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In-silico analysis of cyanide hydratase protein in Bipolaris sorokiniana for pathogenesis management

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Abstract

Wheat, a popular crop globally, faces losses due to biotic stresses. One of the main diseases produced by *Bipolaris sorokiniana* is black point, root rot, crown rot, and spot blotch. These diseases can reduce wheat yields and are caused by soil- or seed-borne inoculum. This study aimed to identify and characterize the Cyanide Hydratase protein synthesized by *Bipolaris sorokiniana*, responsible for causing infectious diseases in wheat. Using BLAST and Clustal Omega programs, the study found over 85% similarity between the 11 cyanide hydratase proteins of *Bipolaris sorokiniana* and other organisms. The study suggests further analysis and validation to understand all virulence factors responsible for wheat disease, enabling effective control and management.

Keywords: *Bipolaris sorokiniana*, *in-silico* study, root rot, crown rot, spot blotch

Introduction

In 2020, world production of wheat is 781 million metric tons (FAOSTAT, 2022) [1]. China was the world's greatest producer of wheat with more than 137 million metric tonnes produced. With a production volume of more than 134 million metric tonnes, the European Union came in second. Wheat is the main cereal crop in the world. Wheat production is influenced by high yielding varieties, chemicals, fertilizers, mechanization, and energy inputs. The Consultative Group on International Agricultural Research (CGIAR) referred to wheat as the century's miracle crop, citing factors such as increased demand, lower prices, convenience, easy growth, availability of new varieties, and higher yields.

The second largest crop after maize is wheat, and in the years 2020–21, India produced 107.6 million tonnes, second only to China's 134.3 million tonnes (WDI, 2022) [2]. A mainstay in the north is wheat. State producers of wheat in India include Uttar Pradesh, Punjab, Madhya Pradesh, Haryana, and Rajasthan. In 2020–21, 35.50 million tonnes of wheat were produced in Uttar Pradesh from 9.85 million hectares of wheat land (Directorate of Economics & Statistics, 2022) [3]. Wheat is grown in Uttar Pradesh's western, eastern, and central areas. India's primary food and energy source is wheat. The second-largest producer of wheat in the world is India. From 6.60 to 107.6 million tonnes, wheat output has increased since independence (WDI, 2022) [2]. Despite having the greatest acreage (35.1%), Uttar Pradesh has the lowest productivity (2.7 tonnes/ha). As a result, it accounts for 35.03 percent of the country's total production. The west of the UP (3.29 million hectares), the east (5.24 million ha), and the centre (0.68 million ha) all cultivate wheat. New pathogenic races of wheat leaf rusts, stripe rust, and stem rust have been introduced into wheat growing areas worldwide (Roelfs, 1989) [4]. Biotic stress in agriculture refers to the impact of living organisms on a plant's body, such as fungi, bacteria, viruses, nematodes, insects, arachnids, and weeds. It differs from abiotic stresses, which include natural factors like sunlight, wind, temperature, heat, salinity, and drought. The types of biotic stresses depend on the climate and the species' resistance to stress. Researchers face challenges in controlling biotic stresses in experimental contexts, as plants lack an adaptive immune system. Stem rust (black rust), leaf rust (brown rust), and stripe rust (yellow rust), all caused by the rust pathogens *Puccinia graminis* f.sp. *Triticis* (Pgt), *Puccinia triticina* (Pt), and *Puccinia striiformis* f. sp. *tritici* (Pst), respectively, continue to endanger worldwide wheat production on a year-round basis (McIntosh *et al.*, 1995; Dean *et al.*, 2012; Hafeez *et al.*, 2021; Mapuranga *et al.*, 2022) [5, 6, 7, 8]. This number includes 20 'soil pathogens' relating to ME12, including foot rot fungi and nematode (Damla *et al.* 2010) [9].

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B. sorokiniana (Sacc.) Shoemaker, (syn. *Helminthosporium sativum* Pammel, King & Bakke, *H. sorokiniana* Sace in Sorokin, and *Drechslera sorokiniana* (Sacc.) Subramanian & Jain, causes diseases on a number of cereals, including wheat (Tunali *et al.*, 2008; Devi *et al.*, 2018; Gulyaeva *et al.*, 2018; Gupta *et al.*, 2018; Jamil *et al.*, 2018; Singh *et al.*, 2019; Villa-Rodriguez *et al.*, 2019; Li *et al.*, 2020)^[10, 11, 12, 13, 14, 15, 16, 17]. *C. sativus* was not reported in nature, except in Zambia (Raemaekers, 1991)^[18]. However, sexual reproduction of *C. sativus* has been rarely reported (Sultana *et al.*, 2018)^[19].

The genus *Bipolaris* has brown conidiophores, mostly simple, producing conidia through the apical pore. The conidia are brown, several-celled (Phragmospore), elliptical, straight, or curved, germinating by one germ tube at each end (Navathe *et al.*, 2020)^[20]. *B. sorokiniana* has olive-brown, ovate conidia, with tapered ends and a prominent basal scar. The conidia are 15 to 28 X 40 to 120 um and have 3 to 10 septa (Wiese, 1987)^[21].

B. sorokiniana attacks different cereals, including wheat, and causes common root rot, spot blotch, and black point diseases. Root rot is one of the most widespread diseases of wheat and it occurs in all areas where wheat is grown. Canada lost approx. *B. sorokiniana* attacks several host plants from different genera and families (Wiese) 1987: Farr *et al.*, 1989)^[21, 23]. The major plant hosts (listed by the genera name) that attacked by *B. sorokiniana* are. *Arthraxon*, *Avena*, *Bouteloua*, *Bromus*, *Buchloe*, *Calamagrostis*, *Calamovilfa*, *Cenchrus*, *Chloris*, *Cynodon*, *Dactylix*, *Dendrobium*, *Dichanthelium*, *Digitaria*, *Echinochloa*, *Elymus*, *Agrohordeum*, *Agropyron*, *Agrostis*, *Ammophila*, *Andropogon* etc. (Farr *et al.*, 1989)^[23].

B. sorokiniana do not have host specialization (forma speciales). However, isolates have been found to differ in their aggressiveness on wheat and barley (Al-Sadi, 2016)^[22]. Common root rot and crown rot of wheat are important diseases in most wheat-growing countries, including China, Australia, Middle East, and Europe. They are characterized by the development of necrotic lesions on the roots, Subcrown, and crown. The lesions are dark brown to black in color. The disease is caused by *B. sorokiniana* (Tunali *et al.*, 2008; Xu *et al.*, 2018; Yue *et al.*, 2018)^[10, 24, 25], which is also associated with other fungi including *Fusarium pseudograminearum*, *F. culmorum*, *Microdochium nivale*, *Pythium spp.*, and *Rhizoctonia cerealis*. Yield and quality of wheat could be reduced by common root rot and crown rot. Common root rot was reported to result in yield losses of 6% to 24% (Wildermuth *et al.*, 1992)^[26]. Yield reduction due to crown rot has been estimated to range from 0 to 89% in New South Wales, Australia (Klein *et al.*, 1991)^[27]. In Queensland (Australia), crown rot caused up to 26% yield loss in some fields, with an overall reduction b=y 5% for the whole state (Burgess *et al.*, 1981)^[28], while a reduction by up to 35% was reported in the Pacific Northwest, North America.

In-silico experiments are computer-based experiments performed on computer chips, using methods like databases, quantitative structure-activity relationships, pharmacophores,

homology models, machine learning, data mining, and network analysis tools. These simulations are used to predict compound reactions with proteins or pathogens, and are commonly used for drug candidate screening using computational bioinformatics tools and servers.

Nowadays, In-silico methodologies have become a crucial part of the drug discovery process. This is mostly because they can impact the entire drug development trajectory. Identifying and discovering new potential drugs with a significant reduction to cost and time (Broggi *et al.*, 2020)^[29]

Materials and Methods

Retrieval of protein FASTA sequence of *Bipolaris sorokiniana* from NCBI

The National Center for Biotechnology Information (NCBI) develops and maintains molecular and bibliographic databases as a part of the National Library of Medicine (NLM). Protein sequence of protein (Cyanide hydratase) in *Bipolaris sorokiniana* was searched through NCBI (<http://www.ncbi.nlm.gov/>)^[30].

Identification and structural characterization of Cyanide hydratase protein in *Bipolaris sorokiniana* from Uni. Prot. KB: The European Bioinformatics Institute (EMBL-EBI), the Swiss Institute of Bioinformatics (SIB), and the Protein Information Resource (PIR) have joined forces to create Uni Prot. <https://www.uniprot.org/>^[31]. More than 100 employees are employed by the three institutes to do a variety of duties, including database management, software development, and support.

Similarity search of Cyanide hydratase protein in *Bipolaris sorokiniana* using BLAST tool

The term "query" refers to a nucleotide or protein sequence, while "subject" refers to nucleotide or protein sequences found in a database. BLAST, which stands for Basic Local Alignment Search Tool, is a group of programmes used to create alignments between these two types of sequences. <https://blast.ncbi.nlm.nih.gov/>^[32]

Phylogenetic analysis of Cyanide hydratase protein in *Bipolaris sorokiniana*

The Clustal omega provides the tool for multiple sequence alignment to show the phylogenetic relationship between the sequences. <https://www.ebi.ac.uk/Tools/msa/clustalo/>^[33]

Results and Discussion

Retrieval of protein FASTA sequence of *Bipolaris sorokiniana* from NCBI.

Total 35723 proteins were appeared in query search from which only pathogenic proteins were screened out for more specificity. The record was downloaded, and the protein sequence was stored in FASTA format in text file. This FASTA format was further used for similarity searching and domain analysis.

Fig 1: All the proteins of *Bipolaris sorokiniana* were searched

Fig 2: The proteins were further screened to identify the pathogenic proteins.

Table 1: Identification of cyanide hydratase protein from UniProtKB

Entry	Reviewed	Entry Name	Protein names	Gene Names	Organism
G8DNT1	unreviewed	G8DNT1_C OCSA	Alpha-amino adipate reductase (EC 1.2.1.31) (EC 1.2.1.95) (L-amino adipate-semialdehyde dehydrogenase)	AAR	<i>Cochliobolus sativus</i> (Common root rot and spot blotch fungus) (<i>Bipolaris sorokiniana</i>)
A0A8H6DWL0	unreviewed	A0A8H6D WL0_COCS A	Cyanide hydratase (CHT) (EC 4.2.1.66) (Cyanide-degrading nitrilase) (Formamide hydrolyase)	GGP41_00395 3	<i>Cochliobolus sativus</i> (Common root rot and spot blotch fungus) (<i>Bipolaris sorokiniana</i>)
A0A6J4DI67	unreviewed	A0A6J4DI6 7_COCSA	Translation elongation factor 1-alpha	TEF1	<i>Cochliobolus sativus</i> (Common root rot and spot blotch fungus) (<i>Bipolaris sorokiniana</i>)
A0A6J4DIE8	unreviewed	A0A6J4DIE 8_COCSA	Translation elongation factor 1-alpha	TEF1	<i>Cochliobolus sativus</i> (Common root rot and spot blotch fungus) (<i>Bipolaris sorokiniana</i>)
A0A6J4DIW0	unreviewed	A0A6J4DI W0_COCS A	Translation elongation factor 1-alpha	TEF1	<i>Cochliobolus sativus</i> (Common root rot and spot blotch fungus) (<i>Bipolaris sorokiniana</i>)
G8DNS9	unreviewed	G8DNS9_C OCSA	Nonribosomal peptide synthetase 6	NPS6	<i>Cochliobolus sativus</i> (Common root rot and spot blotch fungus) (<i>Bipolaris sorokiniana</i>)
G8DNT0	unreviewed	G8DNT0_C OCSA	Polyketide synthase	PKS1 GGP41_00867 6	<i>Cochliobolus sativus</i> (Common root rot and spot blotch fungus) (<i>Bipolaris sorokiniana</i>)

A0A8H6DWL0 - A0A8H6DWL0_COCSA

Function: Catalyzes the hydration of cyanide to formamide. Degradation of cyanide may be important for plant pathogenic fungi in infection of cyanogenic plants.

Catalytic activity: formamide + H₂O → hydrogen cyanide

Chemical Reaction:

```

    formamide      H2O      hydrogen cyanide
    CHEBI:16397   CHEBI:15377  CHEBI:18407
    NH2 / \ O   H - O - N ≡ C H
    |           |   |           |
    H           H   H           N ≡ C H
  
```

Features: Showing features for active site.

Fig 3: Identification and structural characterization of Cyanide hydratase protein from UniProtKB

Names & Taxonomy:

- Protein names: Cyanide hydratase (EC:4.2.1.66)
- Gene names: ORF Names: GGP41_003953
- Organism: Cochliobolus sativus (Common root rot and spot blotch fungus) (Bipolaris sorokiniana)
- Taxonomic identifier: 45130 [NCBI]
- Taxonomic lineage: Eukaryota > Fungi > Dikarya > Ascomycota > Pezizomycotina > Dothideomycetes > Pleosporomycetidae > Pleosporales > Pleosporineae > Pleosporaceae > Bipolaris
- Proteomes: UP000624244 Component: Unassembled WGS sequence

Expression:

- Induction: By cyanide.

Interaction:

- Subunit structure: Oligomer of dimers, forming left-handed helical fibers.

Fig 4: Taxonomic classification expression and Interaction of cyanide hydratase protein

FASTA sequence of Cyanide hydratase protein *Bipolaris sorokiniana* through UniProt

The FASTA sequence or accession no of cyanide hydratase protein was entered in search box of blastp and click on run BLAST. The sequences that produced significant alignment were appeared.

>tr|A0A8H6DWL0|A0A8H6DWL0_COCSA Cyanide hydratase OS-*Cochliobolus sativus* OX=45130
GN=GGP41_003953 PE=2 SV=1
MPLTKYKAAAVTSEPCWFDLEAGVQKTINFINEAGQA
GCK

LVAFPEVWIPGYPYWMWKVNYQQSLPLLKKYRENSLP
IDSEEFRRIRRAARDNQIYVSLGFSEIDHATLYLTQTLID
PMGEVINHRRKIKPTHVEKLVYGDGAGDTFKSVTQTE
LGRLGQLNCWENMNPFLKALNVSEGEQIHIAAWPVYP
GKETLKYPDPATNVSDPASDLVTPAYAIETGTWTLAPF
QRLSVEGLKNN
TPEGVEPETDPTTYNGHARIYKPDGTLVSKPDPDFDGL
LFVDVDLNECHLTKALADFSGHYMRPDLIRLLVDTRR
KELITEADPQGGISTYSTRDRGLNTPLDGGVKKQKVV
AAEAESTNSSAL

The screenshot shows the National Library of Medicine BLAST result page. At the top, it displays the search parameters: Job Title (tr|A0A8H6DWL0|A0A8H6DWL0_COCSA Cyanide hydratase), RID (HH0DEF01016), Program (BLASTP), Database (refseq_protein), Query ID (IclQuery_58724), Description (tr|A0A8H6DWL0|A0A8H6DWL0_COCSA Cyanide hydratase ...), Molecule type (amino acid), Query Length (365), and Other reports (Distance tree of results, Multiple alignment, MSA viewer). A 'Filter Results' section includes fields for Organism, Percent Identity, E value, and Query Coverage, along with 'Filter' and 'Reset' buttons. Below this is a table titled 'Sequences producing significant alignments' with columns for Description, Scientific Name, Max Score, Total Score, Query Cover, E value, Percent Ident, Acc. Len, and Accession. The table lists four entries, each with a checked checkbox in the 'select all' column.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Percent Ident	Acc. Len	Accession
hypothetical protein COCCADRAFT_7345 [Bipolaris zeicola 26-R-13]	Bipolaris zeicola 26-R-13	745	745	100%	0.0	97.81%	366	XP_007715048.1
hypothetical protein COCC4DRAFT_29076 [Bipolaris maydis ATCC 48331]	Bipolaris maydis ATCC 48331	744	744	100%	0.0	98.09%	366	XP_014072527.1
hypothetical protein COCMIDRAFF_37591 [Bipolaris oryzae ATCC 44560]	Bipolaris oryzae ATCC 44560	738	738	100%	0.0	96.72%	366	XP_007688885.1
uncharacterized protein SETTUDRAFT_165747 [Exserohilum turcica Et28A]	Exserohilum turcica Et28A	714	714	100%	0.0	92.88%	365	XP_008030580.1

Fig 5: BLAST result page of Cyanide hydratase protein

Similarity search of Cyanide hydratase protein

Table 2: BLAST search of all Cyanide hydratase proteins in different organisms

Description	Scientific Name	Max score	Total score	Query cover	E value	Percent Identity	Amino acid length	Accession no.
hypothetical protein GGP41_003953	<i>Bipolaris sorokiniana</i>	723	723	100%	0	100	365	KAF5851146.I
hypothetical protein COCCADRAFF_7345 [<i>Bipolaris zeicola</i> 26-R- 13]	<i>Bipolaris zeicola</i> 26-R- 13	709	709	100%	0	97.81	366	XP_007715048.1
hypothetical protein COCMIDRAFF_37591 [<i>Bipolaris oryzae</i> ATCC 44560]	<i>Bipolaris oryzae</i> ATCC 44560	703	703	100%	0	96.72	366	XP_007688885.1
hypothetical protein COCC4DRAFF_29076 [<i>Bipolaris maydis</i> ATCC 48331]	<i>Bipolaris maydis</i> ATCC 48331	692	692	96%	0	98.31	366	XP_014072527.1
uncharacterized protein SETTUDRAFT_16 5747 [<i>Exserohilum turicum</i> Et28A]	<i>Exserohilum turicum</i> Et28A	682	682	100%	0	92.88	365	XP_008030580.1
cyanide hydratase [<i>Stemphylium lycopersici</i>]	<i>Stemphylium lycopersici</i>	680	680	100%	0	91.23	365	KNG51232.1
hypothetical protein B5807 00072	<i>Epicoccum nigrum</i>	667	667	97%	0	92.44	375	OSS54602.1
hypothetical protein G6514_001829 [<i>Epicoccum nigrum</i>]	<i>Epicoccum nigrum</i>	666	666	97%	0	92.44	375	KAG9197345.I
hypothetical protein E8El2_011015 [<i>Didymella heteroderae</i>]	<i>Didymella heteroderae</i>	664	664	98%	0	91.09	368	KAF3044472. L
Cyanide hydratase [<i>Macroventuria anomochaeta</i>]	<i>Macroventuria anomochaeta</i>	663	663	100%	0	89.92	368	XP_033555310.1
uncharacterized protein EK005_004924 [<i>Ascochyta rabiei</i>]	<i>Ascochyta rabiei</i>	662	662	96%	0	92.88	367	XP_038799119. 1

Result of Similarity search using BLAST tool

The BLAST results showed the scores between every query

and subject, gaps and identities which are the basis of similarity search in BLAST tool.

Range 1: 1 to 365		GenPept	Graphics	▼ Next Match	▲ Previous Match
Score	Expect	Method	Identities	Positives	Gaps
759 bits(1959)	0.0	Compositional matrix adjust.	365/365(100%)	365/365(100%)	0/365(0%)
Query 1	MPLTKYKAAAVTSEPCWFDFLEAGVQKTINFINEAGQAGCKLVAFPEWIPGYPYWMWKVN				60
Sbjct 1	MPLTKYKAAAVTSEPCWFDFLEAGVQKTINFINEAGQAGCKLVAFPEWIPGYPYWMWKVN				60
Query 61	YQQSLPLLKKYRENSLPIDSEEFFRRIRRAARDNQIYVSLGFSEIDHATLYLTQTLIDPMG				120
Sbjct 61	YQQSLPLLKKYRENSLPIDSEEFFRRIRRAARDNQIYVSLGFSEIDHATLYLTQTLIDPMG				120
Query 121	EVINHRRRKIKPHTHEVKLVYGDGAGDTFKSVTQTELGRGLQQLNCWENMNPFKLALNVSEGE				180
Sbjct 121	EVINHRRRKIKPHTHEVKLVYGDGAGDTFKSVTQTELGRGLQQLNCWENMNPFKLALNVSEGE				180
Query 181	QIHIAAWPVYPGKETLKYPDPATNVSDPASDLVTPAYAIETGTWTLPQRQLSVEGLKKN				240
Sbjct 181	QIHIAAWPVYPGKETLKYPDPATNVSDPASDLVTPAYAIETGTWTLPQRQLSVEGLKKN				240
Query 241	TPEGVEPETDPTTYNGHARIYKPDGTLVSKPDPDFDGLLFVDVDLNECHLTAKALADFSGH				300
Sbjct 241	TPEGVEPETDPTTYNGHARIYKPDGTLVSKPDPDFDGLLFVDVDLNECHLTAKALADFSGH				300
Query 301	YMRPDLIRLLVDTRRKELITEADPQGGISTYSTRDRRLGLNTPLDGGVKKQKVVAEEAEST				360
Sbjct 301	YMRPDLIRLLVDTRRKELITEADPQGGISTYSTRDRRLGLNTPLDGGVKKQKVVAEEAEST				360
Query 361	NSSAL 365				
Sbjct 361	NSSAL 365				

Fig 6: The alignment result of Cyanide hydratase protein of *Bipolaris sorokiniana* with proteins of other organisms

Multiple Sequence Alignment using Clustal Omega

Multiple sequence alignment was done using Clustal omega by matching the FASTA sequence of cyanide hydratase

protein with multiple sequences to show phylogenetic similarity among the sequences.

CLUSTAL O(1.2.4) multiple sequence alignment	
XP_033555310.1:2-368	MPLTKYKAAAVTSEPCWFDFLEAGVQKTIIFAINAGEAGCKLIAFPPEWIPGYPYWMWKVN
XP_008030580.1:1-365	MPLTKYKAAAVTSEPCWFDFLEAGVQKTIINFIKEAGEAGCKLIAFPPEWIPGYPYWMWKVN
XP_007688885.1:1-366	MPLTKYKAAAVTSEPCWFDFLEAGVQKTIINFIKEAGQAGCKLIAFPPEWIPGYPYWMWKVN
XP_007715048.1:1-366	MPLTKYKAAAVTSEPCWFDFLEAGVQKTIINFIKEAGQAGCKLIAFPPEWIPGYPYWMWKVN
XP_014072527.1:1-366	MPLTKYKAAAVTSEPCWFDFLEAGVQKTIINFIKEAGQAGCKLIAFPPEWIPGYPYWMWKVN
XP_033555310.1:2-368	YQOSLPMLKKYRENSLPIDI SEEFFRKIRRAARDDNQIYVSLGFSEIDHATLYLTQLIDPTG
XP_008030580.1:1-365	YQOSLPMLKKYRENSLPIDI SEEFFRKIRRAARDDNQIYVSLGFSEIDHATLYLTQLIDPTG
XP_007688885.1:1-366	YQOSLPMLKKYRENSLPIDI SEEFFRKIRRAARDDNQIYVSLGFSEIDHATLYLTQLIDPTG
XP_007715048.1:1-366	YQOSLPMLKKYRENSLPIDI SEEFFRKIRRAARDDNQIYVSLGFSEIDHATLYLTQLIDPTG
XP_014072527.1:1-366	YQOSLPMLKKYRENSLPIDI SEEFFRKIRRAARDDNQIYVSLGFSEIDHATLYLTQLIDPTG
XP_033555310.1:2-368	EVINHRRRIKPKTHIVEKLVLYVGDDGSQDTFKS VTOTELGRLLGOLQNLNCWEENRNPFLKSLNVS EGE
XP_008030580.1:1-365	EVINHRRRIKPKTHIVEKLVLYVGDDGSQDTFKS VTOTELGRLLGOLQNLNCWEENRNPFLKSLNVS EGE
XP_007688885.1:1-366	EVINHRRRIKPKTHIVEKLVLYVGDDGSQDTFKS VTOTELGRLLGOLQNLNCWEENRNPFLKSLNVS EGE
XP_007715048.1:1-366	EVINHRRRIKPKTHIVEKLVLYVGDDGSQDTFKS VTOTELGRLLGOLQNLNCWEENRNPFLKSLNVS EGE
XP_014072527.1:1-366	EVINHRRRIKPKTHIVEKLVLYVGDDGSQDTFKS VTOTELGRLLGOLQNLNCWEENRNPFLKSLNVS EGE
XP_033555310.1:2-368	QIHIAAWPVYPGKE TLKYDPATNVADPASD LVTPAYAIEETGTWTLAPFQRMSKEGLRKT
XP_008030580.1:1-365	QIHIAAWPVYPGKE TLKYDPATNVADPASD LVTPAYAIEETGTWTLAPFQRMSKEGLRKT
XP_007688885.1:1-366	QIHIAAWPVYPGKE TLKYDPATNVADPASD LVTPAYAIEETGTWTLAPFQRMSKEGLRKT
XP_007715048.1:1-366	QIHIAAWPVYPGKE TLKYDPATNVADPASD LVTPAYAIEETGTWTLAPFQRMSKEGLRKT
XP_014072527.1:1-366	QIHIAAWPVYPGKE TLKYDPATNVADPASD LVTPAYAIEETGTWTLAPFQRMSKEGLRKT
XP_033555310.1:2-368	TPEGVEPETDPT STVNGHARIVKPDGTLVAKPKDKDFG LLLFDVIDLNECHLT KALADFGSH
XP_008030580.1:1-365	TPEGVEPETDPT STVNGHARIVKPDGTLVAKPKDKDFG LLLFDVIDLNECHLT KALADFGSH
XP_007688885.1:1-366	TPEGVEPETDPT TTVNGHARIVKPDGTLVSKP DKDFG LLLFDVIDLNECHLT KALADFGSH
XP_007715048.1:1-366	TPEGVEPETDPT TTVNGHARIVKPDGTLVSKP DKDFG LLLFDVIDLNECHLT KALADFGSH
XP_014072527.1:1-366	TPEGVEPETDPT TTVNGHARIVKPDGTLVSKP DKDFG LLLFDVIDLNECHLT KALADFGSH
XP_033555310.1:2-368	YMRPDLIRLLV DTRRKELVTEADP QGGIITTY STRERLGLN VPLDA CKKGOK EDVGAAGLK
XP_008030580.1:1-365	YMRPDLIRLLV DTRRKELITEADP PHGGIQAY STRERLGLN PDLGDGHV QKRGVA ADEST
XP_007688885.1:1-366	YMRPDLIRLLV DTRRKELITEADP QGGIITTY STRERLGLN PDLGDGGK HKVKKV VAAA AEAS
XP_007715048.1:1-366	YMRPDLIRLLV DTRRKELITEADP QGGIITTY STRERLGLN PDLGDGV VKKKV VAAA AEAS
XP_014072527.1:1-366	YMRPDLIRLLV DTRRKELITEADP QGGIITTY STRERLGLN PDLGDGV VKKKV VAAA AEAS
XP_033555310.1:2-368	VESSKAL 367
XP_008030580.1:1-365	SATAI-- 365
XP_007688885.1:1-366	ANASA-L 366
XP_007715048.1:1-366	ANASA-L 366
XP_014072527.1:1-366	ANASA-L 366

Fig 7: Multiple sequence alignment of Cyanide hydratase protein in *Bipolaris sorokiniana* using clustal omega

Phylogenetic analysis of Cyanide hydratase protein in *Bipolaris sorokiniana* using Clustal W

The phylogenetic tree was obtained by using Clustal omega tool in which the sequences of Cyanide hydratase protein of different organisms was uploaded in FASTA format and then

submitted to search the multiple sequence alignment. After that the result page shows the various options, among them the option of phylogenetic tree was chosen to obtain the cladogram. The phylogenetic tree showing the similarity between multiple sequences of similar Cyanide hydratase

proteins among different organisms. The branches shown in this tree is showing the phylogenetic similarity between the sequences. The short branch length is showing high phylogenetic similarity whereas longer branch length is showing less similarity.



Fig 8: Phylogenetic tree of Cyanide hydratase protein in *Bipolaris sorokiniana*

Conclusion

Bipolaris sorokiniana is a serious pathogen that can cause significant yield losses and attack most wheat organs. Management strategies should focus on limiting the presence of the fungus in aerial parts of plants and the *B. sorokiniana* inoculum present in soil. The search for biocontrol agents should include antagonistic strains that complement cultural and chemical practices. A study using computational pipelines and analytical software screened 24 pathogenic proteins in *B. sorokiniana*, revealing that they contain only alpha helices in folded manner and convert cyanide into formamide, a virulence factor. The BLAST program showed over 85% similarity between 11 cyanide hydratase proteins of *B. sorokiniana* and other organisms. The study should be further analyzed and validated to understand all virulence factors responsible for wheat disease, enabling effective control and management.

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Annexure

Table 1: FASTA sequence of 24 pathogenic proteins related of *Bipolaris sorokiniana*

S. No.	Accesion No.	Protein name	Amino acid length	FASTA Sequence
1.	QIS9292 9.1	CcqM [<i>Bipolaris sorokiniana</i>]	901	MGKLHCAIAALVLGAPVAISTSVHARLPHTAIVQRAVNDTPVASGILPGSYIVEFANDS ETPESFYASLAAIGVQEPRKDLFSRFFNGVSFQLKNLTSSPSSDSRDGDLMHQLKGLP QVENIWPIRITAHSREKSVAAPQQRVKRQAESQDTFSTHVMTQVDKLHAEVTGK GFLIAIVDGVYDTHPALGGCFPGCLVEVGYDFTGDNYTPTGTNEPKPDDPMDDCV GHGTHVAGTIAAQLKGNTYGFSGAPGVRLGAYRMWGCTATSIDEIEVLAFAVED GANIISYSNGDASGWAQDVRAVIISRIVDGSIPVVVSAGNSGGLGIFYGSTPATGFSATG TGAVSNTKFTPFLERGSYKTSANATANSTREFGFLRGVPFAAGATPLWSAANADDA CKPLPDTPDLSQRIVLLEFKDPRATQCYPQDQGANIAAKGGRFMAYYERTNLTMRD DPYLYADGIEGVIRVPSYAAEDWLSLISQGATVSVTIPSNGSQTHLEELNNEDGGYVS DTLTSWGPTELWESMNPEVVAPGENILSTFPTAMGSYRVMGTGMSAPlVAGVYALLG EVYGKLEPKRLRRLLMHTSKPLAWYHDKTIDPDILAPVPQQGAGLVQAWSAARTTLEI NIDSITLNDTEHFVGTHFTFSVINTGTADEVLELSRKAVTISTIDPKNFLFSPGLISNSIVN AWATVSFSNRITVPAGQSVNVTVDTPPGGVNATLLPVYSGFIAIGKQLNLPYLGVVG SMRSVTVLSSRAAYLAQGYGEAPANASYTIPRDPQNPPWTDRGDIYNMPNVYMYP VGSRLRVLDVLQGNKTLGPLAGFPLTYIPRGEVRAYFNGLMADGRVLEEGTYRMRVM ALHIFGNEDNEGDWDTVETSSFTFKYTA
2.	QIS9292 9.1	CcqM [<i>Bipolaris sorokiniana</i>]	161	MGKLHCAIAALVLGAPVAISTSVHARLPHTAIVQRAVNDTPVASGILPGSYIVEFANDS ETPESFYASLAAIGVQEPRKDLFSRFFNGVSFQLKNLTSSPSSDSRDGDLMHQLKGLP QVENIWPIRITAHSREKSVAAPQQRVKRQAESQDTFSTHVMTQVDKLHAEVTGK GFLIAIVDGVYDTHPALGGCFPGCLVEVGYDFTGDNYTPTGTNEPKPDDPMDDCV GHGTHVAGTIAAQLKGNTYGFSGAPGVRLGAYRMWGCTATSIDEIEVLAFAVED GANIISYSNGDASGWAQDVRAVIISRIVDGSIPVVVSAGNSGGLGIFYGSTPATGFSATG TGAVSNTKFTPFLERGSYKTSANATANSTREFGFLRGVPFAAGATPLWSAANADDA CKPLPDTPDLSQRIVLLEFKDPRATQCYPQDQGANIAAKGGRFMAYYERTNLTMRD DPYLYADGIEGVIRVPSYAAEDWLSLISQGATVSVTIPSNGSQTHLEELNNEDGGYVS DTLTSWGPTELWESMNPEVVAPGENILSTFPTAMGSYRVMGTGMSAPlVAGVYALLG EVYGKLEPKRLRRLLMHTSKPLAWYHDKTIDPDILAPVPQQGAGLVQAWSAARTTLEI NIDSITLNDTEHFVGTHFTFSVINTGTADEVLELSRKAVTISTIDPKNFLFSPGLISNSIVN AWATVSFSNRITVPAGQSVNVTVDTPPGGVNATLLPVYSGFIAIGKQLNLPYLGVVG SMRSVTVLSSRAAYLAQGYGEAPANASYTIPRDPQNPPWTDRGDIYNMPNVYMYP VGSRLRVLDVLQGNKTLGPLAGFPLTYIPRGEVRAYFNGLMADGRVLEEGTYRMRVM ALHIFGNEDNEGDWDTVETSSFTFKYTA
3.	QIS9292 7.1	Ccqk [<i>Bipolaris sorokiniana</i>]	732	MLELYGFYDLLEKHPDVAPKLGAGVGITPNGARILDQIGVWDSMCEYASPVNSGVA LSPKGQTIVFNPHMGEWLEKLFYKIHFLSRHDCLRLIFDKIKQKSNIHLQKEVTRISVG QPGERARVETKDGSIYTADLVIGADGVRSGVRSELWRHADTEKPGYIPKQDKTGIVSL YTAVIGISHDGPLPRGGNARAYNHRLSYFFSEGMERSGEFYWWLCVKNEEPIKGIVPK LSSDTKQALLDKYADDNIGHGLTGGLYKKSTYSTIPLQEFVLEKCFYKNILLIGDAFR KLHPVAGQGANSAVEESAMVADMLWKLRANDALHDTLRVNQALTEFQKERFVRTTA LREDANLVQRMESFDNPVMKFMALHVIPRLPFVVAFLPQLAVSFTPACRMHELPPPKA GMCPFSPDMQAKPNPRSPLATISWIGFLILAACFPLSAHRLLPASSSSILFQSQVQLYIC MMAVSIISGLWVVESYKASLLVSPMSSSLPWILASNYWGWEKTLPVYLCFHVISSQHA VHYYTPQFMTDLGAAKALLPCLAMVYVSIPLTALGSTDKSLFDWWWPVAQYTFPVLVY VSSRILRGIIKA VPQGVVEVFSSIDVPYQRRFFTAIAVVSSVVHVIWGHGAALFNEGIIS

				LLSSPLARAIASLTSLTVAWCAMYMTWELQRISATEVPLAMTWAVILMKTVLLGPAASL AGITCWSKIELEKATSQPLVQEIKEKSYA
4.	QIS9292 6.1	CcqJ [<i>Bipolaris sorokiniana</i>]	246	MPSWAITGASRGIGSADVGNCVQFALIRRQPSAELSKLDSERENLHIKADVTDAQSLSE AAAKVGELTENKLDVFISNACHPGGLDLRFYPTSAFLGKEKEKLKNEIDAPMSVNLAGIS SINCFLPLIRNGELKKIYITSPTGDAEFTRKCGVTVTIGYTATKAAMNLVMSKYAAEKL GEGIKTLALSPGVWVDTDGSTQFPAHLSIDDSVNAHKPHAMYSSGYGPDARGLRNGA TDVSEGEPGSH
5.	QIS9292 8.1	CcqL [<i>Bipolaris sorokiniana</i>]	440	MFFLALLRISDAAQFKSDTSWIVNPACFYFPDRDSTRFTNTWKCDQQAKYPYKEFHQ IWHYAEMLAKSFTTIMADLGQTTLNVLNDNGDLLQYFTANLADFNKLFSDPRFETILR DVAKKPYTAASASQWDLEITPSVYPPHFIVRSLLRALAALYFKF
6.	QIS9292 4.1	CcqH [<i>Bipolaris sorokiniana</i>]	1109	MATETRSQDGWCRLRKKCDEERRPCTECMCLGLDCHGFGTRPAWMMDRGARQKA QAAMKMEKLAQLTKSRRHKQVRTQNDWVAKQPPNSHTERSTIRIDAPDASSGMLMD HEPSSPTQTFGDTSQDEFARSRLAEALEEEDQNTIVSDLALASSDSGSQRIFDDMTFLSG YPVTLAPSRNANFQTSLEGMDFLSEQNFDLPELESLTGSLHMAQGTTAGLGVADDLCE AYLSPPESQISTAYPSYGTSAFILPIGDMEDATLLAYCIEKVFNWQFPFCSTLLSGFSQGY FLWLMSSRPLYLASLALSSSHKSRSQKAVEECSKLRYEDHAGRYNVATEEFHRNLRT PKVADDISMLACTVLLISSSLLQGGKVDWTSHRLRTGTSLIAPWIAQAQHDTNSASPSSL EESSRDFIISSIIRVDILSAINQDSAPGLSHNYKEWFSTRQQFSLAEAVCGCSNWFIVEVLLD VYLLRDWKKKTRAEGLLSLWEUTSKANTIQVDLEKKMTSNMILLRKSKENVEQQQQQ DKDRPLQSGAQGQYDICVVNHTFACAVSVLLEVIVSGAYPRLPEVKQAGRALDALA DIKDDARLLEVLGWPLFVVGVVVEERHGFFRQLLSCQLRNSVTLCGLLDVLEECWKS RASGELEYTWCRNCCSNRVCERGIRLNFDIQTSTPHTGLIDLPPGTQLKFHDESRAIA SGYAVGPQKDKNIETSHTEPTLQTGLPQTLPKYAIPPMEMTSCAEQALVPAQCSSL PSLRVITNHDEGLLMEIFLSKAVPMDCLVASKPFTNITPFYALSQPALYSVMACGE RYLMPTEESLYYEKACQGLEIAKPNADLLCVTISALLHAYETMSDGANKKGSD RTRALIGEAGLSGGNSSLFGACFWAYKLNVSLLDSLVRNSRLTCAERMDMALNAVQS TGTNNGILYCEIDLWAYRMVSVCALKVAALHTEVAQGIVGDSMDRLRQDREQYKGW CDEWASNVPRSMMPLCYIPPPDEDSKSETTHFPQVLFGSSATVARLLYHVSCLLLVR IGVAERSAEAQEACRQIRHAMDICGIASQGEDKYGTLFLDEKAYELFFSLFFCFSWIET R
7.	QIS9292 3.1	CcqG [<i>Bipolaris sorokiniana</i>]	306	MAPKVDTNNHDLVRGVWRLFRLHTIEGLSTVSIGWLALFFYAMQQDLPFALVRRALF GIFATYQITHCVFCLWNIDCERDFDAKVARTRDRPLPSQVTLTEAVIVIFIGLAAAAVA LTYKILGSDVTAIMVPIWGLSTIYPLCKRVIWAPQVVLGLTMAMCVLPPWMAVRPHSG DAGLLPASLFGAIFCWLVYIDLIYASQDRPDDKKAGVKSLAIFLDYKAGLTVLGVA QIVCFVVAEEAQAGVFVWIFGIAVWSASVPWSIISLDLDRKSGGRIFLMNAILGIYM AAVSGINVASPAIWS
8.	QIS9292 2.1	CcqF [<i>Bipolaris sorokiniana</i>]	1944	MDIAAQHVFLFGDQADAPLPMIRRAEKARHSKLNQFLQSAIDNVQLEVSRLTPAER DTIGPFHSLQGLTNALKEKSDRHGLQAMQMSVFIARIGELILHAENDPALLSSTPLLSLG CGGLLPAAAAVAVATNIHELIEVASYLARVNCRAVAISRRSLGIESGTGSWAFLVKGKD IVAQLPDILKQFHREQSIPRHHRAWIAVSTPTWATVFGPPSVLRLRETSAPLRKSDTSE LPAFGAVHAHLAAPEFDDLVDESPILLTRPLKTGYRLLSGSRYAPYDASTLKDLLPQI MLDIFQNETDPSRVFEVAGSYLRKGASPSLYMLGATSYLVLLRSLHTQGFVVDLKTN PPSLQTAELRGGSJVAVVGMMSGQFPGAASVDEMWDVLMRREELHRKIPTERFNADD YLDETGRGLNAITTAYGCFLGSPGLFDHKMFNVSPREAMQMMPGQRLLMHGVTALE DAGLVGGTASADNRRISTYIGDGSSDWRELQQQHGVDKYILQGTQRSFTPGRNLHHF KWEGATFLVDSACGSTASAVERLAYRALINRDCDTAVAGGANIATPFWQSALSKGGFL STTGGCKTFRSDADGYCRGEVGVVVLKLEDALAENDNIISVIRGYARNHSADTVSI TRPHVPAQERAYQAVLHSSGLEPDDISYVEMHGTGTTAGDAELESIVNLAQKSTRA TPLVVGAIKANLGHSEAAGISSLIKASFMRKGVVPPQVGIPKEKMGVFDCLDRGSVLIP GEPVSFRQSVGKTRTMIVNNFDAFLTENNQTNLADLSYTTARRMHSLRSAYTGD SIEIINGLNRDLGTTNNRGDDKTGEPRLAFAFTGNGAHYAGMGADLFKLWQPFKTT SLEKSCMSHGFPFAHVISDPATAMENISTAQVHLSMIALEIALVDVWKLLGISPDLVM GHSIGEYAALYAAAGVLSSTDAMYLVGTRAMLLQDNLEEGAHGMLSISGTQQNIANIVS DESVMVDCEIACHNSPGMVVLGGPRQLAEIEEQLLSASKCKCKLLNVPYAMHSSQL DSILPGIREAARGVCFGTPKIKVISTLTGTEQQHFDGNYLARQTRAEVKFTQAIHCVSQ GLVDSTTLWEIGPAPVCLGLVRSNTSVASHRAMTSMTKEEGGWKSVSSALACLYVA GKTVGWREYHSDFIDSLSLISLPSYSFDRNFWMPTAGSKHEDVQLISSCLHHLVKQE DDGKEQSATFTAMVSQPSLRLMIQGHKLSGITVCPAGVFAEMALTAARYVHTGGSMK TQFPLFSVLDLQIDHPKPKPQPDQSPVIQNVNSKPRQSGDFAVSIMDQAKPSLITSKCCVR ERDEQDFDMTRQQRLGVMLPKISKLMQDAAVGLANRFQGKLFYRLFANLMDYAGQY EGVQEAVNNDFTEALATIRLPKGQDASESCTLSPYWIADLTHLAGFLLNGNPMSSGDD VYIGTHMERMEIVAKDFSPDIVYQSYAYIEHTEGSDDYRGHVYILNGDGVGFLEGARF RKMPRTMLHRLGKVEPAATTSSRSITAEPVNNTSGNNGTNGINGHHKTIESVLVER LMEETGMDESELQPSTFFAEIGVDSLMLCSILADIKAETGVELNASFLLEYPTLGAQR QLRTMEGKRKRERADSATANGTAVVVLNGQNGLSTTKRECNVVLMQAQGHTSSPP LFLMADGAGSAAAAMHLPKLLDVVDGVYAVESPWVRDPAEFTCSFEEAAALYLA RSKQPRGPYLLGGWSGGGVFAYEVARRLLNAGERVGLVVVIDPAPSLQLPSSGVVAE PTMEIIMGMIGLMSIERNFANKAESAESPEAARLKHHMLGTVTCFSRLVPIPMPQPHVRPEHT FVIWATKDVLPKAAFDELPPGLDAWFYPKPRDMGPNGWDALIGDAVEYCQVQGDHF SIMTAPEVLELGQVIQASLEKCKV

9.	QIS9292 1.1	CcqE [<i>Bipolaris sorokiniana</i>]	3173	MAIKRSGSSPSPIAVGIGLRLPGGCHDTKSYWDLNVNQKDARRSIPPERFNIDGFHGKA QGASNLSMRHGYFLDEPVDRFDAAFFSMSQAEVARVDPQQRLLEVMHEALENAGE VNWRGSDIAVYAGSGFQDWLQMQRDMQEGRVYDITGMDDFVFANRVSYEFDLHG PSMTVKAGCSSLALHLACEALNQGDCSGALVGASNLLSPEYFLALDNLGALSPDG SRAFDANANGYARADA VNAIYVKRLDDALRDGNPVRAIRSTA VNADGKTVGLTPS TDAQASLIRRAYERKAGISNP EATPMV ECHGTATGDPLEVA AVVQTFGHQQQTYIGS VKPNIGHGEAAGLSSLIKS VLSLERHTIPPNIKFQT PNP KIPFTEANLVPTKA VPW PQ GRDPRISIDS FGLGGANAHVIEADPSARRPNGVAHINGNTGVSSQRLVFAHTETSL KTMLGKYEAFIKSES FQLTDLAYTLGARRHHHKFRSF CVDGSPL QPAATVRRPENS R LLFIFTGQGAQWSMGRELIGDFPSFRKDIQMQMDKCLAECQPPPWRMEDKISNAEDV NAAEYAQPCTAIQIALVNLLRSWNVHADGVVGHSSGEIAAA YTAGALTMEDAILVA FYRGVTSSQTKPAMA AVGLRDEVNGLLTSGATIACENS RSSVTISGDL SAIKEKTLDR VRQYRAEV LARKLKVDKAYHSKG YPSA VWSKLISTIKSPHD DLSLPIPFSSVTGKETRD ASLLGANWYKS N M ENPVLFLSA VESA LESTQDFGMALE LGPHS AL SGPF RQICKDRNK IVTYDSCL TRASDSTS KSLLSAVGRLFCQGV KDFAAMNGA TTMSSLPPYPWTHDTSY WHESRISREIRTRAHPEHELLGARVIGGNDLEPTWRKMLSKEV P WLSDHVVAGDVVF PAAGYITMATEAMRQLSSYPGSFTIRAL SIGSAMPLNNGKPT EIMTRLQPLR LTDNQDS AWFDFS VMSYD GTRWSRHCSEIRIAHALDSSAAGLNLSAPECKREVATAK WYQA AK SVGLEYGPSFQGLQDVFYDL SRGCTS ATLQSTEQYYGLFHPTTIDQLLQCCILGSVKGH ERLLNR TALPVYIEEMSIGAGDYLNNLHCDAYTLFSGHDTLSA HGHIGV RGGV LALK KG IQFRLLDMHTSTAEDPLKELHLL EWRPDIDLTSDLQIVRQDHDLSSCLEVERLNILC VLESTRILKHLDSSH HHFQRFKAWNEEYIDLKLRNGSRVVKDVNQILEMKPEKRAGAI TALTEEAILTPARDIALAIVRIFNDVEGIFTGA VEP LAVLLKDNL MEIYNFFNMLEYRD FFRLLGHKNRGTLRVVEIGAGTGGFTSTILPALMDSTGDCLFSTYTYTDI SGGFKAKE RFG EYSGIEYTVLDISKDPASQGLELGSYDLVVAANVLHATPDLVQTMKNCRSLLRTG GRLFMLELCSEAKWVNYIMGTLPGWWLGEADGRPNEPYIQGEQWKVILQ RAGFRSTT AIMDQKPYQLDNII VASADD DVATPSKALNLLVRDSNQPSA IANTFLLEFRQAGYDVTL CSLREQPISP VDTV SLLIDGPRSFFEDLDEKGLRGII RFITNNRGQQMLWLTGPAQVST ENPHHAMVGLARTLRL ELGSHFATIELDIDAGPA LF GTVVKVFDQIQRQSKV DVA DC EFALVN GTVQVPRFLTRTADQV VPIPDDQVFRKLHMGKPGI LASLQWQEGLRDSSLGE GEVEINRSSA VTHQDVL FASGA VHGKQDLG FECAGIVSRTSTS GTDLQVGDRVLCWS SGSLATHARVN STCCIKLPDSL SFNDA VTMPTAYAS MIRGLDNSSLAAGETV LIHSAS SPMGLAGI QIARMQGAE IFV TAKTEAEKEFLVNEQDI QTSHV FSSLDNSF VAGIMQATN ARGV D VV VNL LSGD L HESW KCV AAGGNMIELS GRD ITGH GKL DM TIFGGN RGF YGI NIPALITQKPSLAPRLLKATMDLYTGGSIKPI SPIKG CTPSNIKQAFQQLHADSDQIGSVV LEFPDDPQSLIVA E SCSDEI QFRKDRS YV LIGGLGGL RSA VVLA ERGAGCI FLSRSA SAGAESRSLVQELNVLGCETQIATGSV TDATA VDRL VANA AKPIA GVLH LALV KDEA LLDMAFDSWRAATEAKVQGTWNLHRALEGQPLEFFV LASSIYI QGQNPQANYAA AS TFLDAFVQFRQKLGLPASVIDLGVMADGVYV SERPA ILES K RAGA QLLCENDFLRSL QLGIRASSAPPLPTDLISGYVNRAQFVVGIGQHPPDARGLGLKRAKDSH QGLG KRA TTQDSTGEGSGD KLRQFLEGAKRDPSSL NDEM GATQFLA A QVWTENK MGQK ASWE ASTNGKVSLDTTEDQKTDYSKWL NDEQGRQ SWRYLESDEEAKWPQTTAEKHF LG AECLKG LLI FGD NSSLDL GLADLGVD S LVA IELQS WVIQS FATHITI LE LT KASIV A LAVSERSTMFL DIMS VADD PYNLQ DLPVPK AQTPL QAAQGA VS YFSQ LQMP SGQ WA SECTGP HFIL PCVILA GYVTG THLPAGY AVEI RYLLAS QRVADGGWGWHA EAHSSA IATALNYVVLRLLGANR DDP RLVKARELLHTFGGATHVPGIGKFWLCVLGV MKWEC VNPFLPELWLSSDSDPAAPSKWYHLTRTNFTSMSYVWSKQWSYDGD AVTEQLK D ELY TQPYDTIDFAGH RSSLA A VDN NYPKWWL VN LMN WVT VTVYLPYLRKPTT AYS GERK VWDLIMAEDKNT EYIGLSP ISKAGN LIAS YIEGPQGSSVRAH RRTMTQYFWMTK DGM ACNLS DGIQVW DTS LAVQ ALCAAGAGADPKFH STL VRAH AFLEDHQL DDV P DQDK CYRW PRKG GGPWFSTRYQGYMISECTGEGLRSV MQLQ EM SHLD LAQPIPE QRL HD A D CLLN LQNDTGGF VYE KRTGSPK LAW LEMGEFVGKTMV TYDF VECTA AVS ALLSFSK LYPDYRAADIEATTVQGLSFIKK SQR PGW HGAWGV SFTYAGMFA LETL A LAG ETY TTSEASRRGCDFLVN KQKA DGGW GEYLSLQ REEYVQH QESQVQTA WVC M ALM HA GYPEMEPVK RGLK LIMTRQSKGQWYQEA LEGGV GDG
10.	QIS9292 0.1	CcqD [<i>Bipolaris sorokiniana</i>]	359	MVFESTPEANDTACVPLDGIEITKWDTGELITRTAANPVGKQNAVHGDMLALLAR AQELSNVEIRLGARVVDV DIEATVAFLSDGQRVAGDLIIAADGVKSTLAKVCPPEAV VPLPTGEAAYRFTLSRDLLES PRLQELVQR SWATRWDGSPR HVVAYPVQNH RLLNV VLIHPDNGDAEESWTSVTDKQNVLTDYQGWDPTLLKVALAPPEVPNFRMFYIYPPAPV WVKGSTILLGDACHAMLPYLGQGVQA VEDA TAIATVLSLIENRQQLPLALRAYESSR KERVDQIQAATYRAREQLHLD RGDAQAARDLERKAASNTGQNSDVVKMQHSYWTW DAAGVAEKT LAALIVA
11.	QIS9291 9.1	CcqC [<i>Bipolaris sorokiniana</i>]	755	MGDFEKSHDFKVVIVGGSVAGLVL AHLHKAGIDYIVLEGRDHIDPQVGASIGLFSNGS RILDQLGVFKSILECTEPLK WYDMLTQGQD LVRCCDSLQLIEARTGYPVTFLERRQVL QKLHQLAPEQSKILTSKKVISVRTLPDGVEVHCGDGSIFTGDIVAGADGVHSQIRREM W RHAKSDGALKHLKNDEKAMFADYRCLYGMSSPVPGLRETSIYRSFNKNWSFLVVVGK DERCFWFVFEKLSRTYRLPNLPRYTESDQAEFVKPFM KRHV SQGVTFDALWHRK TAA TAALEEAQYQHWTYGRFVCLGDSIHKMTPNIGQGGNWAIESAA ALTNKLYAMMRT TQRPSFVECDTLSDYEQSRQV RTKEVCTMAGFATRLEAFDKFWHKL MALYVVPRAG

				DMLVDVHCQSVAAGAHMLDFLPPPPELSRQDTIFQAVEFDRRGLHVAWRVLGATPLA FCFAARHTLEPTGSVLVANQDAKAQVAMLSHMGDFPQLQVIALIESARRGNNSMGIAA LWPLFVLAGYGSTAYAFPVYFFLQYILSPPSRYAAADNRLVPTHYARSAVAATVMG YFLVAYGAASQQKYAVYAHWYLLPTMAVLHPLFASFLTNTTFTDRVQNPRADMK YLLVSYMSGIFTAITHVYSWALSPWGTLTNILSILTRQGTLLLEEIGPEKSIQRHHVL MLGSGLFWTLHLWDLKSTGRLKAGWLKVVGALVSMVMFGPGTALVLGAWRE AVLARKTAPH
12.	QIS9291 8.1	CcqB [<i>Bipolaris sorokiniana</i>]	198	MSKIALITGSTRTPRGTDVAGWVHEVIKSRPEDKLEVEPLSIADFNLPIFDEPVMPAM VPAMKQFTKDHSKKWSEAIASFQGYIFVIPEYNGMSGATKNAIDYLYNEWPGKPVA VISYGHGGERANKHLSSESLEVMKMKVAPTKVQLAFAPEGTDVFAAINDGVLGEDTK KAWTEAGAKDQILKAFGEVKEILVQ
13.	QIS9291 7.1	CcqA [<i>Bipolaris sorokiniana</i>]	520	MLDISLIKSLQHPGARLAINFILTIAITLIRRIGSRCLAKYRLRALPLVNGYGLFESDKRS KENFFFNA QSLLNAGYAKSPRAFRVQTDNSQVVLAPECVSEIRNDNRNFNTQLLADDLGHIPAAFT NFSPHNGLNDM AKEVLTKKLNPSSLGLVTKDVEATLAFRNHWTDDTEWHSIMKG_TILEIVAQLSSRV FLGPCLRNPawan LRITVDYTTNLFFGVEALKKWHSHFLRPVWRFVPEVRKVRQQIEEAIRLIQPVVDKRTA ESGSSAASLNK VKYTDQAVWANELAGGRPYHPALLQLQGFLSLAAIHTTDQLCQTLYDLCAYPEYIEPLR KELVTKLESGM TKAGLYKLKLMDSFMKESQRLKPGASLLMRRLVMEDVTLSNGVFLPRGIQIGFPLRSH FDPKAYPEPDVF DGYRFVKMAGDPEKEMLRHVFSTPEHLAFQFGKHSCPGRFFAAIEVKIALCHILLKYD FKLAEGETPTV MKMGWALIADPMAQFIHKRESIDESILIT
14.	KAF584 7262.1	hypothetical protein GGP41_003539 [<i>Bipolaris sorokiniana</i>]	261	MPVELRKRAAAPAPAPPACKKAPAKAKKADGEKTVVEKVQDAVVEKAЕAVKKAV VSKTNGASTAKSSAPKVGDIDLASFGGEIETNDGKTS LAKLVEESKAGVVLFTYPKA STPGCTTQVCLFRDSYTPLTATGLSIYGLSSDSPKANTTFKTKQKLPYSLLCDPAQTLIS AIGFKKAPRGTTTRGVFVVDKQGKVLAEPGGPAATVEVVKKLVGDAEKVPAKEEEVE AKEKEEDKKVAETADEVADSAAKVDAPAAA
15.	BCG062 53.1	Translation elongation factor 1-alpha, partial [<i>Bipolaris sorokiniana</i>]	8	EAAELGKG
16.	BCG062 52.1	Translation elongation factor 1-alpha, partial [<i>Bipolaris sorokiniana</i>]	10	FEKEAAELGK
17.	BCG062 51.1	translation elongation factor 1-alpha, partial [<i>Bipolaris sorokiniana</i>]	10	EKEAAELGKG
18.	BCG062 50.1	translation elongation factor 1-alpha, partial [<i>Bipolaris sorokiniana</i>]	10	EKEAAELGKG
19.	XP_0077 02024.1	uncharacterized protein COCSADRAFT_1 21709 [<i>Bipolaris sorokiniana</i> ND90Pr]	261	MPVELRKRAAAPAPAPPACKKAPAKAKKADGEKTVVEKVQDAVVEKAЕAVKKAV VSKTNGASTAKSSAPKVGDIDLASFGGEIETNDGKTS LAKLVEESKAGVVLFTYPKA STPGCTTQVCLFRDSYTPLTATGLSIYGLSSDSPKANTTFKTKQKLPYSLLCDPAQTLIS AIGFKKAPRGTTTRGVFVVDKQGKVLAEPGGPAATVEVVKKLVGDAEKVPAKEEEVE AKEKEEDKKVAETADEVADSAAKVDAPAAA
20.	EMD627 28.1	hypothetical protein COCSADRAFT_1 21709 [<i>Bipolaris sorokiniana</i> ND90Pr]	261	MPVELRKRAAAPAPAPPACKKAPAKAKKADGEKTVVEKVQDAVVEKAЕAVKKAV VSKTNGASTAKSSAPKVGDIDLASFGGEIETNDGKTS LAKLVEESKAGVVLFTYPKA STPGCTTQVCLFRDSYTPLTATGLSIYGLSSDSPKANTTFKTKQKLPYSLLCDPAQTLIS AIGFKKAPRGTTTRGVFVVDKQGKVLAEPGGPAATVEVVKKLVGDAEKVPAKEEEVE AKEKEEDKKVAETADEVADSAAKVDAPAAA
21.	AER360 18.1	phosphopantetheinyl transferase [<i>Bipolaris sorokiniana</i>]	375	MVDQDDNGAAGSSFTCWLLDTRSIWPGTKITDSEAAREALSLVSLEERENITRKYHIAD ARMSLGSALLKRLFVHRMLGIPWKDIRFGRKRDPKHGKPIALLPPPQHGPAPLEFNISH QAGLVALVGCKTDELDAEVGVDIVCVNERDEYRIDKEGFDGWIDMYAEIYSQEELF DLKYNVDSFPLLDGTMTQEIIGRHDRCARHKQLSITLPNGEKRVFDSLLELIADKRR FYTFWCYKEAYIKLDGEALLAQWIPRLEFKNVRAPRAGTPARCSTHGTWGERISDAEV

				WFTMKADGKGPAVGKGMKRDESKRLDDTRVEIQAFDEKFMIKGVAAKERTDAVVDG NRQKLPEVLTQFQALHLDDEIMSVARVA
22.	AER360 17.1	alpha- amino adipate reductase [<i>Bipolaris sorokiniana</i>]	1181	MTMASLTKGSIPTADLHWSDFKGPIHEIFASNARKHPDRPCVETATGKTPERKFTY KHIFEATSVLAHHLVQSGVQRGEVVMMIFAHRGVDLVVAIMAVLAAGATFSVLDPLYP DRQCIVLEVSQPRALVIIDKATREAGPLSDQVRDYIKDNLQLRTEVPALELKDDGTLVG GTKDGKDILDEQQQLRTELPGVLVGPDSTPTLSFTSGSEGKPKGVKGRHFSLTHYFPW MTEMFLSENDKFTMLSGIAHDPIQRDFITPLFLGAQLLVPSKEDIQHEKLAEMWRQY GATVTHLTPAMGQILVGGASA VFPSSLHSFFVGDLIJKRDCRRLQNLAPNVRIVNMYG TTETQRASVYYELPSCSEAPFDLDTIGEVIPAGRGMNSNVQLL VVNREDRNQICKPGESG EIYVRAGGLAEYLGGLPDLTATKFINNWFDQQKWIDEDKKQVESQGAPEPWREFYK GPRDRLYRSGDLGHYSEDGNVHCTGRVDSQVKIRGFRIELGEIDSHLSAHPVLVRENVT LKRDAYEETLVSYIVPEMKRWYDWLEERGAKESDSTSMTLLKRFKYLRRDDVR EHLKKKLPAYAVPSVIVPLVRFPLNPNGKIDRPALPFPDPADLAAGARRPSQLGAALT PTEKAMARIWAELLGDRGVTA DSIGGSDFFDLGGHSIAQQLFFKIRQEWDKIDVPM TIFQYPTLRLGSANIDQAMDPIGLRLDTAEALEDDPDDEA YSADARDLANKLTEFNTR ELNPKEEVHTFLTGATGFLGAYILRDLISRPGVTVL VRAQDIDAALGRVRQTCTAYGI WEDGWESRLEPLVGDLKENGEANTWNKLVDSDVVIHNGALVHWVLPYSRLRG PNVLSTMALSMCAAGKAKKFGLVSSSTSVLDTDYFVSLSEKSLAEGGTGVPEADDLEG ARKGLGTGYGQSKWAAEYLTRQAGKKGGLSGCVCIRPGYVLDPEY GTTNTDDFLVRM LKGCIQLESRPDITNTINMVPVTHARVVVASSFNPPVAPLGAQVTSHPRITFNEFLGA LEKFGYNVPLVPPYAEWKQRMESYVADRSGTKEENALLPLYHFVTGDLPADTKAPELD DKNAAEALKKDQEWTSQGGAVTEDTVSVVSYLIELGFMPRPEKKGIKELVMS RLTDAMREGMKLVGGRRV
23.	AER360 16.1	polyketide synthase [<i>Bipolaris sorokiniana</i>]	2153	MDVLIFGDQTADQYPLLRKACTWKNNATLTFDRISVVIREEVQKLPRTQRDQIPNFL TTWDLVEAYYAKGLKIPIEIESCMVTIAQLAHYIGYFAENPTELPNPSNTRVGLCTGL AGSVVASARSLSELLPLATEAVRAFAGTCVGAKEALEQSSTS KDSWSTIVTNISED AAKDAIAAFHEEQKIPTLAQAYISA VSTMALTISGPPATTKRLLEKEAFKSSARVPIPVY APYHASHLYSQADIDRILDKDAIRHLQQFRPVALVHSATGKCQTATNTLELVRTALH EMLVEPVRWDSLLSEVVSQVTSASNAQCSVAFGVT SITNSLASALKNGGSAITVRD QSAWVPADHDSRGRTQNDKIAIVGMSGRFGAANPEALWDLLERGLDVHREVPADRF DAKAHC DPGSKGKKNKSHTPYGCFIDEPLFDPRFFNMSPREAQTDPMGRLALTTAYE ALEMSGYVPNRTPSTKLERIGTFYQTSDDWREINASENIDTYFITGGVRAFAPGRINY YFKFSGPSYSVDTACSSSLAAIQLACTSLWAGDCDTACAGGLNVLTNPDI FSGLSKGQF LSKTGSCKTYDNDADGYCRGDGCGSVLKYEDAIAKDNLGCLGAATNHSAEAV SITHPHAGAQEYLYNKVLSNAGVDAHDISYVEMHGTGTQAGDGIEMTSVTNAFAPRH RQRTPEQTHLGAIKANIGHGEAASGINSLVKVLMMMKKNAIPANVGIKGVMNKTFP KDLAQRNVHIETTQVAWPRKGGEKRKIFLNNFAAGGNTAVILEDGPLPEEPKGVDPR TMHMVTVSARSIA SKKNINLDFVDENPSVTLPSLAYTTARRIQHNYRVAFCVSDM SKVKDGLRAQLKDTYSPLPMVPTKTAFTFTGQGSQYTGLGQKLYEDLETFKNDIQLD KLARLHSLPSIPLLTGADVATLSPVVQLGMACIQVALARMWGAWGVRPIAVIGHSL GEYAALHVAGVISASDMVFLVGRRAQILEEECTANTHGMLAVKGSVDAIKVALGDK MTEIACMNGPEETVLCGTVDVVDSTNELLASKGFSTKLNVPFAFHSAQVEPILEKFK AAADSVTFNKPVVPVMSPLNGDIIVEAGIIGPDLARHARETVNFWTALTNGQEQLF DAKTAWLEVGAHPVCSGMVKSSLGGSPVAAGSLRRNEDPWKTLSNTLTTMYLAGVY IDFNEYHRLFNDAHQMYTLPTYAFDSKKYWLDPYHNWTLTKGEVLQAAVSAIEA APVVEAPSKLSTSCHKIVREDLHANS GTVVVQSDLSDPDKL KATITGHQVNGTPLTPSS LYADQAMTVADYLYQQLRPGTETPGNVCMSMEVTKTLIPQYPPPATGQHLQIEGNADL ETSQVKITFR TVSADGSKILA EHAVGIVKYEDVNAWKEE WGRQIYMVQSQIDMLQQK LATGAAHKVLRGMA YKLFKALV TYADNYRGMEEVILDGKQTEATASVQFQTTEADG DFLCSPYWI DLSAHL SGFIVN ASDHLDSENSVYISHGWSKIA GKL SPEKKYRSYVRM QPAPGNISVGDVYIMDGAIEI GMVMGLKFQNIPR RALNIMMPPAGKAASAPA AKA KPVPKALPTAAPVKTHA AVPKAVKAPKAVKVAAPAGVTSKVMNIVAQEIDVDMSEL VDEAAFENLGVDSLLSLTISARFREELDMDIPSTLFTDCSTV GELKKHFSQFDGTTIVED DSSVTSDEPSGAPTPFEAPAGQSDSTPASSAGSDDGHDDIKPSAATEGGASLARKLVAE EMGV DVSEITDDL LTDIGMDSLMSLTILGSMREATGRDLPADFLTVNVTIKDIETALG MRPQPKAAKPATKASSKAPQ LSEVNKKLASLPDVSNLPPASSVLLQGNPKTATKKFL VPDGS GSATSYISIPNIPS MAVA YGLNC PFMK CPEK WTC VEGV SRLYLNEIKR RQPA YLVGGWSAGGV MAYEVAQQLVNAGEK VESL VLIDAPCPVALDPLPARLH IFFDQI GTGKPGGT PSLPHFASAIQNLKDYDPTPMNP KIAPPV LAIWC TDGVCPNP EDPR GEGEDPAPMKWLLNNRTDFSDNGWAQLLPKENFQYAVMGGNHFTMMKGDHGVTLG KLIQQGLKL
24.	AER360 15.1	nonribosomal peptide synthetase 6 [<i>Bipolaris sorokiniana</i>]	1939	MHLPNGRENTVRFSGVSEEPAAKKVQSSNGFNAQEHALQSENPTGAKHTNRNASK DIHVHLPNGTDRDLD TVLIAWAILIQRYQRDV FQQFTWGRKEQENAATQCISVADIDW ANQQTAA SLRTKISSVKS NQFTLNETT LFDNGTNE EWT FQVLLNVQ QDTL HATSTWQT TTMSRHQA VSQLHFFASILEALFKD VDHPLS DFHN VSEDE DDLW SWN TPLQPELRC HEKV SERA ALHPEKIA IDAWD GTL TYGQIEDYSDK LAKL RLL DDSSN RIIPV LFEK TSVAVLA IMKSGACF ALLDPAQPEG RLRAV VQQVNAKLFSSKAQ STLA ARVAPA IPI SKSF NKIFS PTA E QPST TIPP VSPD QP LYI QFTSG STGV PKG CIL THS QYTSG AVGYYPHSRV LDFAS YAFD VCI DSML TLA HGATL CTPS DERRM NDMSG AMR DM FAGMTPS VARTL DV DIL NN L E SIAL G GEGV SIS DAM SWG QR RVN AY GP SEATV GATI

		NDNVAAKPYITMGKRKGCALWLTDPENHNKLVPVAGVGELLIEGPIVGNGYLNNSKT KEVFIEDPEFLLKGSKSYPGRHGRYKTGDLVRFDPDGNGEPIFVGRQDQQVKLRGQRI ELAEIEFNMQKLPPDTQLAAEVIKPSGGEQTLVAFLVEQKKNGMRHLDGNVFGSFTN KFQSALRDMTKQLFVDLPSYMIPCAYPLWKMPPLLVSCKTDRKRLREIGASVTRQDLRR FNSAVSEKKEVTTEMELKLQLSQLWAKLLGGDADFSANDNFSMGGDSLRAMRLVAAAR DEGIVLSVPDIMLNPTLSAMAEKAKPVSAEETNEVHPFSMIGKDWDADAARQESRLCG VDVANVEDVYPCTPLQEGLIALSAKFQDAYVAQRVATLPAKTAVLKKAFTDAVEGS PILRTRIVNVTRGLFQVVLKDQLVREYGTDVSEYRLDRNEPMMDLGTALFRYGLVKE PESDKINFVITMHAVYDGWSMPLFDHVNRNFGLHTERSTSFKHFIKLISLDPADAQ QYWKDCLEGTSQHFPPLPQKGYTTQADSLLEHYVTVPSAHSKLTATIIRGAVALVS SLYMGHPDIVGETLTGRSAPVPGIEQIEGPMITTVPIRVRLSLDRPIAEYLQKIHQTVKQ IPHEHLGLQNIRRLSKDARVACDLRTGLVLPKEDEDWGTVDMDNPATFLPANDAEG AREALKFNTALMLVCTLEENGFLVMASFDSNCISKETMERVLAVLGRIVHAFLGNPES KLGDVAVLDPAEARDAEAMRPRVMSDSALGMSPVDPGPESMDAGLKELSPNEEKLRSI LGRILGIKETDIRSSDSFFDLGGSIVAMRLVSDARAQGLNLTVAQVFQSSLSDLAAASA SNEREDKLAEILSRILGIAKTDIKSNDSSFFELGGDSIGAMRLVSDARAQGLNITVAQVFQ SKSLAELASSAGEETPSQPKVVDAPFIALGKDANLHSPDRVGLYLENQEWEITNIYPT RPLQQLAVEGTVDLPRYSLRYELIKFATPIDRQKLEQACQELVARNEVLRTVFVKDNE LTGVVVLSSLKVPYTETAVPEGEDADTFIQAGIQQDIEAPKPYGSSFVAFNLFTHTNSAS TLVFRISHAQYDEICLPILFEQLSTLYSGTTVPETVPFSKHVNHHVLDNIPKAIPYWKNL LSGSEMTVLKPITPLTHRGPADIYREFDISGRPANITIGSLPTAAWALVLSRRLNRTDVV FGEVVSGRNVGAPNADRIFGPTWQYIPFRVAFSKWSYLDLLRYVQDQHMTSAAYES MGFSEIVQNCTDWADKVQWFDTVHQAPA WVEELPFGNGVEAKFQTLYPHGEPLR EWKCQAFVKDGGRKLGIEIVTFEEWIGEAEGVLEEVGKALECLMEGRAGESVF
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