACT

The promoter region of the β-1,3-glucanase gene (*DbGluc*) was isolated from carnivorous plant species *Drosera binata* and sequenced. Subsequently, a DNA fragment of 1154 bp upstream of the transcription start site was searched for the presence of motifs identical to *cis*-element motifs previously reported in the PLACE database. The analysis revealed that the *DbGluc* promoter contains a TATA-box sequence 35 nucleotides upstream of the transcription start site. Moreover, three functional groups of light-, tissue- and stress-responsive putative *cis*-elements were identified within the investigated DNA sequence. The group of stress-responsive elements (MYBCORE, ACGTATATERD1, CURECORECR, WRKY-box, WBOXATNPR1, GT1) is very characteristic for promoters of genes encoding pathogenesis-related proteins, including β-1,3-glucanases. These hydrolases may play a role not only in reaction to biotic and abiotic stress but also during the digestive processes in carnivorous species.

Keywords: β-1,3-glucanase, *cis*-acting elements, *Drosera binata*, *in silico* analysis, proximal promoter, transcriptional regulation

INTRODUCTION

Control of gene transcription in eukaryotic organisms, including plants, is multistage, where each stage is regulated by specific factors. For example, epigenetic changes involving DNA methylation or modification of histones can result in either silencing or superactivation of selected DNA templates **(Habu *et al*., 2001; Emanuele *et al*., 2011).**
Gene transcription itself is conditional upon the availability of genes in euchromatin and involves the preinitiation complex formation, RNA polymerase II recruitment, the transition to an initiation site, elongation using RNA polymerase II, and finally termination. In addition, this process is controlled by numerous specific regulatory protein transcription factors – mediators of intracellular and extracellular signals. Transcription factors interact with the *cis*-regulatory sequences (*cis*-elements) occurring in the promoter sequence and *via* active complexes they modulate adjacent gene expression **(Meshi and Iwabuchi, 1995**; **Bilas *et al*., 2016)**.
In *Arabidopsis thaliana*, the length of potential *cis*-elements - motifs varies from 4 to 10 nucleotides **(Kaur *et al*., 2017**). Depending on the type, these are present in different copy numbers as well as at variable distances and orientations in relation to the gene **(Venter and Botha, 2010)**. Their prediction in plant promoters and subsequent verification involves both bioinformatics and co-expression based approaches. The former is based on the fact that *cis*-regulatory sequences are conserved in orthologous gene promoter sequences across diverging species. The latter considers the specific expression profiles of genes that are conditioned by the occurrence of *cis*-elements - the binding sites for transcription factors **(Ibraheem *et al*., 2010; Korkuc *et al*., 2014)**. Such *in vitro* practical verification of new potential *cis*-element function is usually performed using plant transformation techniques and subsequent transgene expression analysis **(Hernandez-Garcia *et al*., 2014)**.
Currently, available databases, for example PLACE **(Higo *et al*., 1999)** and PLANTCARE **(Lescot *et al*., 2002),** contain a collection of various *cis*-regulatory sequences described in earlier studies that can be used as a tool for *in silico* analysis of unknown promoter sequences.
Endo-β-1,3-glucanases (EC 3.2.1.39) belong to Glycoside hydrolase family 17, whose role is to catalyse the hydrolysis of the glycosidic bond in the β-1,3-glucans - structural components of the cell walls of bacteria, yeasts, fungi, lichens and plants **(Manners *et al*., 1973)**. In plants the genes for β-1,3-glucanases form highly complex and diverse gene families with dozens of members **(Xu *et al*., 2016)**. The crucial role of plant β-1,3-glucanases has been reported in a wide range of physiological and developmental processes and in plant defence **(Mauch *et al*., 1988; Wojtkowiak *et al*., 2012)**. Their participation in the digestion processes of carnivorous plants has also been confirmed **(Michalko *et al*., 2013)**. Recently β-1,3-glucanase gene sequence (*DbGluc*) (MN481115) has been isolated from the poorly-investigated carnivorous plant *Drosera binata* genome. As individual β-1,3-glucanases have specific expression profiles, in order to derive a potential role of the *DbGluc* in *D. binata* plants, *in silico* analysis of the corresponding promoter sequence was the focus of this study. For this, a 1154 bp DNA sequence upstream of the transcription start site of *DbGluc* was searched for the occurrence of putative *cis*-regulatory elements, using the PLACE database, and their role as potential binding sites for transcription factors modulating the specific gene expression profile in plants is discussed in light of the available literature.

MATERIAL AND METHODS

Plant material

Plants of *D. binata* were cultivated aseptically on MS media (Duchefa, The Netherlands) supplemented with 2% (w/v) sucrose and 0.8% (w/v) agar at 24 °C ±2 °C with 16/8 photoperiod and light intensity of 50 µmol m^{-2} s^{-1}.

Isolation of promoter, sequence amplification and sequencing

The *D. binata* genomic DNA was extracted from the green tissues (1 g) according to **Bekesiova *et al*. (1999)**. The β-1,3-glucanase gene (MN481115) was isolated using the Genome Walker kit (Clontech, Mountain View, CA, USA). Subsequently, the adjacent promoter sequence was re-amplified in 25 µl solution containing 100 ng template, 15 pmol P1 (5´-TGATCACCGTTGAACATCTTCCAT-3´) and P2 (5´-TGATCACCGTTGAACATCTTCCAT-3´) primers, 200 µmol dNTP, 1 x PCR buffer and 1U FIREPol DNA polymerase (*Solis BioDyne*, Tartu, Estonia). The first step was performed at 94 °C for 3 min and was followed by 35 cycles of denaturation at 94 °C for 30 s; annealing at 63 °C for 30 s, extension at 72 °C for 90 s and the final step was performed at 72 °C for 7 min. The PCR product was cloned into pJet 1.2 (Thermo Fischer Scientific, USA) and sequenced using an

ABI Prism 310 genetic analyser with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, ThermoFischer Scientific, Inc.).

***In silico* analysis of *DbGluc* promoter region**

The homology search tool of the PLACE database (https://www.dna.affrc.go.jp/PLACE/?action=newplace) (**Higo *et al*., 1999)** was used for scanning of *cis*-element motifs present in the *DbGluc* promoter sequence.

RESULTS AND DISCUSSION

To predict the biological functions of β-1,3-glucanase isolated from *D. binata*, the adjacent promoter sequence of length 1154 bp upstream of the transcriptional start site was assessed for the presence of motifs identical to the previously reported *cis*-elements in the PLACE database **(Higo *et al*., 1999)**. As plant promoter architecture is poorly explored, the region approximately 1000 bp upstream of the transcriptional start site is usually analysed for the occurrence of *cis*-regulatory motifs that may be involved in the control of gene expression **(Picot *et al*., 2010; Yamamoto *et al*., 2007)**.

Out of 460 motifs and their variants available in the PLACE database putative 67 different binding sites were identified within the *DbGluc* promoter sequence. The list of the most relevant potential *cis*-regulatory elements is shown in Table 1.

The search revealed a putative TATA-box 35 nucleotides upstream of the transcription start site of the *DbGluc* promoter. This agrees with the site of most plant TATA box-promoters that contain the element for the RNA binding complex, which are found at a distance from 25 to 45 bp upstream of the transcription start site **(Shahmuradov *et al*., 2005)**. **Sawant *et al*. (1999)** attributed this sequence preferentially to highly expressed plant genes. Later, the responsibility of this motif for accurate transcription initiation and selectivity of gene expression in plants was proven **(Srivastava *et al*., 2014)**. Study of the *Arabidopsis* genome showed that approximately 29% of promoters contain this motif **(Molina and Grotewold, 2005; Jopcik *et al*., 2014)**.

The closest CAAT-box with a defined role in the enhancement of gene transcription **(Sawant *et al*., 1999)** is positioned 59 nucleotides upstream of the core TATA box. Overall, 11 copies of CAAT tetranucleotide were identified within both strands of the promoter sequence.

Subsequently, the other putative binding sites for transcription factors were classified into three functional groups: light-, tissue- and stress-responsive *cis*-elements.

The group of light-responsive elements involved twelve GATA-BOXes, one I-BOX, four T-BOXes and two CIRCADIAN motifs within both strands of the *DbGluc* promoter. The presence of a higher number of repeats for GATABOX (GATA sequence) is not surprising, as this motif is required for high level, light-regulated and tissue-specific gene expression **(Abu El-Heba *et al*., 2015)**. Another GATA-related motif I-box (GATAA sequence) has been found in light-regulated genes such as the CAB (chlorophyll A/B binding protein) or GAPDH (glyceraldehyde-3-phosphate dehydrogenase) genes. Regulation of gene expression is performed *via* interaction with GATA transcription factors that belong to the group of DNA binding proteins distinguished by a zinc finger motif **(Reyes *et al*., 2004; Liu *et al*., 2019)**. T-box and CIRCADIANLELHC *cis*-elements have also been described as light-regulated elements in plants **(Hwang *et al*., 1994; Chan *et al*., 2001., Chen *et al*. 2020)**. Deletion of some of these elements may strongly reduce promoter activity **(Giuliano *et al*., 1988; Reyes *et al*., 2004)**.

The group of tissue-specific *cis*-elements is represented by fifteen DOFCOREZM, thirteen CACTFTPPCA, five RAV1AAT, seven ROOTMOTIFTAPOX1, four POLLEN1LELAT52 and four GTGANTG10 motifs within the *DbGluc* promoter sequence.

Table 1 The *cis*-regulatory elements with putative functions identified within both strands (+), (-) of the 1154-bp *DbGlu1* promoter sequence predicted using the PLACE database. The position of the TIS is +1 and the nucleotides upstream from this site are negatively numbered.

***Cis*-element**	**Location**	**Signal sequence**	**Putative function**
TATA	-35(+)	TATA	Core promoter element about -30 bp upstream from the transcription start site
CAAT	-94 (+), -524 (+), -636(+), -674 (+), -843 (+), -994 (+), -177 (-), -256 (-), -518 (-), -807 (-), -1103 (-)	CAAT	Common *cis*-acting element in promoter and enhancer regions
GATABOX	-197 (+),-389 (+),-617 (+),-755 (+),-976 (+),-1106 (+), -195 (-), -216 (-), -268 (-), -383 (-), -775 (-), -835 (-)	GATA	Light-responsive elements
IBOXCORE	-776(-)	GATAA	
T-BOXATGAPB	-141(+), -164 (+), -360 (+), -1032 (-)	ACTTTG	
CIRCADIANLELHC	-201 (+), -902 (+),	CAANNNNATC	
DOFCOREZM	-169 (+), -226 (+), -1031 (+), -108 (+), -1083(-), -20(-), -104(-), -117(-), -140(-), -163(-),-359(-),-488(-),-686(-), -783(-), 1852(-)	AAAG	Core site of Dof proteins (endosperm-specific expression)
CACTFTPPCA	-142 (+), -230 (+), -373 (+), -443 (+), -646 (+), -929 (+), -986 (+), -69 (-), -263 (-), -284 (-), -297 (-), -973 (-), -1136(-)	YACT	mesophyll-specific gene expression in C4 plants
RAV1AAT	-151(+), -319 (+), -17 (-), -175(-), -873 (-)	CAACA	Root-specific elements
ROOTMOTIFTAPOX1	-179 (+), -292 (+),-809 (+),-815 (+),-1105 (+), -1146(+), -802 (-)	ATATT	
POLLEN1LELAT52	-564 (+), -1082 (+), -671 (-), -718 (-)	AGAAA	Pollen-specific elements
GTGANTG10	-68 (+), -1049 (+), -1135 (+), -408 (-)	GTGA	
ARR1AT	-137 (+), -160 (+), -324 (+), -500 (+), -93 (-), -354 (-), -641 (-), -826 (-),-896 (-)	NGATT	Cytokinin-responsive element
GAREAT	-309 (-)	TAACAAR	GA-responsive element
MYCCONSENSUSAT	-530 (+), -653 (+), -530 (-), -653 (-)	CANNTG	Dehydration-, cold- and ABA-responsive elements
MYBCORE	-319 (-), -505(-)	CNGTTR	Dehydration responsive element
ACGTATERD1	-233 (+), -1062 (+), -233 (-), -1062 (-)	ACGT	Dehydration early responsive element
GT1CONSENSUS	-123 (+),-378 (-), -1007 (+), -1089 (-),	GRWAAW	Salinity stress
WRKY71OS	-67(+), -129 (+), -254 (+), -1048 (+), -1068 (+), -203(-), -314 (-), -877 (-), -881 (-), -1028 (-), -1060 (-)	TGAC	Biotic-, abiotic-stress and germination-responsive element
WBOXATNPR1	-255 (+), -1069 (+), -203 (-)	TTGAC	SA-induced element, disease responsive element
CURECORECR	-231 (+), -647 (+), -231 (-), -647 (-)	GTAC	Copper-responsive element

Legend: W = A or T; Y = C or T; R = A or G; K = G or T

The tetranucleotide AAAG (DOFCOREZM motif) has been described as a target binding site of Dof transcription factors, involved in photosynthetic carbon assimilation and light-mediated gene regulation. In addition, DOF *cis*-regulatory elements have been found in genes participating in many other processes such as seed germination and maturation, accumulation of seed storage proteins, photoperiodic flowering and dormancy **(Kim *et al*., 2010; Kushwaha *et al*., 2011)**. The CACTFTPPCA motif represented by a tetranucleotide YACT was abundantly distributed within the analysed promoter sequence. As a key component of a 41-bp MEM1 module, this was described in mesophyll-specific expression in C4 plants **(Gowik *et al*., 2004)**. The *DbGluc* promoter sequence contains several RAV1AAT *cis*-regulatory motifs (CAACA), target site for RAV1 transcription factor. Its expression has been reported to be up-regulated by touch-related mechanical stimuli **(Kagaya and Hattori, 2009).** In addition, RAV1 acts as a negative regulator of ABA in seed germination and seedling greening rates **(Feng *et al*., 2014)**. The occurrence of pentanucleotide ATATT denoted as ROOTMOTIFTAPOX1 *cis*-element in the promoter of the peroxidase gene has been reported to be responsible for a distinctive expression profile in roots of wheat **(Hertig *et al*., 1991)**. Finally, POLLEN1LELAT52 and

GTGANTG10 elements have been found to control pollen-specific expression of the indie-β-mananas gene in tomato **(Filichkin *et al*., 2004; Tein Dung *et al*. 2016)** and late pollen gene g10 in tobacco **(Rogers *et al*., 2001)**, respectively.
The third group of *cis*-elements are putative target sequences of regulatory proteins responding to biotic and abiotic stress, seed dormancy/germination and senescence **(Wu *et al*., 2009; Wang *et al*., 2015; Zhao *et al*., 2017;)**. As shown in Table 1, two MYBCORE, four ACGTATATERD1, four CURECORECR, eleven WRKY-box, three WBOXATNPR1 and four GT1 *cis*-elements were identified, within both strands of the *DbGluc* promoter.
The MYBCORE sequence was described as a binding motif for plant MYB proteins involved in water stress gene expression **(Zahur *et al*., 2009)**. GT1 *cis*-element target sequences for GT1 transcription factors play a role in response to stress caused by pathogens and high salinity **(Park *et al*., 2004; Zhao *et al*., 2017)**. The ERD1 (ACGT) domain has been reported to be required in dehydration stress and dark-induced senescence **(Simpson *et al*., 2003)**. WBOXATNPR1 *cis*-element (TGAC) is a part of a WRKY-box (TTGAC) that is recognized by WRKY transcriptional factors regulating the plant's response to a wide range of stresses (biotic/abiotic stress factors, senescence, germination**) (Abu El–Heba *et al*., 2015; Lancikova and Ziarovska, 2020)**.
When the *cis*-elements of the *DbGluc* promoter and β-1,3-glucanase and chitinase of *D. rotundifolia*, another carnivorous species, were compared **(Durechova *et al*., 2014; Michalko *et al*., 2017)**, the group of stress-responsive *cis*-elements was abundant within all three promoters. It is noteworthy that the role of many genes for pathogenesis-related proteins involved in defence processes was transformed and many of these genes are extensively utilized in the digestive processes within Droseraceae family **(Durechova *et al*., 2013; Jopcik *et al*., 2017; Schulze *et al*., 2012)**.

CONCLUSION

Herein, the β-1,3-glucanase promoter region of carnivorous plant *D. binata* was successfully isolated and sequenced. *In silico* analysis of the promoter sequence revealed the presence of numerous light-, tissue- and stress-responsive *cis*-elements. The group of stress-responsive elements is predominantly characteristic of pathogenesis-related proteins, including β-1,3-glucanases, that may participate in the digestive processes in carnivorous species as well. However, only the detailed expression profile of the β-1,3-glucanase gene in individual plant tissues/organs, under various stress conditions and during the digestion processes can reveal the real role of this gene in *D. binata* plants.

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REFERENCES

Abu El-Heba, G. A., Hussein, G. M., Fahmy, I. F., Abdou, S. M., Faisal, A., Taha, O., Abdallah, N. A. (2015). Impact of *cis*-acting elements' frequency in transcription activity in dicot and monocot plants. *3 Biotech*, 5**,** 1007-1019. https://doi.org/10.1007/s13205-015-0305-6
Bekesiova, I., Nap, J. P., & Mlynarova, L. (1999). Isolation of high quality DNA and RNA from leaves of the carnivorous plant *Drosera rotundifolia*. *Plant Molecular Biology Reporter, 17*(3), 269-277. https://doi.org/10.1023/A:1007627509824
Bilas, R., Szafran, K., Hnatuszko-Konka, K., Kononowicz, A. K. (2016). *Cis*-regulatory elements used to control gene expression in plants. *Plant Cell, Tissue and Organ Culture*, 127**,** 269-287. https://doi.org/10.1007/s11240-016-1057-7
Chan, C. S., Guo, L., Shih, M. C. (2001). Promoter analysis of the nuclear gene encoding the chloroplast glyceraldehyde-3-phosphate dehydrogenase B subunit of *Arabidopsis thaliana*. *Plant Molecular Biology*, 46**,** 131-141. https://doi.org/10.1023/a:1010602031070
Chen, L. Y., Xin, Y. H., Wai, C. M., Liu, J., & Ming, R. (2020). The role of *cis*-elements in the evolution of crassulacean acid metabolism photosynthesis. *Horticulture Research*, *7*(1). https://doi.org/10.1038/s41438-019-0229-0
Durechova, D., Matusikova, I., Moravcikova, J., Jopcik, M., & Libantova, J. (2013). Isolation and characterization of chitinase gene from the untraditional plant species. *Journal of Microbiology, Biotechnology and Food Sciences*, 2 (Special issue 1) 1405-1413
Durechova, D., Matusikova, I., Moravcikova, J., Jopcik, M., Libantova, J. (2014). *In silico* analysis of chitinase promoter isolated from *Drosera rotundifolia* L. *Journal of Microbiology, Biotechnology and Food Sciences*, 3**,** 71-73.
Emanuele, M., Costa, S., Ragusa, M. A., Gianguzza, F. (2011). Chromatin dynamics of the developmentally regulated P-lividus neural alpha tubulin gene. *International Journal of Developmental Biology*, 55**,** 591-596. https://doi.org/10.1387/ijdb.103264me
Feng, C. Z., Chen, Y., Wang, C., Kong, Y. H., Wu, W. H., Chen, Y. F. (2014). *Arabidopsis* RAV1 transcription factor, phosphorylated by SnRK2 kinases, regulates the expression of *ABI3*, *ABI4*, and *ABI5* during seed germination and early seedling development. *Plant Journal*, 80**,** 654-668. https://doi.org/10.1111/tpj.12670
Filichkin, S. A., Leonard, J. M., Monteros, A., Liu, P. P., Nonogaki, H. (2004). A novel endo-beta-mannanase gene in tomato LeMAN5 is associated with anther and pollen development. *Plant Physiology*, 134**,** 1080-1087. https://doi.org/10.1104/pp.103.035998
Giuliano, G., Pichersky, E., Malik, V. S., Timko, M. P., Scolnik, P. A., Cashmore, A. R. (1988). An evolutionarily conserved protein binding sequence upstream of a plant light-regulated gene. *Proceedings of the National Academy of Sciences of the United States of America*, 85**,** 7089-7093. https://doi.org/10.1073/pnas.85.19.7089
Gowik, U., Burscheidt, J., Akyildiz, M., Schlue, U., Koczor, M., Streubel, M., Westhoff, P. (2004). *Cis*-regulatory elements for mesophyll-specific gene expression in the C-4 plant *Flaveria trinervia*, the promoter of the C-4 phosphoenolpyruvate carboxylase gene. *Plant Cell*, 16**,** 1077-1090. https://doi.org/10.1105/tpc.019729
Habu, Y., Kakutani, T., Paszkowski, J. (2001). Epigenetic developmental mechanisms in plants: molecules and targets of plant epigenetic regulation. *Current Opinion in Genetics & Development*, 11**,** 215-220. https://doi.org/10.1016/s0959-437x(00)00182-9
Hernandez-Garcia, C. M., Finer, J. J. (2014). Identification and validation of promoters and *cis*-acting regulatory elements. *Plant Science*, 217**,** 109-119. https://doi.org/10.1016/j.plantsci.2013.12.007
Hertig, C., Rebmann, G., Bull, J., Mauch, F., Dudler, R. (1991). Sequence and tissue-specific expression of a putative peroxidase gene from wheat (*Triticum-aestivum* L). *Plant Molecular Biology*, 16**,** 171-174. https://doi.org/10.1007/bf00017928
Higo, K., Ugawa, Y., Iwamoto, M., Korenaga, T. (1999). Plant *cis*-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Research*, 27**,** 297-300. https://doi.org/10.1093/nar/27.1.297
Hwang, S. B., Herrin, D. L. (1994). Control of *lhc* gene-transcription by the circadian clock in *Chlamydomonas reinhardtii*. *Plant Molecular Biology*, 26**,** 557-569. https://doi.org/10.1007/bf00013743
Ibraheem, O., Botha, C. E. J., Bradley, G. (2010). *In silico* analysis of *cis*-acting regulatory elements in 5' regulatory regions of sucrose transporter gene families in rice (*Oryza sativa* Japonica) and *Arabidopsis thaliana*. *Computational Biology and Chemistry*, 34**,** 268-283. https://doi.org/10.1016/j.compbiolchem.2010.09.003
Jopcik, M., Moravcikova, J., Matusikova, I., Bauer, M., Rajninec, M., Libantova, J. (2017). Structural and functional characterisation of a class I endochitinase of the carnivorous sundew (*Drosera rotundifolia* L.). *Planta*, 245**,** 313-327. https://doi.org/10.1007/s00425-016-2608-1
Jopcik, M., Moravcikova, J., Matusikova, I., Libantova, J. (2014). Spacer length-dependent protection of specific activity of pollen and/or embryo promoters from influence of *CaMV 35S* promoter/enhancer in transgenic plants. *Plant Cell Tissue and Organ Culture*, 118**,** 507-518. https://doi.org/10.1007/s11240-014-0503-7
Kagaya, Y., Hattori, T. (2009). *Arabidopsis* transcription factors, RAV1 and RAV2, are regulated by touch-related stimuli in a dose-dependent and biphasic manner. *Genes & Genetic Systems*, 84**,** 95-99. https://doi.org/10.1266/ggs.84.95
Kaur, A., Pati, P. K., Pati, A. M., Nagpal, A. K. (2017). *In-silico* analysis of *cis*-acting regulatory elements of pathogenesis-related proteins of *Arabidopsis thaliana* and *Oryza sativa*. *Plos One*, 12. https://doi.org/10.1371/journal.pone.0184523
Kim, H. S., Kim, S. J., Abbasi, N., Bressan, R. A., Yun, D. J., Yoo, S. D., Kwon, S. Y., Choi, S. B. (2010). The DOF transcription factor Dof5.1 influences leaf axial patterning by promoting *Revoluta* transcription in *Arabidopsis*. *Plant Journal*, 64**,** 524-535. https://doi.org/10.1111/j.1365-313X.2010.04346.x
Korkuc, P., Schippers, J. H. M., Walther, D. (2014). Characterization and Identification of *cis*-regulatory elements in *Arabidopsis* based on single-nucleotide polymorphism information. *Plant Physiology*, 164**,** 181-200. https://doi.org/10.1104/pp.113.229716
Kushwaha, H., Gupta, S., Singh, V. K., Rastogi, S., Yadav, D. (2011). Genome wide identification of *Dof* transcription factor gene family in sorghum and its comparative phylogenetic analysis with rice and *Arabidopsis*. *Molecular Biology Reports*, 38**,** 5037-5053. https://doi.org/10.1007/s11033-010-0650-9
Lancikova, V., Ziarovska, J. (2020). Evaluation of the fatty acid desaturase 3 genes stability in flax seeds harvested from radio-contaminated area near Chernobyl. *Journal of Microbiology Biotechnology and Food Sciences*, *9*(4), 816-819. https://doi.org/10.15414/jmbfs.2020.9.4.816-819
Lescot, M., Dehais, P., Thijs, G., Marchal, K., Moreau, Y., Van De Peer, Y., Rouze, P., Rombauts, S. (2002). PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Research*, 30**,** 325-327. doi: 10.1093/nar/30.1.325
Liu, Y. L., Patra, B., Pattanaik, S., Wang, Y., Yuan, L. (2019). GATA and phytochrome interacting factor transcription factors regulate light-induced vindoline biosynthesis in catharanthus roseus. *Plant Physiology*, *180*(3), 1336-1350. https://doi.org/10.1104/pp.19.00489
Manners, D. J., Wilson, G. (1973). Studies on beta-glucanases. Some properties

of a bacterial endo-beta-(1-3)-glucanase system. *Biochemical Journal*, 135**,** 11-18. https://doi.org/10.1042/bj1350011
Mauch, F., Mauch-Mani, B., Boller, T. (1988). Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and beta-1,3-glucanase. *Plant Physiology*, 88**,** 936-942. https://doi.org/10.1104/pp.88.3.936
Meshi, T., Iwabuchi, M. (1995). Plant transcription factors. *Plant and Cell Physiology*, 36**,** 1405-1420. https://doi.org/10.1093/oxfordjournals.pcp.a078903
Michalko, J., Socha, P., Meszaros, P., Blehova, A., Libantova, J., Moravcikova, J., Matusikova, I. (2013). Glucan-rich diet is digested and taken up by the carnivorous sundew (*Drosera rotundifolia* L.): implication for a novel role of plant beta-1,3-glucanases. *Planta*, *238*(4), 715-725. https://doi.org/10.1007/s00425-013-1925-x
Michalko, J., Renner, T., Meszaros, P., Socha, P., Moravcikova, J., Blehova, A., Libantova, J., Poloniova, Z., Matusikova, I. (2017). Molecular characterization and evolution of carnivorous sundew (*Drosera rotundifolia* L.) class V beta-1,3-glucanase. *Planta*, 245**,** 77-91. https://doi.org/10.1007/s00425-016-2592-5
Molina, C., Grotewold, E. (2005). Genome wide analysis of *Arabidopsis* core promoters. *Bmc Genomics*, 6, 25. https://doi.org/10.1186/1471-2164-6-25
Park, H. C., Kim, M. L., Kang, Y. H., Jeon, J. M., Yoo, J. H., Kim, M. C., Park, C. Y., Jeong, J. C., Moon, B. C., Lee, J. H., Yoon, H. W., Lee, S. H., Chung, W. S., Lim, C. O., Lee, S. Y., Hong, J. C., Cho, M. J. (2004). Pathogen- and NaCl-induced expression of the *SCaM-4* promoter is mediated in part by a GT-1 box that interacts with a GT-1-like transcription factor. *Plant Physiology*, 135**,** 2150-2161. https://doi.org/10.1104/pp.104.041442
Picot, E., Krusche, P., Tiskin, A., Carre, I. & Ott, S. (2010). Evolutionary analysis of regulatory sequences (EARS) in plants. *Plant Journal*, 64**,** 165-176. https://doi.org/10.1111/j.1365-313X.2010.04314.x
Reyes, J. C., Muro-Pastor, M. I., Florencio, F. J. (2004). The GATA family of transcription factors in *Arabidopsis* and rice. *Plant Physiology*, 134**,** 1718-1732. https://doi.org/10.1104/pp.103.037788
Rogers, H. J., Bate, N., Combe, J., Sullivan, J., Sweetman, J., Swan, C., Lonsdale, D. M., Twell, D. (2001). Functional analysis of *cis*-regulatory elements within the promoter of the tobacco late pollen gene g10. *Plant Molecular Biology*, 45**,** 577-585. https://doi.org/10.1023/a:1010695226241
Sawant, S. V., Singh, P. K., Gupta, S. K., Madnala, R., Tuli, R. (1999). Conserved nucleotide sequences in highly expressed genes in plants. *Journal of Genetics*, 78**,** 123-131. https://doi.org/10.1007/bf02924562.
Shahmuradov, I. A., Solovyev, V. V. & Gammerman, A. J. 2005. Plant promoter prediction with confidence estimation. *Nucleic Acids Research*, 33**,** 1069-1076. https://doi.org/10.1093/nar/gki247.
Schulze, W. X., Sanggaard, K. W., Kreuzer, I., Knudsen, A. D., Bemm, F., Thogersen, I. B., Braeutigam, A., Thomsen, L. R., Schliesky, S., Dyrlund, T. F., Escalante-Perez, M., Becker, D., Schultz, J., Karring, H., Weber, A., Hojrup, P., Hedrich, R., Enghild, J. J. (2012). The protein composition of the digestive fluid from the venus flytrap sheds light on prey digestion mechanisms. *Molecular & Cellular Proteomics*, 11**,** 1306-1319. https://doi.org/10.1074/mcp.M112.021006
Simpson, S. D., Nakashima, K., Narusaka, Y., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K. (2003). Two different novel *cis*-acting elements of *erd1*, a *clp*A homologous *Arabidopsis* gene function in induction by dehydration stress and dark-induced senescence. *Plant Journal*, 33**,** 259-270. https://doi.org/10.1046/j.1365-313X.2003.01624.x
Srivastava, R., Rai, K. M., Srivastava, M., Kumar, V., Pandey, B., Singh, S. P., Bag, S. K., Singh, B. D., Tuli, R., Sawant, S. V. (2014). Distinct role of core promoter architecture in regulation of light-mediated responses in plant genes. *Molecular Plant*, 7**,** 626-641. https://doi.org/10.1093/mp/sst146
Tien Dung, N., Moon, S., Van Ngoc Tuyet, N., Gho, Y., Chandran, A. K. N., Soh, M.-S., et al. (2016). Genome-wide identification and analysis of rice genes preferentially expressed in pollen at an early developmental stage. *Plant Molecular Biology*, *92*(1-2), 71-88. https://doi.org/10.1007/s11103-016-0496-1
Venter, M.; Botha, F. C. (2010). Synthetic promoter engineering. *Plant Developmental Biology-Biotechnological Perspectives*, 393-414. https://doi.org/10.1007/978-3-642-04670-4_20
Wang, H., Meng, J., Peng, X., Tang, X., Zhou, P., Xiang, J., Deng, X. (2015). Rice WRKY4 acts as a transcriptional activator mediating defense responses toward *Rhizoctonia solani*, the causing agent of rice sheath blight. *Plant Molecular Biology*, 89**,** 157-171. https://doi.org/10.1007/s11103-015-0360-8
Wojtkowiak, A., Witek, K., Hennig, J., Jaskolski, M. (2012). Two high-resolution structures of potato endo-1,3-beta-glucanase reveal subdomain flexibility with implications for substrate binding. *Acta Crystallographica Section D-Structural Biology*, 68**,** 713-723. https://doi.org/10.1107/s090744491200995x
Wu, X., Kishitani, S., Ito, Y., Toriyama, K. (2009). Accumulation of raffinose in rice seedlings overexpressing *OsWRKY11* in relation to desiccation tolerance. *Plant Biotechnology*, 26**,** 431-434. https://doi.org/10.5511/plantbiotechnology.26.431
Xu, X., Feng, Y., Fang, S., Xu, J., Wang, X., Guo, W. (2016). Genome-wide characterization of the beta-1,3-glucanase gene family in *Gossypium* by comparative analysis. *Scientific Reports*, 6. https://doi.org/10.1038/srep29044
Yamamoto, Y. Y., Ichida, H., Abe, T., Suzuki, Y., Sugano, S. & Obokata, J. (2007). Differentiation of core promoter architecture between plants and mammals revealed by LDSS analysis. *Nucleic Acids Research*, 35**,** 6219-6226. https://doi.org/10.1093/nar/gkm685
Zahur, M., Maqbool, A., Irfan, M., Barozai, M. Y. K., Rashid, B., Riazuddin, S., Husnain, T. (2009). Isolation and functional analysis of cotton universal stress protein promoter in response to phytohormones and abiotic stresses. *Molecular Biology*, 43**,** 578-585. https://doi.org/10.1134/s0026893309040086
Zhao, H., Jiang, J., Li, K., Liu, G. (2017). *Populus simonii* x *Populus nigra* WRKY70 is involved in salt stress and leaf blight disease responses. *Tree Physiology*, 37**,** 827-844. https://doi.org/10.1093/treephys/tpx020