

Original Research Article

Motives of Trypsin Inhibitor Gene Promoters Sequence from *Vigna Unguiculata* That Respond to Environmental Factors are Analyzed with Bioinformatics

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ABSTRACT

The function and mechanism of regulation of gene transcription process at the molecular level can be studied by promoter analysis. In this study, the analysis of TI gene promoter from *Vigna unguiculata* as model plant. This study aims to find a promoter motives on *Vigna unguiculata*. Gene promoter sequences obtained from NCBI with accession code number AY573237.1, DQ417204.1, and AJ271752.1. The promoter sequences are analyzed using the PlantCARE program online to obtain the regulator element. The results obtained are the sequence of the promoter of the TI gene has a motive in responding to several environmental factors, both biotic and abiotic that is responsive to light, responsive to the presence of gibberellin hormone, responsive to heat stress, responsive to defense, and also responsive to daily plant circadian mechanism.

Keyword: Promoters, Trypsin Inhibitor Gene, *Vigna unguiculata*

INTRODUCTION

In the data base, Trypsin Inhibitor (TI) genes are commonly found in Fabaceae, particularly *Vigna* and *Phaseolus* (Primandiri et al, 2016). The TI gene is important for further review because TI plays an important role in plant response to stress and development (Singh et al., 2009). TI genes are able to regulate plant resistance from both biotic and abiotic factors (Sanchez et al., 2004; Srinivasan et al., 2009). TI is detected in salt-deficient plants (Shan et al, 2008; Srinivasan et al., 2009; Kidric et al, 2014), drought stress (Kidric et al, 2014), tolerance for insect attack (Fan & Wu, 2005; Srinivasan et al., 2009; Anandhan et al., 2010; Mendoza-Blanco & Casaretto, 2012), and ABA and jasmonate applications (Sanchez et al., 2004;

Srinivasan et al., 2009).

The function and mechanism of regulation of gene transcription process at the molecular level can be studied by promoter analysis. Promoters are part of the DNA where RNA polymerase attaches and transcription factors to initiate the transcription process of a gene (Campbell et al, 2008). Promoter is one of the regulator of gene expression, so the promoter can be analogous as switch on a gene. To predict the promoter several computational methods have been developed (Qiu, 2003). These methods include approaches based on certain motives (such as TATA box, CAAT box, etc.) as the basis of the search. Other methods are certain base-sequence approaches that use certain basic sequence differences (such as triplet base pair

preferences in the initial area of transcription, hexamer frequency in area 5' along 100 base pairs, etc.) as a basis for differentiating the promoter region from the non-promoter region. The success rate of promoter prediction by computation method varies between 15-54%.

With the increasing number of genes that have been successfully sequenced, it facilitates the determination of the promoter region by aligning the complete mRNA or cDNA with equivalent genomic DNA. With this approach, the identification rate of promoter identification can reach more than 80% (Qiu, 2003). After the promoter identified, the promoter can be isolated. In this study, an analysis of sequences of gene promoter TI to reinforce the TI gene is important for further study.

MATERIALS AND METHODS

Gene promoter sequences of *TI* were obtained from NCBI with accession code numbers AY573237.1, DQ417204.1, and AJ271752.1. All of samples from *Vigna unguiculata*. Promoter sequence samples were collected and analyzed using PlantCARE online

(<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) to find regulatory elements.

RESULT

Promoters are specific DNA sequences that play a role in controlling the transcription of genes located in upstream of the structural part of a gene. Analysis of sequence motive of TI gene promoter in silico has been done. Motives promoter gene TI obtained quite diverse. The promoter sequence has a motive in responding to several environmental factors, both biotic and abiotic. For example responsive to light, responsive to the presence of gibberellin hormone, responsive to heat stress, responsive to defense, and also responsive to the daily circadian mechanism of plants. In addition, to ensure that the collected sequence is a promoter sequence, the results of the in silico analysis also found some general motives of promoter sequence markers. For example, BoxI and Box4 motives, each found in sequence collection samples AY573237.1 and AJ271752.1.

Table 1 Motif of Trypsin Inhibitor Gene Promoters

| Accession Code | Genes Motive | Annotation |
|----------------|--|--|
| AY573237.1 | Box I ¹ , GAG-motif ¹ , TCT-motif ¹ , GARE-motif ² , HSE ³ , TC-rich repeats ⁴ | 1) light responsive element 2) gibberellin-responsive element |
| DQ417204.1 | Box I ¹ , GARE-motif ² , TC-rich repeats ⁴ | 3) heat stress responsiveness |
| AJ271752.1 | Box 4 ¹ , GAG-motif ¹ , GT1-motif ¹ , circadian ⁵ | 4) defense and stress responsiveness 5) circadian control |

DISCUSSION

The promoter is generally located on the 5'-flanking region. The promoter region is divided into 2, the proximal promoter region and the distal promoter region. The proximal promoter region serves as a transcriptional initiation site and the distal promoter region functions in the regulation of gene expression (Campbell *et al.*, 2008). Promoters have regulatory sequences that can recognize transcription factor binding sites (TFBS) for special expressions in specific developmental and environmental phases (Butler & Hannapel, 2012; Bacha *et al.*, 2015). Short-regulator sequences are

often called cis-regulatory elements (CREs) (Won *et al.*, 2009, Wittkopp & Kalay 2012).

Sequence motive of gene promoter of TI obtained from NCBI with accession code AY573237.1, DQ417204.1, and AJ271752.1 was analyzed using PlantCARE program on line obtained result that is 5 cis element responsive to light that is BoxI, GAG-motif, TCT-motif, Box 4, and GT1-motif, a cis element responsive to gibberellins that is GARE-motif, one cis element responsive to heat stress that is HSE, one cis element responsive to defense and stress that is TC-rich, and one cis element control circadian ie circadian .

BoxI, GAG-motif, TCT-motif, Box 4, and GT1-motifs that are part of a light-responsive promoter. The results of the study Bacha et al. (2015) obtained BoxI and GT1-motif on *susy* promoter sequences on tomato plants for response to light. BoxI, GAG-motif, TCT-motif, Box 4, and GT1-motif are also found on the promoter of the BBX gene in tomatoes (Chu et al., 2016). Light is a major factor that controls the circadian rhythm of various life processes such as growth, development, and stress response in plants such as biotic stress response (Arpaia et al, 1993).

Motif lain yang ditemukan adalah GARE-motif yang responsif terhadap giberelin. Hasil penelitian Heidari *et al.* (2015) GARE-motif ditemukan di daerah regulator gen *AT3G19850*, *AT2G15960* dan *AT3G45780* yang terkait dengan elemen responsif giberelin. Motif GARE tunggal pada beberapa promoter dapat meregulasi transkripsi hormonal secara langsung pada tingkat tinggi karena bekerja sama dengan elemen cis-acting lainnya (Rogers *et al.*, 1994).

Another motive found is GARE-motif that is responsive to gibberellins. GARE-motives were found in the *AT3G19850* gene regulatory area, *AT2G15960* and *AT3G45780* associated with the gibberellin responsive element (Heidari et al., 2015). A single GARE motif in some promoters can regulate hormonal transcription directly at high levels as it cooperates with other cis-acting elements (Rogers et al., 1994).

HSE (heat stress responsiveness element) that is responsive to heat stress. HSE was identified from *AtFER2*, *AtFER3* and *AtFER4* gene promoters at *A. thaliana* (Zang et al., 2017), a promoter of the tomato BX gene (Chu et al., 2016). TC-rich repeats are found in NAC genes in nuts (Wu et al., 2016), PtMPC gene promoters in poplar (Chen et al, 2013), PbCBL1 gene promoters in pear (Xu et al., 2015).

CONCLUSION

Sequence of the gene promoter of TI has a motive in responding to several environmental factors, both biotic and abiotic. For example responsive to light, responsive to the presence of gibberellin hormone, responsive to heat stress, responsive to defense, and also responsive to the daily circadian mechanism of plants. Therefore, the TI gene is an important gene candidate to be studied further in the breeding process as a source of genes for assembling superior plants that tolerant of biotic, abiotic, and high production.

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How to cite this article: Primandiri PR, Amin M, Zubaidah S et al. Motives of trypsin inhibitor gene promoters sequence from *vigna unguiculata* that respond to environmental factors are analyzed with bioinformatics. *International Journal of Research and Review*. 2017; 4(6):68-71.
