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Specific up-regulation of transcription factors in the fungus *Parastagonospora nodorum* suggests a role in pathogenicity on wheat

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Abstract: The necrotrophic fungus *Parastagonospora nodorum*, the causal agent of stagonospora nodorum blotch (SNB), is responsible for significant economic losses of wheat worldwide. Despite the presence of a high number of TF-encoding genes within the genome of *P. nodorum*, very little is known about their expression profile and their functions during cellular and biological processes. In this study, ten putative TF genes in *P. nodorum* isolate SN15 were selected, four of which shared homology with well-known fungal TFs involved in pathogenesis, and the other six genes were located in putative secondary metabolite biosynthetic gene clusters (BGCs). The expression profile of these candidate TF genes was investigated using both semi-quantitative and quantitative RT-PCR assays under *in vitro* and *in planta* conditions. The results revealed that six candidate TF genes exhibited the highest expression levels in the minimal media lacking both nitrogen and carbon sources as well as during the early stages of infection. These findings suggest that these six candidate TFs may play an important role in the pathogenicity of *P. nodorum*.

Keywords: Glume blotch, QPCR, Expression profile, Minimal media

INTRODUCTION

Parastagonospora nodorum, the causal agent of stagonospora nodorum blotch (SNB) or glume blotch, is a necrotrophic fungal pathogen that causes significant yield loss in many wheat-growing areas worldwide (Duba *et al.* 2018; Oliver *et al.* 2012). *P. nodorum* is a model organism for studying the pathogenesis of necrotrophic pathogens (Oliver *et al.* 2012). Genome mining of *P. nodorum* has revealed that it harbors a large number of genes involved in the production of pathogenicity factors such as proteinaceous host-specific toxins known as necrotrophic effectors (NEs) and extracellular enzymes (Hane *et al.* 2007; Oliver *et al.* 2012). In addition, *P. nodorum* possesses over 40 biosynthetic gene clusters (BGCs) responsible for the production of secondary metabolites (SMs), which may play a crucial role in the fungus survival under stress conditions (Chooi *et al.* 2014). Transcription factors (TFs) are one the most important players in the regulation of gene expression through their interaction with RNA polymerase II. TFs are required for many cellular and biological processes such as morphogenesis, sexual and asexual development, nutrient utilization, virulence, toxin production, and stress response in fungi (Lin 2017). TFs are generally categorized by their DNA-binding domains. The zinc finger family is the largest family of fungal TFs, which is classified into three sub-classes Cys4, Cys2His2 (C2H2), and Zn2Cys6 (C6) (John *et al.* 2021).

Genome mining of *P. nodorum* revealed that it harbors 486 putative TFs which represent almost 3 % of the total number of annotated genes. A limited number of TFs have been functionally characterized in *P. nodorum* (Lin 2017). Most characterized TF genes in *P. nodorum* play key roles in the regulation of NE genes expression.

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For instance, the TF gene *SnStuA*, homologous to the characterized TF genes *StuAp* in *Aspergillus nidulans* and *FoStuA* in *Fusarium oxysporum*, plays an important role in regulating the expression of the NE gene *SnTox3*, carbon metabolism, and sporulation in *P. nodorum* (IpCho *et al.* 2010). The TF gene *PnPf2* is involved in the regulating two NE genes, *SnToxA* and *SnTox3* (Rybak *et al.* 2017), and *PnCon7* plays a significant role in the regulation of the NE genes *SnToxA*, *SnTox1*, and *SnTox3* (Lin *et al.* 2018). *ElcR* is a functionally characterized TF gene embedded in a SM BGC in *P. nodorum*. This TF controls the expression of this BGC, which is similar to the *CTB* gene cluster involved in the synthesis of cercosporin in *Cercospora nicotianae* (Chooi *et al.* 2017). Our study aimed to assess the expression profile of ten candidate TF genes in *P. nodorum* isolate SN15, including four TFs with homology to the well-known TF genes in other fungi involved in pathogenesis, and other six TF genes located in SM BGCs. Expression analysis was performed under *in vitro* and *in planta* conditions using semi-quantitative and quantitative RT-PCR assays.

MATERIALS AND METHODS

Isolate, media, and growth conditions

The *P. nodorum* reference isolate SN15 was grown on Czapek-Dox V8 agar ($NaNO_3$, 2 g/L; KCl, 0.5 g/L; $MgSO_4$, 0.5 g/L; $FeSO_4$, 0.01 g/L; K_2HPO_4, 1 g/L; sucrose, 30 g/L; $CaCO_3$, 3 g/L; V8 juice, 200 ml; agar, 15 g/L). The plates were incubated at 22 °C until the pycnidia and pycnidiospores were produced.

Selection of TF genes

The TF genes were selected based on homology searches of well-known characterized fungal TFs against the amino acid sequence of *P. nodorum* SN15 using the Blastp search tool in the NCBI database. The protein hits with e-value < 10^{-4} were chosen. The candidate TF genes located in the BGCs were retrieved from previously identified BGCs (Chooi *et al.* 2014). The presence of the TF genes in the BGCs was confirmed by predicting BGC regions using fungiSMASH(https://fungismash.secondarymetabolites. org/) (Blin *et al.* 2023) with default parameters. To generate *in vitro* and *in planta* expression data for ten selected TF genes, primers were designed using Primer 3 software (https://primer3.ut.ee/) (Untergasser *et al.* 2012) (Table 1).

Table 1. Primers used for the expression analysis of candidate transcription factor-encoding genes (TFs) in *Parastagonospora nodorum* isolate SN15

Primer name	Gene ID	Primer Sequence (5′-3′)	Expected region (bp)
Pn_Vf19	JI435_037800	F: CTGTGAGGGTGAGGTCTTCT R: GGGGAATGGGTGAAGGTGAT	170
Pn_MetR	JI435_049640	F: AGTCGACGTTACTGCTGAGC R: GTGTCAAGCAGAGGTGTGGA	162
Pn_AtfA	JI435_056770	F: AACCCAAACTGCGAGGACC R: GGTTGCGCATTGGAGGTTG	179
Pn_Crz1	JI435_152130	F: GCAACAGCAACAGCGTGG R: CGTTGTGTACAGCTTGTGACA	164
Pn_06678	JI435_066780	F: AAAGCAGCTCCGAGGAAGAT R: TCAGCCAGTGTCTCTCTAGC	171
Pn_07131	JI435_071310	F: ACATGCAGTACCTACACACCG R: TCAGGGGTCTCATAGTAGTACGT	160
Pn_08284	JI435_082840	F: CTGTTTGGTGATTTTGCGCG R: GCAGGCATGAGTGAAGATCA	171
Pn_12614	JI435_126140	F: TCACGCCTAGGGAAACATACT R: CCGCTGGTGAAATCGAGGTA	164
Pn_13024	JI435_130240	F: ATGCCATCCAAGTCCCGAG R: GTGCACTCAGAGTTCGTGAC	175
Pn_14844	JI435_148440	F: CTACCGGGGATGAGAATGCA R: CCACATCATGACAGTCTGGC	162

***In vitro* growing conditions**

To stimulate *P. nodorum* to produce pathogenicity factors, the fungal biomass grown in liquid-rich media was transferred into the minimal media lacking nitrogen and carbon sources. To do so, four 250 ml flasks each containing 100 ml rich liquid media Czapek-Dox V8 broth were inoculated with 2 ml of *P. nodorum* SN15 spore suspension (10^6 spore/ ml). The flasks were placed in a shaking incubator (150 rpm) at 22 °C for four days. The fungal biomasses were washed using a stream of sterile DEPC-distilled water. The biomass collected from one flask was stored at -80 °C for RNA extraction as a control (fungi grown in rich media).

The other three biomasses were transferred into three 250 ml flasks containing 100 ml B5 minimal media (Gamborg *et al.* 1968) lacking nitrogen (-N, by omitting KNO_3 and $(NH_4)_2SO_4$ from B5 medium), lacking carbon (-C, by omitting sucrose from B5 medium) and lacking both nitrogen and carbon sources (-NC, by omitting KNO_3, $(NH_4)_2SO_4$, and sucrose from B5 medium) and kept under the same conditions (shaking incubator, 150 rpm, 22 °C) for 24-h. The fungal biomasses grown in the aforementioned minimal media (B5-N, B5-C, and B5-NC) were washed with DEPC-distilled water followed by storing at -80 °C for further RNA extraction.

***In planta* infection assay**

To conduct *in planta* experiments, the SNB-susceptible wheat cv. Grandin was inoculated with the *P. nodorum* SN15 under greenhouse conditions as described previously (Liu *et al.* 2004). Briefly, the 14-day-old wheat seedlings of cv. Grandin grown in 8 cm diameter pots containing sterile soil, leaf mold, and peat moss (1:1:1) were inoculated with the pycnidiospore suspension (10^6 spore/ ml) supplemented with 0.15 % Tween-20 using a hand-held sprayer until run-off. To provide high relative humidity (RH), the plants were covered with transparent polyethylene bags for 48-h in darkness. Afterward, the pots were kept under 85 % RH25-27 °C, and a photoperiodic regime of 16-h light/ 8-h dark. The SNB-inoculated leaves were collected in three biological replicates at 0-7, and 10 days post-inoculation (DPI). The uninoculated wheat leaves were collected as controls. Samples were frozen immediately using liquid nitrogen and transferred to a -80 °C freezer for further investigations.

RNA extraction, semi-quantitative, and quantitative RT-PCR assays

Total RNA was extracted from both fungal biomass and SNB-inoculated wheat leaves using the CTAB method (Orek 2018) with some modifications as described previously (Rahimi Tamandegani *et al.* 2021). DNA contamination was removed using DNase I, RNase-free (Thermo Fisher Scientific Inc., Pub. No. MAN0012000) according to the manufacturer's instruction. One microgram of total RNA primed with random hexamer was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription kit (Yekta Tajhiz Azma, Cat No. YT4500).

The Semi-quantitative RT-PCR was carried out for the *P. nodorum* biomass collected from rich medium Czapek-Dox V8 broth and minimal media (B5-N, B5-C, and B5-NC) and the SNB-inoculated wheat samples at 0-7 and 10 DPI. The experiment was performed in a 15 µL reaction containing 1 µL of cDNA, 1x *Taq* DNA Polymerase 2x Master Mix RED (Ampliqon, Cat. No. A190301), and 0.5 µM of each primer under the following conditions: initial denaturation at 96 °C for 2 min, 35 cycles of denaturation at 96 °C for 45 s, annealing at 57 °C for 45 s, and extension at 72 °C for 50 s with a final extension at 72 °C for 10 min. The PCR products were resolved on a horizontal 1.2 % agarose gel in 1x TBE buffer and visualized by ethidium bromide staining.

The quantitative RT-PCR was performed for the*P. nodorum* biomass collected from the minimal media (B5-N, B5-C and B5-NC) and the SNB-inoculated wheat samples at 0, 1, 3, 5, 7 and 10 DPI in a 20 µL reaction containing 1 µL of diluted (1/5) cDNA, 1x SYBR Green qPCR MasterMix (Yekta Tajhiz Azma, Tehran, Iran, Cat No. YT2551) and 0.2 µM of each primer using a 48-well StepOne RealTime PCR System (Applied Biosystems StepOne, Foster City, CA, USA). The thermal cycle parameters were: 95 °C, 5 min; 40 cycles 95 °C, 20 s; 60 °C, 30 s and 72 °C, 35 s.

The reactions were performed in three biological and two technical replicates. The relative expression level of each TF gene was normalized against the *EF1-α* gene as an endogenous control and was calculated with the $2^{\Delta Ct}$ method (Schmittgen & Livak 2008). The qPCR results were statistically analyzed in a completely randomized design with one-way ANOVA and the t-test least significant differences (LSD) ($p < 0.05$) using SAS software v9.4.

RESULTS

Candidate TF genes

Ten putative TF-encoding genes in *P. nodorum* SN15 were selected to evaluate their expression profiles under *in vitro* and *in planta* conditions. Four of the genes shared a high homology with well-known TF genes in other fungal species involved in pathogenesis. The other six candidate TF genes were located in predicted secondary metabolite BGCs (Table 2).

Semi-quantitative RT-PCR assay

The semi-quantitative RT-PCR assay was carried out to screen the expression profile of ten candidate TF genes in *P. nodorum* SN15 grown in both rich and carbon and/or nitrogen starvation media and during the *P. nodorum* infection on wheat at 0-7 and 10 DPI.

Table 2. Candidate transcription factor (TF) genes in *Parastagonospora nodorum* isolate SN15

N0.	Locus tag	TF family	Orthologue (synonyms)	Reported in fungal species	Regulated pathway	Regulatory functions	Reference
1	JI435_037800	C2H2	Msn2, Vf19, Msn1, GzC2H045, MoMsn2/ Tdg1, VmSeb1, VdMsn2, ZtVf1, AbVf19	*Alternaria brassicicola, Aspergillus fumigatus, Fusarium graminearum, Magnaporthe oryzae, Verticillium dahliae, Zymoseptoria tritici*	Regulating cell wall-degrading enzyme genes	Sporulation, hyphal growth, enzyme secretion, abiotic, stress tolerance	(John *et al*. 2021; Mohammadi *et al*. 2017; Srivastava *et al*. 2012)
2	JI435_049640	bZIP	MetR, bZIP_Ab0007, MoCys-3, AaMetR	*Alternaria alternata, A. brassicicola, M. oryzae*	Cysteine and methionine metabolism	Oxidative stress tolerance, pathogenicity, hyphal growth, sporulation	(Gai *et al*. 2019)
3	JI435_056770	bZIP	Atf1, BcAtf1, CpTf1, FgAtf1, Foatf1, MoAtf1, VDAG_08676, AtfA	*Botrytis cinerea, Claviceps purpurea,* F. *graminearum, Fusarium oxysporum, M. oryzae, V. dahliae*	Stress-activated protein kinase (SAPK) sensing pathways	Sporulation, virulence, oxidative stress tolerance, nitrogen metabolism	(Fang *et al*. 2017; John *et al*. 2021)
4	JI435_152130	C2H2	Crz1, CrzA, BcCrz1, FgCrz1A, GzC2H013, PdCrz1, VdCrz1	*A. fumigatus, B. cinerea, Colletotrichum graminicola, F. graminearum, M. oryzae, Penicillium digitatum, V. dahlia*	Ca2+/calcineurin signaling	Ionic/cell wall stress response, sporulation, hyphal growth, SM biosynthesis	(Chen *et al*. 2019; John *et al*. 2021)
5	JI435_066780	C6			In highly reducing PKSs (HR-PKSs) BGC		(Chooi *et al.* 2014)
6	JI435_071310	C6			In nonribosomal peptide synthetases like (NRPS-like) BGC		(Chooi *et al.* 2014)
7	JI435_082840	C2H2			In nonreducing polyketide synthases (nrPKSs) BGC		(Chooi *et al.* 2014)
8	JI435_126140	bZIP			In class II terpene synthases (TS) BGC		(Chooi *et al.* 2014)
9	JI435_130240	C6			In HR-PKSs BGC		(Chooi *et al.* 2014)
10	JI435_148440	C6			In NRPS BGC		(Chooi *et al.* 2014)

Results showed that six TF genes out of ten,

including four genes homologous to the well-known TF genes in other fungal species (locus tags JI435_037800, JI435_049640, JI435_056770, and JI435_152130) and two genes located in the SM BGCs (locus tags JI435_071310 and JI435_082840) were expressed under *in vitro* and/or *in planta* conditions (Fig. 1).

The expected amplicons were generated for all candidate TFs sharing homology with well-known TFs. The TF gene *PnVf19* (locus tag JI435_037800) showed a constitutive expression profile under all *in vitro* conditions and at all time points post-inoculation (*in planta*) (Fig. 1a). The TF genes *JI435_071310* embedded in an NRPS-like BGC produced the expected fragments at all time points post inoculation and displayed a distinct amplicon under exposure to the carbon and nitrogen starvation (Fig. 1c). The TF gene *JI435_082840* located in nrPKS BGC showed the lowest expression profile amplifying the expected fragment only at 1, 6 and 7 DPI as well as under both carbon and nitrogen deficiency (Fig. 1f). The expected amplicon was not amplified for other four TF genes embedded in SM BGCs (locus tags JI435_066780, JI435_126140, JI435_130240, and JI435_148440) suggesting that they may not play a significant role in the pathogenicity of *P. nodorum*. Therefore, these genes were excluded from further studies.

Quantitative RT-PCR assay

The six TF genes showing amplification patterns in the semi-quantitative RT-PCR assay were subjected to expression analysis using a quantitative RT-PCR assay. The qPCR experiment was carried out to explore the expression profile of TF genes under carbon and/or nitrogen starvation conditions and during *P. nodorum* infection at 0, 1, 3, 5, 7, and 10 DPI on wheat plants. All TF genes were up-regulated in the absence of both nitrogen and carbon sources (Fig. 2).

Under *in planta* conditions, the candidate TF genes were mostly expressed during the early stage of infection in comparison with the late stage (Fig. 3). The expression level of most candidate TF genes showed a gradual increase from zero reaching to the highest expression level at 3 DPI, followed by a gradual decrease at 7 DPI and finally the expression level for most TF genes reached to the lowest level at 10 DPI (Fig. 3).

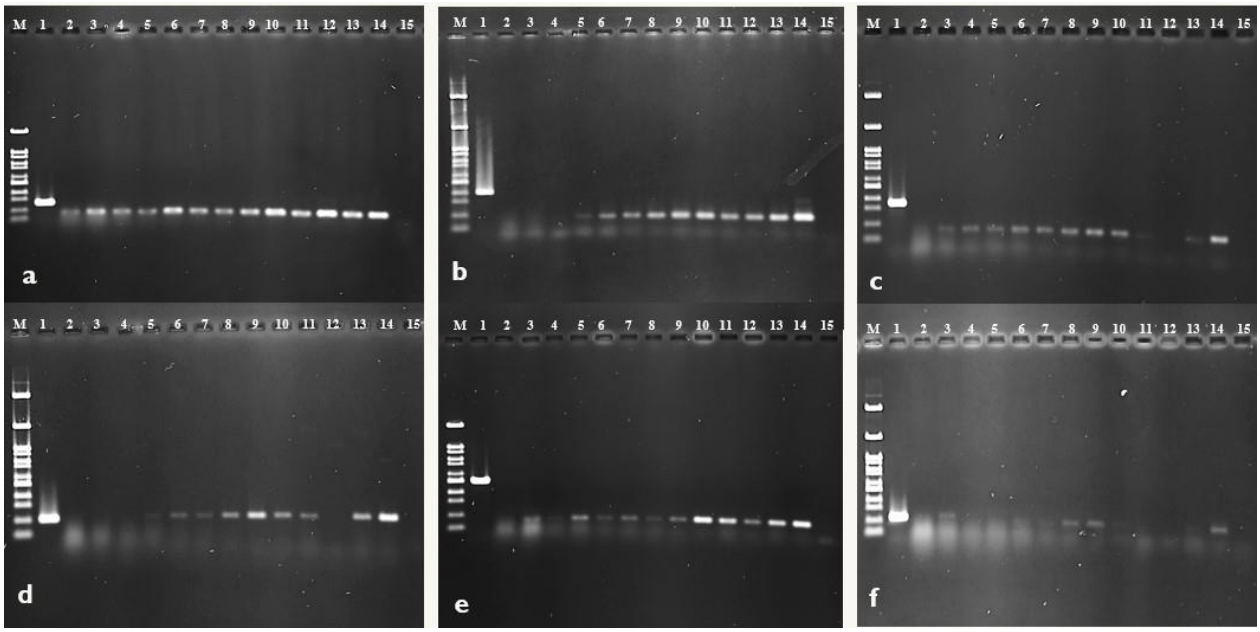

Fig. 1. RT-PCR pattern of candidate transcription factor genes in *Parastagonospora nodorum* SN15 under *in vitro* (grown in rich Czapek-Dox V8 broth and B5 minimal media lacking carbon and nitrogen sources) and *in planta* (N days post inoculation); a. *PnVf19* (locus tag JI435_037800); b. *PnAtfA* (locus tag JI435_056770); c. Locus tag JI435_071310 in NRPS-like BGC; d. *PnCrz1* (locus tag JI435_152130); e. *PnMetR* (locus tag JI435_049640); f. locus tag JI435_082840 in nrPKS BGC; (1) genomic DNA, (2) 0 DPI, (3) 1 DPI, (4) 2 DPI, (5) 3 DPI, (6) 4 DPI, (7) 5 DPI, (8) 6 DPI, (9) 7 DPI, (10) 10 DPI, (11) Rich media, (12) Minimal media lacking nitrogen, (13) Minimal media lacking carbon, (14) Minimal media lacking nitrogen and carbon, (15) water control (M) 100 bp DNA marker**.**

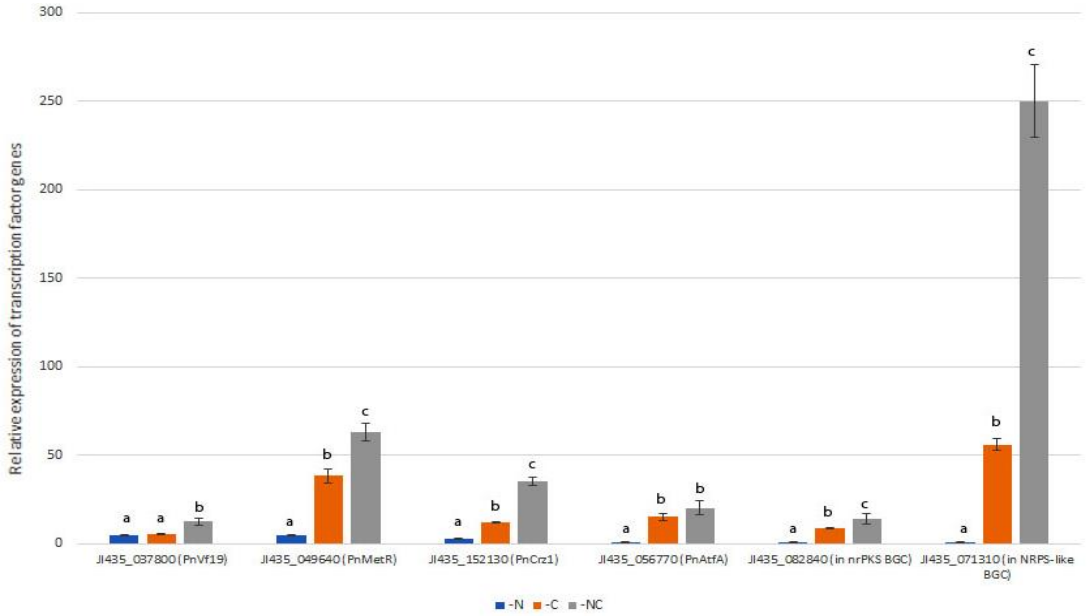

Fig. 2. Expression profile of candidate TF genes in *Parastagonospora nodorum* SN15 under *in vitro* conditions using quantitative RT-PCR assay. The x-axis represents the different nutrient starvation conditions including minus nitrogen (-N), minus carbon (-C) and minus both nitrogen, and carbon (-NC) sources used in B5 minimal media. The y-axis shows the relative expression of candidate TF genes normalized to the housekeeping gene *EF1-α*. All TF genes are highly expressed under a lack of both nitrogen and carbon sources (-NC). The t-test least significant differences (LSD) ($p < 0.05$) was used for statistical analysis. Different letters mean significantly different values ($p < 0.05$).

SNB symptom development

The SNB symptom development on wheat c.v. Grandin was monitored throughout 10 DPI. The visibly noticeable symptoms appeared at 2 DPI as small yellow spots progressing to brown spindle-shaped necrotic lesions surrounding with a yellow halo at 3-5 DPI. The necrotic lesions were extended to large irregular necrotic lesions at 7 DPI followed by fully collapsed leaf tissues covered by pycnidia at 10 DPI (Fig. 4).

DISCUSSION

This study was performed to analyze the expression profile of ten candidate TF-encoding genes in *P. nodorum* under *in vitro* and *in planta* conditions using semi-quantitative and quantitative RT-PCR assays. Based on the expression profile, six candidate TF genes were found to be expressed under nutrient starvation conditions (*in vitro*) as well as the early stage of infection (*in planta*). Starvation for nutrients serves as an environmental stress signal mimicking *in planta* conditions which effectively triggers the pathogen to enhance the production of virulence factors (Lin 2017). The findings of the previous studies support the link between nutrient starvation and the expression of effector genes in fungal plant pathogens. For instance, Avr9 is one of the effector proteins produced by the apoplast-colonizing fungus *Cladosporium fulvum* during infection on tomato plants. It is reported that *Avr9* is highly up-regulated under nitrogen deprivation, however, the expression of other effector genes was not induced by nitrogen level (Thomma *et al.* 2006).

Similarly, the genome-wide analysis of gene expression showed that nitrogen-limiting conditions induce the expression of genes related to nitrogen metabolism during the rice infection by *Maganaporthe oryzae* (Donofrio *et al.* 2006). The impact of nutritional starvation on the expression of two NE genes *SnToxA* and *SnTox3* in *P. nodorum* has been addressed showing that the *SnToxA* was up-regulated in minimal media lacking carbon source while the expression of *SnTox3* was induced in Fries media containing nitrogen sources (Lin 2017).

Here, the B5 medium without carbon and nitrogen sources was used to mimic *in planta* conditions stimulating the TF genes expression. The expression of candidate TF genes was unaffected by nitrogen starvation; however, the TF genes were highly up-regulated under the lack of carbon and both carbon and nitrogen sources.

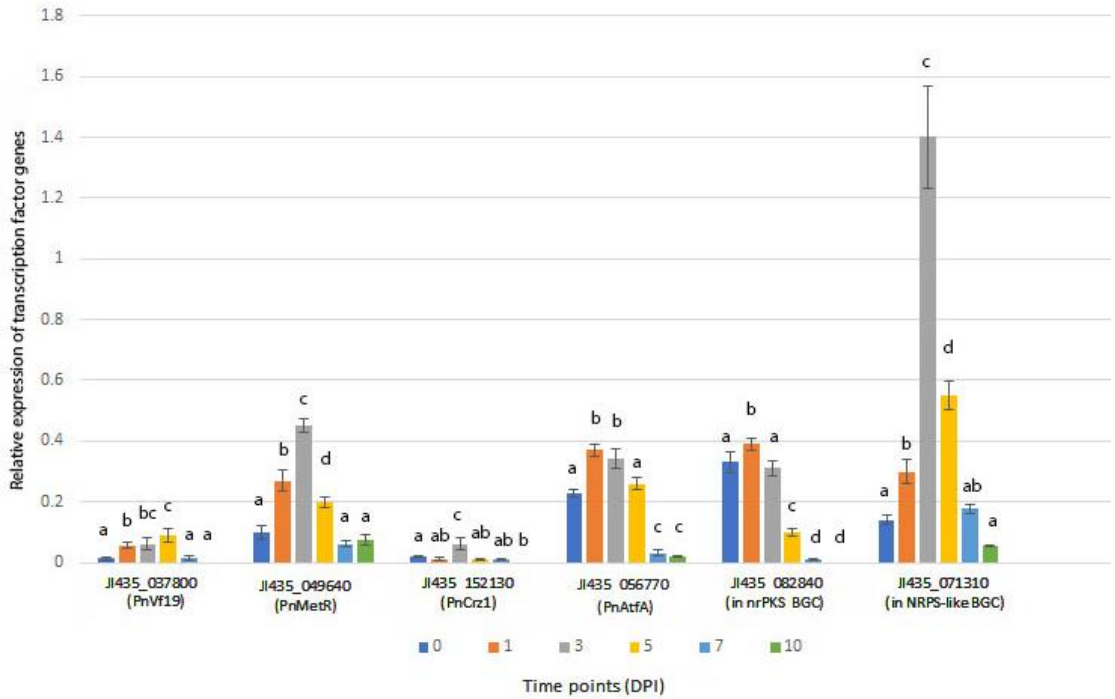

Fig. 3. Expression profile of candidate TF genes in *Parastagonospora nodorum* SN15 under *in planta* conditions using quantitative RT-PCR assay. The x-axis represents the days post inoculation (DPI) of *P. nodorum* isolate SN15 on wheat cultivar Grandin. The y-axis shows the relative expression of candidate TF genes normalized to the housekeeping gene *EF1-α*. The expression level of candidate TF genes mostly peaked at three days post-inoculation (DPI). The t-test least significant differences (LSD) ($p < 0.05$) was used for statistical analysis. Different letters mean significantly different values ($p < 0.05$).

Fig. 4. Development of stagonospora nodorum blotch (SNB) symptoms by *Parastagonospora nodorum* SN15 on wheat cultivar Grandin at 0, 2, 3, 5, 7 and, 10 days post-inoculation.

Hence, the notable upregulation of TF genes observed in minimal media lacking nitrogen and carbon sources strongly suggests their possible involvement in the pathogenicity of *P. nodorum*.

Under *in planta* conditions, the expression of almost all candidate TF genes increased gradually from zero to 3 DPI, maximized at 3 DPI, and decreased gradually at 5-10 DPI. This expression pattern is consistent with the pattern of SNB symptom development at the same time points. *P. nodorum* is a necrotrophic fungal pathogen that kills plant cells by producing toxins and cell wall-degrading enzymes before the invasion to facilitate absorbing plant nutrients (Oliver *et al.* 2012). Therefore, the high expression of candidate TF genes in the early stages of infection and its correlation with the SNB symptom development suggests the possible role of the candidate TF genes in the pathogenicity of *P. nodorum*.

Four of the candidate TF genes share high homology with well-known fungal TFs playing an important role in regulating a wide range of cellular and biological processes in the fungal life cycle including hyphal growth, sporulation, virulence, and stress tolerance response. This similarity suggests a potential shared role in *P. nodorum*. The absence or very low expression of candidate TF genes embedded in BGCs might be explained by the fact that, unlike primary metabolites, secondary metabolites are not essential for growth or reproduction and they most likely contribute to the organism's adaptation to its environment (Mosunova *et al.* 2021). Nevertheless, two of the candidate TFs in BGCs showed an expression profile under *in vitro* and *in planta* conditions. The TF gene *JI435_071310* (in NRPS-like BGC) was highly expressed at 3 DPI and under nitrogen and carbon deficiency showing its possible role in the pathogenicity of *P. nodorum*. The low expression of TF gene *JI435_082840* (in nrPKS BGC) during the infection process indicates that this TF may not play a significant role in virulence which could be explained by its location in an SM BGC. However, its expression under a lack of nitrogen and carbon sources indicates that it still could serve as an important protection factor under stress conditions.

This study significantly contributes to the understanding of several crucial TF genes in *P. nodorum*, providing fundamental knowledge in their expression profile under different *in vitro* and *in planta* conditions. The high expression level of these TFs under nutrient starvation and early stages of infection suggests that they may play an important role in the pathogenicity of *P. nodorum*. However, the precise function of these genes remains to be determined, necessitating further validation through functional characterization using reverse genetics approaches, such as gene knockouts.

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افزایش اختصاصی بیان ژن‌های فاکتور نسخه‌برداری در قارچ *Parastagonospora nodorum*، بیانگر نقش آنها در بیماری‌زایی روی گندم

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چکیده: فاکتورهای نسخه‌برداری به عنوان عوامل تنظیم کننده بیان ژن در بسیاری از فرایندهای حیاتی سلول مانند ریخت‌زایی، تولیدمثل، بیماری‌زایی و پاسخ به تنش‌های محیطی در قارچ‌ها ایفای نقش می‌کنند. قارچ مرده پرور (نکروتروف) *Parastagonospora nodorum*، عامل سوختگی خوشه گندم، سبب خسارات اقتصادی قابل ملاحظه‌ای در جهان می‌شود. علی‌رغم حضور تعداد زیادی از ژن‌های کد کننده فاکتورهای نسخه‌برداری در ژنوم قارچ *P. nodorum*، اطلاعات چندانی در زمینه الگوی بیان این ژن‌ها و همچنین نحوه عملکرد آن‌ها طی فرایندهای بیولوژیکی و سلولی در دسترس نیست. از این رو در این پژوهش، ده ژن فاکتور نسخه‌برداری شامل چهار ژن دارای شباهت با فاکتورهای نسخه‌برداری دخیل در بیماری‌زایی در سایر قارچ‌ها و همچنین شش ژن واقع در خوشه‌های ژنی بیوسنتز متابولیت‌های ثانویه در قارچ *P. nodorum* (جدایه SN15) انتخاب شدند و الگوی بیان آن‌ها با استفاده از آزمایشات نیمه‌کمی و کمی RT-PCR تحت شرایط *in vitro* و *in planta* مورد بررسی قرار گرفت. نتایج نشان داد که شش ژن بیشترین سطح بیان در محیط فقیر فاقد منابع نیتروژن و کربن و همچنین طی مراحل اولیه بیماری‌زایی دارند که بیانگر نقش احتمالی این فاکتورهای نسخه‌برداری در بیماری‌زایی قارچ *P. nodorum* است.

کلمات کلیدی: فاکتورهای نسخه‌برداری، QPCR، الگوی بیان، محیط حداقل

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