FUNGAL GROWTH REGULATING GENE CLUSTERS HOMOLOGY WITH PLANT GROWTH HORMONE AND STRESS IN FUNGUS THEIR ADAPTATIONS UNDER AZOLE

Fatima Ismail
University college of Veterinary and Animal Sciences
The Islamia University of Bahawalpur, Pakistan 63100.
Email: fatima.ismail@ymail.com

***ABSTRACT*:** *Gibberellin is the plant hormone required for growth and development in plants and fungi. Growth hormone induce fungal growth in normal conditions. Whereas, fungal Gibberelline pathway responses in stress was not well known. This study has focused on azole adaptive role in Gibberelline (GA) biosynthesis gene cluster in Neurospora crassa. GA synthesis carried out by number of gene clusters such as CYP450, ggs2 and CPS/KS. GA biosynthesis gene cluster in Gibberella fujikuroi has been used as model. Study focused on three genes such as NCU05376 cytochrome p450 a gene of GA biosynthesis and two transcriptional factors NCU04158 and NCU09068 in Neurospora crassa. It was found out that the gibberelline a growth hormone gene cluster have three active genes in the fusarium graminearium GA pathway such as cytochrome P450, ggs2 and CPS/KS while in comparison with the Neurospora crassa P450 have homolog NCU5376, ggs2 have three homolog genes NCU01427, NCU02305 and NCU01175. CPS/KS has one NCU09272. The ketoconazole drug sensitivity test of the gene revealed similar phenotype except GATA transcription factor that shown azole sensitive phenotype as compare the wild type. The GATA transcriptional factor upregulated the transcriptional responses under stress. That predict the GATA transcriptional factor as a potent gene stress regulation gene in fungi and plants under stress conditions.*

1. INTRODUCTION

Gibberellin is the plant hormone required for growth and development of plants. Gibberelline (Gas) belong to the diterpenoids first isolated from the rice pathogene fungus where it was involved in super elongation of the rice shoots [1-2]. Later in 1956 Gibberelline was detected from higher plants which concluded (Gas) as plant hormone[4,5].The complex system of pathways comprised of four major enzymes GA1,GA3,GA4,GA7 whereas, the initial pathway starts from acetyl-Coa by mevalonic acid pathway and the fundamental isoprenoid unit unit isopentenyl diphosphate (IPP) to subsequent production of trans-geranylgeranyl diphosphate (GGDP) to GA12- aldehyde and then converted to tetra cyclic hydrocarbon ent-kaurene and later modified to GA12-aldehyde by sequential oxidation of C-19, C-7 and C6 while in plants ent-kaurene biosynthesis from GGDP require two diterpene cyclase enzyme(CPP Synthase CPS and ent-kaurene synthase KS) however, in fungi only one cyclase work for both above steps**.** After formation of GA12-aldehyde the pathway vary from plants to fungi as GA12-aldehyde is 3β –hydroxylated to GA14-aldehyde,which further oxidized on C-7 for the formation of GA14 [6,7].The consequent conversion of GA14 to GA4 by 20 oxidations steps is equivalent to biosynthesis of GA9and GA 20 in plants.

However the desaturation of GA4 C-1,2 effects to the formation of GA7 and converts to the key product in Fungus *F.fujikuroi* GA3 via 13 hydroxylation while,

GA1 formation is formed by 13-hydroxylationof GA4 [8]. According a study on F.fujikuroi the major difference of plants and fungi is at site of conversion of GA4 to GA1. According to studies genes involved in fungal secondary metabolites, GA genes formed of seven gene clusters [9]. First gene cluster DES catalyze the GA4 to GA 7 by the formation of double bond in C-1,2 and have oxoglutarate dependent dioxgenase and also reported as a widely available gene in different species[10].While four of these cluster comprised of enzymes characterized as cytochrome P450 monooxygenases (P450-1to P450-4) .Sixth cluster comprehends on pathways specific geranylgerenyl diphosphate synthase genes(ggs2) which is responsible for GGDP in pathway ,while cpk/ks is a single bifunctional encoding gene as ggs2.Studies in f.fujikuroi shows following gene clusters involved in GA biosynthesis. Deletion of ggs2 in *F.fujikuroi* shows the total loss of ggs2 activity which confirmed that one other gene can complement ggs2 in gene cluster while the cps/ks is also reported as having the close linkage of ggs2 also probably share the same promoter.

In fungi one terpene cyclase exists while all oxidative process are catalyzed by multi cytochrome P450. DES is the only gene catalyze the GA4 as a desaturase and this gene do not have high homologies in any other fungus other than F.fujikuroi [11]. Studies suggested meab gene transcriptional upregulation of GA biosynthesis in Fujikuroi. The transcription factors of transcription factors nmr (N metabolite regulator) is an alternative gene in *Neurospora crassa*. Another gene AreA a major N regulation gene in Saccharomyces cerevisiae studied as an affected gene in Ga biosynthesis. Deletion of mepB shown significant degradation in GA biosynthesis.

2. MATERIALS AND METHODS

Gibberelline synthesis pathway genes in *Fusarium graminearum* and its homologous genes in *Neurospora crassa* .

Homolog genes of the Gibberelline biosynthesis pathway were retrieved from the broad institute homolog gene Data bases in comparison with *fusarium graminearium* and the model fungi *Neurospora crassa* as mentioned in table 1.

2.2. Culture media

The gibberelline biosynthesis genes homologs in *Neurospora crassa* were obtained from the Fungal Genetics Stock Center, The University of Kansas. Vogel's minimum medium [12], with 2% (w/v) sucrose for slants and Petri plate inoculations and 2% glucose for the liquid culture medium was used. All the fungal cultures were incubated at 28°C with light. Antifungal drugs were added as needed.

2.3. Drug sensitivity test of the mutants corresponding to GA Bio synthesis by drug screening test.

Three of the most common antifungal azole drugs such as Ketoconazole, itraconazole and fluconazole were solubilized in dimethyl sulfoxide (DMSO) and added to the sterilized medium aseptically afore preparing agar plates. The azole antifungals (ketoconazole, concentration was 20mg/ml. The final concentration of dimethyl sulfoxide DMSO was less than 0.25% (v/v). The culture plates (D9 cm) were inoculated with 2µl of the conidial suspension in control and with antifungal drugs and kept at 28°C for 66 hours.

2.4. RNA EXTRACTION AND DIGITAL GENE EXPRESSION PROFILING ANALYSIS.

Gene transcriptional levels of the gibberelline pathway homolog genes were examined for adaptations under azole stress in the wild-type strain was carried out by digital gene expression (DGE) profiling (13). The wild-type strain was inoculated into 20ml liquid culture medium in a plate ((D9 cm) and kept in the incubator for 24 h at 28°C in darkness to get mycelial mass on the apparent side of the liquid medium. Mycelial mass was taken into small pieces (D10 mm) and inoculated in the liquid culture medium (2 pieces/100 ml). Liquid Cultures was incubated in the shaking incubator at 28°C at 180 rpm for 12 h. Ketoconazole was added in the culture medium with the 2.5 mg/ml final concentration. Mycelia mat was harvested after 24 hour of incubation. The total RNA was extracted and submitted for the DGE investigation as explained by (14).

Table.1. Neurospora crassa isolates used in the study.

Neurospora crassa deletion strains	*Neurospora crassa (wild type)*
NCU09068,NCU05376,NCU04158	*4600(Wild type)*

Gibberelline pathway genes in *Neurospora crassa.*

3. RESULTS

3.1. Gibberelline gene cluster Homologs in *Neurospora crassa.*

We occasionally found a gene related to cytochrome P450 belong to GA synthesis work as cytochrome p450 in gene cluster of GA biosynthesis and two transcriptional factors NCU04158 and NCU09068 in *Neurospora crassa*. We further find out the gibberelline gene cluster homologs in Neurospora crasaa and fusarium graminearium. It was found out that the gibberelline a growth hormone gene cluster have three active genes in the pathway such as cytochrome P450, ggs2 and CPS/KS while in comparison with the Neurospora crassa P450 have homolog NCU5376, ggs2 have three homolog genes NCU01427, NCU02305 and NCU01175. CPS/KS has one NCU09272 as mention in figure2.

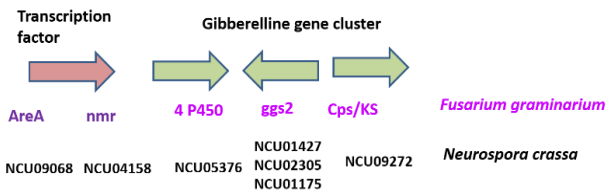

Fig. 2 Gibberellin gene cluster homolog in *Fusarium graminarium* and *Neurospora crassa* a model fungi

.

3.2. Drug sensitivity

Focusing GA synthesis we screened out the available GA homologous mutant with azole and found similar responses as compare to wild type in *Neurospora crassa* homologous mutants. However the transcription factor sensitivity results shown sensitiveness to azole in contrast to wild type. We determined the adaptation of the gibberelline a growth hormone in ketoconazole stress. On the base of transcription response to KTZ as compare to wild type.

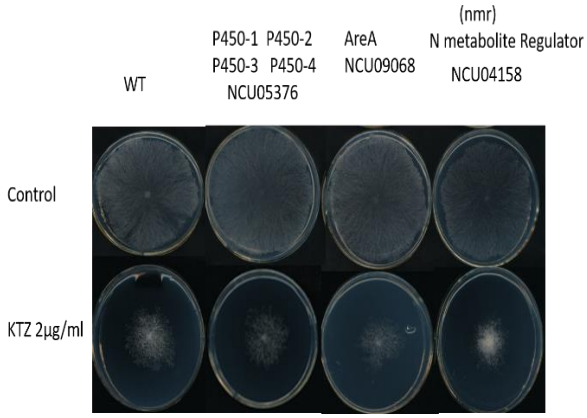

Fig 2. Drug sensitivity tests of the transcription factors NCU09068, NCU04158 and NCU05376 cyp450 homolog of Gibberelline biosynthesis pathway.

3.3. Transcriptional Regulations under azole stress

Ketoconazole stress response was further analyzed by RNA sequencing that resulted the in NCU09068 **0.56** fold reductions after azole stress in wild type. While the response of a cytochrome p450 NCU05376 (a potent gene of GA pathway) could not determine any significance in transcriptional regulations. However the GATA transcriptional factor NCU4158 resulted in 42.5 fold upregulated azole transcriptional response. Gene transcriptional responses were also repeated by RT-PCR for azole responsive transcriptional regulation that also find the same response such as NCU09068 and NCU05376 resulted in non-significant while NCU04158 showed increased levels of gene transcriptional levels in *Neurospora crassa* wild type as mentioned in figure 3.

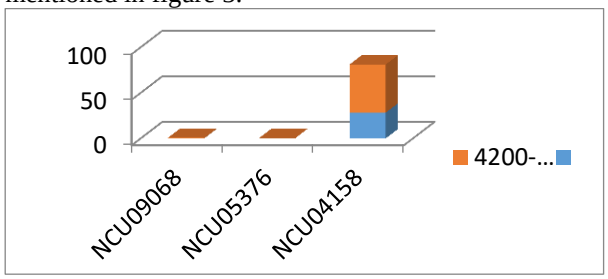

Fig. 3. Ketoconazole Transcriptional levels of transcription factors NCU09068, NCU04158 and NCU05376 cyp450 homolog of Gibberelline biosynthesis pathway.

4. DISCUSSIONS

Gibberelline is a plant growth hormone which have also been studied in various fungal groups. Study has focused on the azole adaptive role in Gibberelline biosynthesis gene cluster in fungi [4]. GA synthesis in fungi is carried out by number

of genes. However, study focused on three genes such as cytochrome p450 a gene of GA biosynthesis and two transcriptional factors NCU04158 and NCU09068 in *Neurospora crassa*. Gibberelline cluster gene has been acquired on fusarium fujikori homology [7]. Such as cytochrome P450, ggs2 and CPS/KS while in comparison with the *Neurospora crassa* P450 have homolog NCU5376, ggs2 have three homolog genes NCU01427, NCU02305 and NCU01175. CPS/KS has one NCU09272. In order to find the gibberelline growth hormone adaptability under ketoconazole stress. Drug sensitivity was carried on. The ketoconazole drug sensitivity test of three gene revealed similar phenotype except GATA transcription factor that shown sensitive phenotype as compare the wild type. The ketoconazole regulated transcriptional responses were also carried and found nonsignificant transcriptional responses in both cyp450 gene NCU05376 and Gibberelline transcriptional factor gene AREA NCU09068 in wild type. However, the GATA transcriptional factor NCU04158 upregulated the transcriptional responses under stress in *Neurospora crassa* wild type. Which means GATA transcriptional factor is involved in promoting growth in fungi under stress conditions.

5. CONCLUSION

By reviewing more and more diversified and specialized proteins for predicting more potent targets in manipulating the effective antifungal drugs. Study has focused on the azole adaptive role in Gibberelline biosynthesis gene cluster (A growth hormone in plant and fungi). GA synthesis carried out by number of genes but study focused on three genes such as cytochrome p450 a gene of GA biosynthesis and two transcriptional factors NCU04158 and NCU09068 in *Neurospora crassa*. We further find out the gibberelline gene cluster homologs in *Neurospora crasaa* and *fusarium graminearium.* It was found out that the gibberelline a growth hormone gene cluster have three active genes in the pathway such as cytochrome P450, ggs2 and CPS/KS while in comparison with the *Neurospora crassa* P450 have homolog NCU5376, ggs2 have three homolog genes NCU01427, NCU02305 and NCU01175. CPS/KS has one NCU09272. The ketoconazole drug sensitivity test of three gene revealed similar phenotype except GATA transcription factor shown sensitive phenotype as compare the wild type. Furthermore the ketoconazole regulated transcriptional responses were significantly upregulated that suggested that the GATA transcriptional factor upregulated the transcriptional responses under stress. Which means GATA transcriptional factor induce growth rate in fungi and plants under stress conditions. Hence, we suggest the overexpression of the GATA transcription in *Neurospora crassa* order to observe the effect of other physiological stresses in fungi.

REFERENCES

[1]. Kawaide, H. (2006). Biochemical and molecular analyses of gibberellin biosynthesis in fungi. Bioscience, biotechnology, and biochemistry, 70(3), 583-590.

[2]. Fuska, J., Kuhr, I., Podojil, M., & Ševčík, V. (1961). The influence of the nitrogen source on the production of gibberellic acid in submerse cultivation ofGibberella fujikuroi. Folia Microbiologica, 6(1), 18.

[3]. Tudzynski, B. (2005). Gibberellin biosynthesis in fungi: genes, enzymes, evolution, and impact on biotechnology. Applied Microbiology and Biotechnology, 66(6), 597-611.

[4]. Podust, L. M., J. Stojan, T. L. Poulos, and M. R. Waterman**.** 2001. Substrate recognition sites in 14α-sterol demethylase from comparative analysis of amino acid sequences and X-ray structure of *Mycobacterium tuberculosis* CYP51. J. Inorg. Biochem.87**:**227-235.

[5]. Radley, M. 1956. Occurences of substances similar to gibberellic acid inhigher plants. Nature 178: 1070–1071.

[6]. Kawaide, H. et al. (1997) ent-Kaurene synthase from the fungus Phaeosphaeriasp.L487 – cDNA isolation, characterization, and bacterial expression of a bifunctional diterpene cyclase in fungal gibberellin biosynthesis. J. Biol. Chem. 272, 21706–21712.

[7]. Hedden P, Phillips AL, Rojas MC, Carrera E, Tudzynski B (2001)Gibberellin biosynthesis in plants and fungi: a case of convergent evolution? J Plant Growth Regul 20:319–331.

[8]. MacMillan J (1997) Biosynthesis of the gibberellin plant hormones.Nat Prod Res 14:221–243.

[9]. Tudzynski B, Hölter K (1998) Gibberellin biosynthetic pathway in Gibberella fujikuroi: evidence for a gene cluster. Fungal Genet Biol 25:157–170.

[10]. Hedden P, Phillips AL, Rojas MC, Carrera E, Tudzynski B. (2001) Gibberellin biosynthesis in plants and fungi: a case of convergent evolution? J Plant Growth Regul 20: 319–331.

[11]. Tudzynski, B., Mihlan, M., Rojas, M.C., Linnemannstöns, P., Gaskin, P., Hedden,P., 2003. Characterization of the final two genes of the gibberellinbiosynthesis gene cluster of Gibberella fujikuroi: des and P450–3 encode GA4 desaturase and the 13-hydroxylase respectively. J. Biol. Chem. 278,28635–28643.

[12]. Vogel, H. J. (1956). A convenient growth medium for *Neurospora* (Medium N). *Microb. Genet. Bull.* 13, 42–43.

[13]. Nielsen, K. L., Høgh, A. L., and Emmersen, J. (2006). DeepSAGE – digital transcriptomics with high sensitivity, simple experimental protocol and multiplexing of samples. *Nucleic Acids Res.* 34, e133.

[14]. Sun, X., Zhang, H., Zhang, Z., Wang, Y., and Li, S. (2011). Involvement of a helix-loop-helix transcription factor CHC-1 in CO(2)-mediated conidiation suppression in *Neurospora crassa*. *Fungal Genet. Biol.* 48, 1077–1086