cek turnitin jurnal agrium.docx

by 1 1

Submission date: 20-Aug-2025 10:37AM (UTC-0500)
Submission ID: 2730957854
File name: cek_turnitin_jurnal_agrium.docx (1.99M)
Word count: 2506
Character count: 14675

Isolation of Drought-Resistance Gene Encoded *GmNAC-D* in Dering-1 Variety of Soybean

Abstract

Drought is one of the most critical abiotic stresses limiting crop growth and productivity, causing substantial yield losses in major crops such as soybean (Glycine max L.*). The No Apical Meristem (NAC) transcription factors (TFs) are plant-specific proteins that have been widely reported to play key roles in enhancing drought tolerance across various plant species. This study aimed to isolate and characterize a soybean NAC gene through a molecular approach. A NAC family gene was successfully isolated from drought-tolerant soybean cultivar Dering-1 and designated GmNAC-D (D = Dering-1). The gene consists of a 762 bp open reading frame encoding 253 amino acids. Database analysis revealed that GmNAC-D shares high homology with GmNAC02, a known stress-responsive gene in soybean. Expression analysis across different tissues demonstrated that GmNAC-D is most abundantly expressed in stem tissue, followed by flowers and roots. Amino acid sequence analysis further confirmed its high similarity to GmNAC02, although with distinct expression profiles. The differential expression patterns of GmNAC genes suggest functional diversification during plant growth and development. Collectively, these findings expand the repertoire of soybean NAC transcription factors and provide a valuable genetic resource for the development of transgenic soybean with enhanced drought tolerance.*

Keywords: *Transcription factor, GmNAC-D, soybean, drought-resistant.*

INTRODUCTION

Drought is one of the major environmental constraints that can suppress plant productivity through various physiological and biochemical processes, such as photosynthesis, respiration, nutrient metabolism, ion absorption and translocation (Oguz *et al.*, 2022; El Haddad *et al.*, 2022). The adverse effects of drought on soybean plants have become a major concern since this legume is considered one of the most important crops providing the largest sources of vegetable oil, protein, and macronutrient minerals for human consumption and livestock feed. The numerous benefits contained in soybeans as a high-quality food source have led to a steadily increasing demand for this crop from year to year (Bantacut, 2017).

Plants possess various defense mechanisms that enable them to cope with drought-induced stress. In response to such conditions, they activate biochemical, physiological, and morphological pathways (Pamungkas *et al.*, 2022). At the biochemical level, plants enhance the production of antioxidant compounds, chlorophyll content, proline accumulation, as well as various hormones and other secondary metabolites. Physiologically, plants change stomatal activity, osmotic balance, photosynthesis, transpiration, water transport and leaf water content. In addition, morphological alterations such as reduced leaf area and number, accelerated leaf senescence, increased root length, and shifts in growth stages may also occur. All these changes are triggered by several molecular mechanisms, including the upregulation of gene expression (Conesa *et al.*, 2016).

Great efforts have been made by researchers over the past decade to understand drought tolerance mechanisms in plants, including in maize (Hammad *et al.*, 2017; Ghahfarokhi *et al.*, 2015), mung bean (Jincy *et al.*, 2021), wheat (Jin *et al.*, 2018; Khan *et al.*, 2019), sorghum (Sarshad *et al.*,

2021; Sanjari *et al*., 2021), rice (Yang *et al*., 2019) and, soybean (Dong *et al*., 2019). When plants are exposed to environmental stresses such as drought, their initial response is the activation of a set of genes involved in defense mechanisms that function to enhance tolerance against unfavorable conditions (Zhang *et al*., 2022). The defense mechanisms against stress are largely regulated by genes involved in physiological and metabolic processes, among which are Transcription Factors (TFs) and the cis-elements of target genes located in their promoters (Zuo *et al*., 2023). Transcription factors bind to specific DNA sequences and are involved in regulating the transfer of genetic information from DNA to RNA (Hao *et al*., 2021). Therefore, the identification of novel TFs holds great potential for overcoming key limitations in the development of transgenic soybean varieties with enhanced drought tolerance (Gahlaut *et al*., 2016).

NAC TFs are plant-specific transcription factors and are proteins involved in various processes, including flowering, senescence, seed development, secondary cell wall thickening, and responses to stresses caused by both biotic and abiotic factors (Li *et al*., 2023). One of the important roles of NAC TFs that has been investigated over the past decades is their contribution to enhancing resistance against both biotic and abiotic stresses. Stress-responsive NAC TFs are largely encoded by members of the ATAF subfamily, including StNAC262 in potato plants (Zhang *et al*., 2018), *Brassica napus*, *BnNAC485* (Ying *et al*., 2014), and Arabidopsis, NAC transcription factors from the ATAF subfamily such as ATAF1, ATAF2, ANAC019, ANAC055, and ANAC072, also known as ERD11 (Responsive to Dehydration Stress) play crucial roles in regulating plant responses to environmental stresses (Jensen *et al*., 2010), and ANAC032 is involved in regulating tolerance to drought and salinity stress, as well as in the signaling pathways of jasmonic acid and abscisic acid (ABA). Overexpression of the ANAC032 protein in Arabidopsis not only enhances plant tolerance to drought and salinity stress but is also associated with sugar biosynthesis, which is likely closely linked to the photosynthetic process (Zhang *et al*., 2022).

The identification and cloning of cDNA for NAC proteins in soybean revealed the presence of 32 different NACs, seven of which encode stress-inducible genes (Melo *et al*., 2018). In this study, one NAC gene was successfully isolated from a drought-tolerant soybean variety (var. Dering-1), which shows high homology with Arabidopsis NAC genes that have been proven to confer drought-tolerant phenotypes (Ermawati *et al*., 2024). The existence of this gene represents a novel source of genetic diversity that can be utilized for the development of superior drought-tolerant soybean varieties through genetic engineering.

MATERIALS AND METHODS

Experiment Site

The research was conducted at the Biosains Laboratory and Greenhouse, Politeknik Negeri Jember, from June to November 2024.

Planting and Stress Treatment

The soybean variety used in this study was Dering-1, which is known for its drought tolerance (BRMP, Malang). Planting was carried out in the greenhouse of Politeknik Negeri Jember under day/night temperatures of approximately 31°C/25°C, using pots (Ø 25 cm) filled with a soil-compost mixture at a 1:1 ratio. Two plants were grown in each pot. Drought stress treatment was applied when the plants were about three weeks old, at which point soil moisture content had decreased to around 30% of field capacity, by withholding irrigation for 7 consecutive days.

RNA Isolation and cDNA Synthesis

Total RNA was isolated from 100 mg of various plant tissues, such as roots, stems, leaves, and flowers, using the RNeasy Plant Mini Kit (Qiagen). The RNA concentration was determined using a UV-Vis spectrophotometer (Amersham Biosciences). The first-strand cDNA was prepared from 1 µg of

total RNA using Transcriptor First Strand cDNA Synthesis Kit (Roche, USA) according to the manufacturer's instructions.

PCR and Cloning of *GmNAC-D*

The *GmNAC-D* gene was amplified from cDNA derived from stem, leaf, root, pod, and flower tissues subjected to drought stress using the Core Kit Taq DNA Polymerase (Roche, USA). Primers were designed based on the Arabidopsis NAC gene (*At1g77450*) with the following sequences: Forward: 5'-GCGGATCCATGATGAAATCTGGGGCTGATT-3', Reverse: 5'-GCTCTAGATCAGAAAGTTCCCTGCCTA ACC-3'. The PCR conditions were 94°C for 3 min, followed by 30 cycles at 94°C for 30 sec, 60°C for 30 sec, 72°C for 1 min and a final extension at 72°C for 5 min. The PCR products were separated by electrophoresis on a 1% (w/v) agarose gel and visualized under a UV illuminator (Biometra, Germany). The amplified fragments were subsequently cloned into the pGEM-T vector (Promega, USA) as previously described by Prasetyo *et al.*, (2018) and sequenced using Sanger dideoxy sequencing technology (The 1st BASE, Malaysia).

Phylogenetic Analysis

The amino acid sequences of six *GmNAC* genes obtained from the NCBI GenBank database were analyzed for sequence homology using ClustalX (Thompson *et al.*, 1997), with the following parameters: gap open penalty = 10 and gap extension penalty = 0.2. A phylogenetic tree was then constructed using the neighbor-joining method with the online EMBL-EBI software (www.ebi ac.uk/).

RESULTS AND DISCUSSION

Isolation of *GmNAC-D*

To investigate the involvement of genes from the NAC transcription factor (TF) family in regulating drought stress, gene isolation was performed using the drought-tolerant cultivar Dering-1 as the genetic source. The gene isolation was not performed through conventional cDNA library screening, but instead utilized literature studies and bioinformatics databases, including GenBank (www.ncbi.nml.nih.gov/) and the Plant Transcription Factor Database (http://planttfdb.cbi.pku.edu.cn/). Previous studies highlighting the role of NAC TFs in Arabidopsis, particularly *ANAC032*, were also taken into consideration. Notably, overexpression of *ANAC032* has been reported to confer enhanced tolerance to drought and salinity stress, providing a strong rationale for its use as a reference in soybean gene isolation (Ermawati *et al.*, 2024). Based on database information, the NAC family in soybean has been identified to comprise 180 members, of which 32 full-length open reading frames (ORFs) have been characterized (Melo *et al.*, 2018). Building upon this knowledge, primers were designed from the Arabidopsis NAC gene sequences as templates to amplify and synthesize NAC genes from drought-stressed soybean plants.

Gene isolation process shown that a putative fragment of 762 bp was obtained and designated as *GmNAC-D* (D = Dering-1) (Figure 1). Sequencing and bioinformatic analysis using BLAST (www.ncbi.nml.nih.gov/) revealed that *GmNAC-D* contains an open reading frame (ORF) of 762 bp, encoding 253 amino acids (Figure 2). Two nucleotide differences were detected when compared with the Arabidopsis *NAC32* sequence; however, these substitutions were synonymous and did not alter the amino acid composition, indicating that *GmNAC-D* and Arabidopsis *NAC32* share an identical amino acid sequence (data not shown).

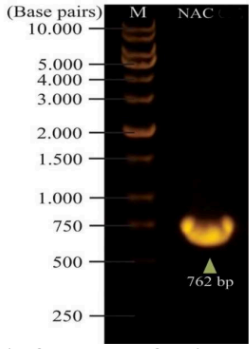

Figure 1. The *GmNAC-D* gene, with a fragment size of 762 bp, was confirmed by electrophoresis of a 20 µl PCR product on a 1% agarose gel.

```
                                                          TTCTTTTGCGGATTCCC
ATGATGAAATCTGGGGCTGATTTGCAATTTCCACCAGGATTTAGATTTCATCCTACGGAT    60
 M  M  K  S  G  A  D  L  Q  F  P  P  G  F  R  F  H  P  T  D    20
GAGGAGCTAGTCCTCATGTATCTCTGTCGTAAATGCGCGTCGCAGCCGATCCCTGCTCCG   120
 E  E  L  V  L  M  Y  L  C  R  K  C  A  S  Q  P  I  P  A  P    40
ATTATCACCGAACTCGATTTGTACCGATATGATCCTTGGGACCTTCCCGACATGGCTTTG   180
 I  I  T  E  L  D  L  Y  R  Y  D  P  W  D  L  P  D  M  A  L    60
TACGGTGAAAAGGAGTGGTATTTTTTCTCACCAAGAGATCGAAAGTATCCAAACGGTTCA   240
 Y  G  E  K  E  W  Y  F  F  S  P  R  D  R  K  Y  P  N  G  S    80
AGACCCAACCGTGCAGCTGGTACTGGATATTGGAAAGCTACCGGAGCTGATAAACCAATA   300
 R  P  N  R  A  A  G  T  G  Y  W  K  A  T  G  A  D  K  P  I   100
GGTCGTCCTAAACCGGTTGGTATTAAGAAGGCTCTAGTGTTTTACTCGGGAAAACCTCCA   360
 G  R  P  K  P  V  G  I  K  K  A  L  V  F  Y  S  G  K  P  P   120
AATGGAGAGAAAACCAATTGGATTATGCACGAATACCGGCTCGCTGACGTTGACCGGTCG   420
 N  G  E  K  T  N  W  I  M  H  E  Y  R  L  A  D  V  D  R  S   140
GTTCGTAAGAAAAACAGTCTAAGATTGGACGATTGGGTATTGTGTCGTATATATAACAAG   480
 V  R  K  K  N  S  L  R  L  D  D  W  V  L  C  R  I  Y  N  K   160
AAAGGTGTCATCGAGAAGCGACGAAGCGATATCGAGGACGGGTTAAAGCCTGTGACTGAC   540
 K  G  V  I  E  K  R  R  S  D  I  E  D  G  L  K  P  V  T  D   180
ACGTGTCCACCGGAATCTGTGGCGAGATTGATCTCCGGCTCGGAGCAAGCGGTGTCACCG   600
 T  C  P  P  E  S  V  A  R  L  I  S  G  S  E  Q  A  V  S  P   200
GAATTCACGTGTAGCAACGGTCGGTTGAGTAATGCCCTTGATTTTCCGTTTAATTACGTA   660
 E  F  T  C  S  N  G  R  L  S  N  A  L  D  F  P  F  N  Y  V   220
GATGCCATCGCCGATAACGAGATTGTGTCACGGCTATTGGGCGGGAATCAGATGTGGTCG   720
 D  A  I  A  D  N  E  I  V  S  R  L  L  G  G  N  Q  M  W  S   240
ACGACGCTTGATCCACTTGTGGTTAGGCAGGGAACTTTCTAATCTAGAGCAAAA        762
 T  T  L  D  P  L  V  V  R  Q  G  T  F  *                    253
```

Figure 2. The predicted nucleotide and amino acid sequences of *GmNAC-D* are shown, with the putative nuclear localization signal (NLS) highlighted within the boxed region.

Database analysis revealed that *GmNAC-D* contains a conserved NAC domain, which is a characteristic feature of NAC transcription factors, and shares high similarity with other members of the NAC family. However, the C-terminal region exhibited a high degree of variation within the NAC family (Figures 2 and 3). According to Li *et al.* (2021), in addition to exhibiting high variation in the C-terminal region, a large number of repetitive amino acids particularly serine, proline, and threonine were also identified. Sequence prediction further revealed a putative nuclear localization signal (NLS) with the motif **PRDRKYPNGS** (Mohanta *et al*., 2020), located in the N-terminal region of *GmNAC-D* (Figure 2). As reported by Bian *et al.* (2020), the NAC domain contains an NLS whose primary function

is to facilitate the translocation of proteins from the cytoplasmic matrix into the nucleus. Moreover, this domain can form homo- or heterodimers, enabling the protein to interact with DNA.

Bioinformatics Analysis of the *GmNAC-D* Protein

From the soybean database, 32 genes encoding NAC proteins have been independently identified in full length (Melo *et al*., 2018). However, only six NAC genes (*GmNAC1–GmNAC6*) are listed and accessible in the GenBank database. The relationship between these six *GmNAC* genes and *GmNAC-D*, based on the high degree of domain conservation, is shown in Figure 3A. The N-terminal region of *GmNAC* proteins exhibits a very high level of homology among members, whereas the C-terminal region, known as the Transcriptional Activation Region (TAR), consists of simple repeated amino acids and is typically enriched in serine/threonine, glutamine and proline residues (Li *et al*., 2021). According to Bian *et al*. (2020), NAC proteins generally possess two relatively independent domains: the N-terminal domain, which consists of approximately 151–159 amino acids, and the C-terminal TAR, which is comparatively more diverse.

A total of 13 motifs have been identified within the TAR region of Arabidopsis and rice NAC proteins, which are thought to be associated with the structural and functional specificity of individual NAC members (Ooka *et al*., 2003). The phylogenetic tree of *GmNAC-D* (Figure 3B) revealed a close relationship with *GmNAC02*, which is classified within the cluster of stress-responsive genes (Tran *et al*., 2009). Considering that *GmNAC-D* shares a high degree of similarity with *ANAC032* from Arabidopsis, it is plausible that this protein may play a comparable functional role in plants.

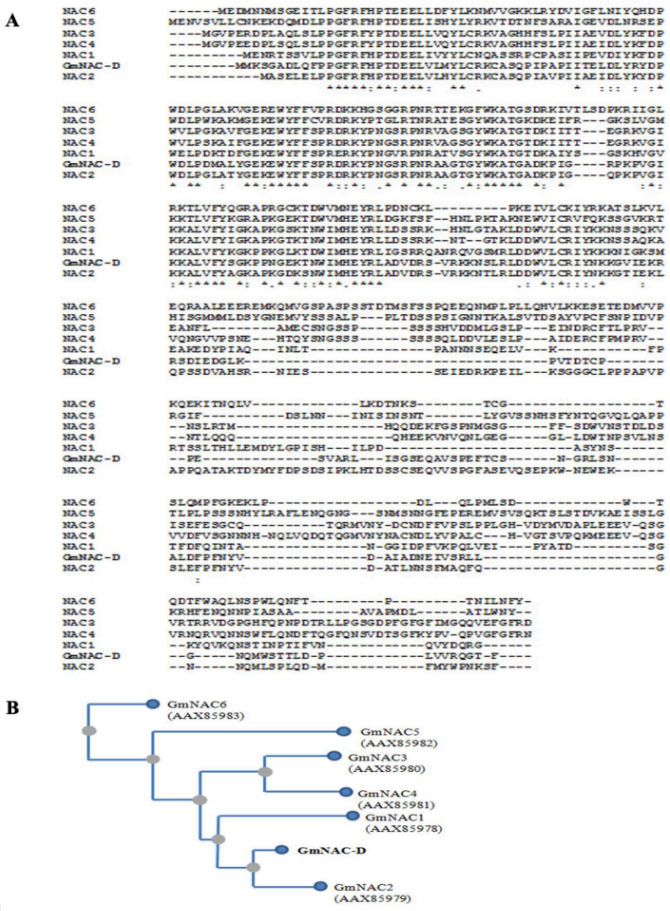

Figure 3. Comparison of Amino Acid Sequences and Phylogenetic Tree of *GmNAC-D* and other *NAC* Proteins. (A) Amino acid sequence comparison of *GmNAC-D* with *GmNAC1* (AAX85978.1), *GmNAC2* (AAX85979.1), *GmNAC3* (AAX85980.1), *GmNAC4* (AAX85981.1), *GmNAC5* (AAX85982.1), and *GmNAC6* (AAX85983.1). Identical amino acids are indicated with an asterisk (*). (B) Phylogenetic tree showing the relationship of *GmNAC-D* with the six other *GmNAC* proteins.

Expression of *GmNAC-D*

Previous studies have reported that tissue-specific expression of NAC transcription factors (TFs) plays a critical role in plant growth and development (Li *et al.*, 2018). Root development, in particular, is strongly correlated with mechanisms of stress tolerance, and organ-specific gene expression

profiling provides valuable information for identifying candidate genes with potential applications in improving drought resistance, especially when exploited in transgenic crop development systems (Amin *et al*., 2024). To support this evidence, expression pattern analysis of *GmNAC-D* was carried out across several soybean tissues. Repeated PCR-based expression assays revealed that *GmNAC-D* exhibited the highest expression in stems, followed in descending order by flowers, pods, roots, and leaves (Figure 4B). Interestingly, this expression profile differs from that of *GmNAC02*, a closely related homolog with high sequence similarity to *GmNAC-D*, which was previously reported to show the highest expression in roots (Tran *et al*., 2009).

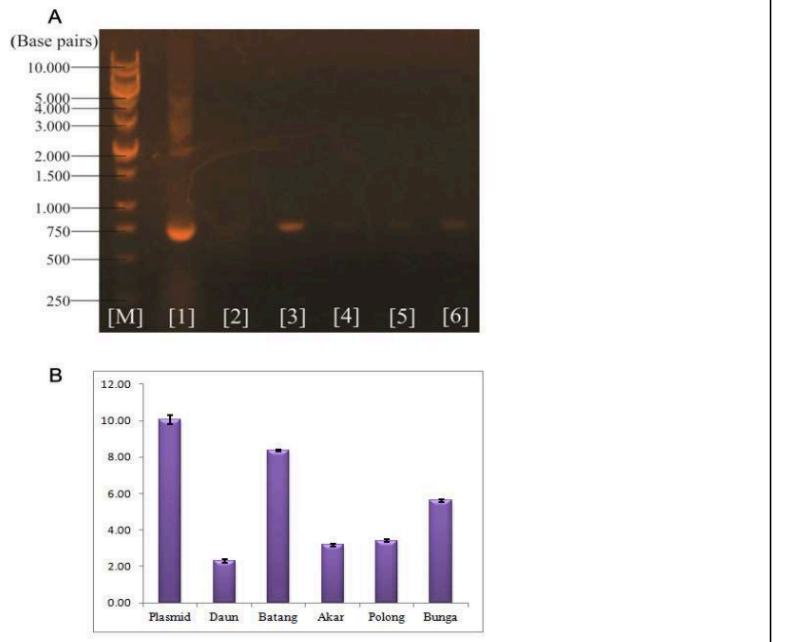

Figure 4. Expression of *GmNAC-D* in various plant tissues. Plasmid: *GmNAC-D* (1), leaf (2), stem (3), root (4), pod (5), and flower (6). A 10 µl aliquot of the PCR product was subjected to electrophoresis on a 1% agarose gel.

The differential expression profiles of *GmNAC* genes indicate that their products are involved in diverse functional roles during plant growth and development. More detailed functional characterization of *GmNAC-D* is required, particularly in light of the preliminary findings from this study, which suggest its potential involvement in abiotic stress responses. These results imply that *GmNAC-D* could serve as a promising candidate gene for genetic manipulation aimed at enhancing tolerance to environmental stresses. The integration of molecular, bioinformatic, and functional genomics approaches will be crucial to fully elucidate the role of *GmNAC-D*. Such efforts not only advance fundamental understanding of NAC transcription factors but also hold promise for **developing climate-resilient crops** through targeted genetic improvement.

CONCLUSIONS

This study successfully isolated the *GmNAC-D* gene from drought-tolerant soybean (*Glycine max*) var. Dering-1. The gene comprises a 762 bp open reading frame (ORF) encoding 253 amino acids, with strong sequence homology to *GmNAC02*, a stress-responsive NAC transcription factor. Phylogenetic analysis further confirmed its close relationship with members of the stress-responsive gene cluster, while bioinformatics analysis revealed conserved features typical of NAC proteins, including a nuclear localization signal and the presence of a characteristic NAC domain. Expression profiling indicated that *GmNAC-D* is expressed in multiple tissues, with the highest expression detected in the stem. However, overall, the findings from this preliminary study are not yet sufficient to confirm the direct involvement of *GmNAC-D* in drought tolerance. Further detailed functional analyses are required to elucidate the precise regulatory role of the *GmNAC-D* protein in plant drought stress responses.

ACKNOWLEDGMENT

This research was funded by the Ministry of Research, Technology, and Higher Education through the National Innovation System (SINas) program in 2018.

cek turnitin jurnal agrium.docx

ORIGINALITY REPORT

17%	12%	12%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	link.springer.com Internet Source	3%
2	koreabreedjournal.org Internet Source	2%
3	www.science.gov Internet Source	1%
4	Le-Bin Song, Yong-Xin Jiao, Zhi-Qiang Xu, Dan-Xuan Zhu, Yong-Shi Yang, Ji-Fu Wei, Jin-Lyu Sun, Yan Lu. "Identification of Pla a 7 as a novel pollen allergen group in Platanus acerifolia pollen", International Immunopharmacology, 2023 Publication	1%
5	Liu, Yongchang, Jie Sun, and Yaorong Wu. "Arabidopsis ATAF1 enhances the tolerance to salt stress and ABA in transgenic rice", Journal of Plant Research, 2016. Publication	1%
6	Juan Carlos Lacal, Frank McCormick. "The ras ", CRC Press, 2017 Publication	1%
7	www.biorxiv.org Internet Source	1%
8	Gijing Zhang, Tong Li, Lijie zhang, Wenxuan dong, Aide Wang. "Expression analysis of NAC genes during the growth and ripening of apples", Horticultural Science, 2018 Publication	1%

9	Hendelman, A., R. Stav, H. Zemach, and T. Arazi. "The tomato NAC transcription factor SlNAM2 is involved in flower-boundary morphogenesis", Journal of Experimental Botany, 2013. Publication	1 %
10	dokumen.pub Internet Source	1 %
11	Submitted to Higher Education Commission Pakistan Student Paper	1 %
12	www.plantphysiol.org Internet Source	1 %
13	Dongyou Liu. "Molecular Detection of Human Fungal Pathogens", CRC Press, 2019 Publication	<1 %
14	www.sumobrain.com Internet Source	<1 %
15	www.wjgnet.com Internet Source	<1 %
16	journals.lww.com Internet Source	<1 %
17	www.slideshare.net Internet Source	<1 %
18	Dongyou Liu. "Molecular Detection of Human Viral Pathogens", CRC Press, 2019 Publication	<1 %
19	Jeyabalan Sangeetha, Devarajan Thangadurai, Saher Islam. "Beneficial Microbes for Sustainable Agriculture and Environmental Management", CRC Press, 2020 Publication	<1 %

20	Melinda Moy, Huaijun Michael Li, Ray Sullivan, James F. White, Faith C. Belanger. "Endophytic Fungal β-1,6-Glucanase Expression in the Infected Host Grass", Plant Physiology, 2002 Publication	<1 %
21	R Sjahril, Kasmiati, M Riadi, I Ridwan, I Jamaluddin, N J Panga. "Flow cytometry analysis on colchicine induced polyploid of Katokkon peppers (Capsicum chinense Jacq.)", IOP Conference Series: Earth and Environmental Science, 2021 Publication	<1 %
22	Submitted to University of Newcastle Student Paper	<1 %
23	bioone.org Internet Source	<1 %
24	www.ncbi.nlm.nih.gov Internet Source	<1 %
25	Dongyou Liu. "Molecular Detection of Human Parasitic Pathogens", CRC Press, 2019 Publication	<1 %

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		