Morphology, Anatomy and Gene Expression Analysis of Cellulose Synthase (*CesA*) and Phenylalanine Ammonia-lyase (*PAL*) Genes from Napier Grasses Cultivar Pakchong 1 and Giant King Grass

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Abstract

In Thailand, there are many cultivars of Napier grass (*Pennisetum* spp.) which have high potential to develop as plant material for bioethanol production. However, the efficiency for bioethanol production depends on the ratio of cellulose to lignin content of each cultivar. Thus, this research aim to investigate the morphology and the expression of genes involved in cellulose (*CesA5* and *CesA7*) and lignin (*PAL*) synthesis of the two cultivars of Napier grass i.e. Giant King Grass (KG) and Pakchong 1 (PC1). The results showed that the leaf margin was clearly different while other morphological characteristics were not significantly different. mRNA expression analysis showed that relative gene expression of *CesA7* gene in KG was significantly higher than the expression in PC1, while *CesA5* and *PAL* gene expression levels were not different. Thus, KG might have higher cellulose content and will be selected for further study.

Introduction

Crude oil is a type of non-renewable energy, the need for alternative sources of energy is expected in the coming years. Among alternative bioenergy resources, lignocellulosic materials such as corn, sugarcane and grass have been identified as the prime source of biofuels [1].

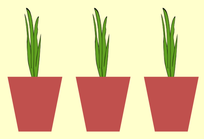
Napier grasses (*Pennisetum* spp.) are being used to produce biofuel and consists of many cultivars. It is also a non-food crop so it is better to used as lignocellulosic material for bioethanol production. Since the efficiency for bioethanol production depends on the ratio of cellulose to lignin content. Thus, the cultivar that showed higher expression ratio of *CesAs/PAL* gene will be selected for further evaluation as a potential lignocellulosic material.

Objective

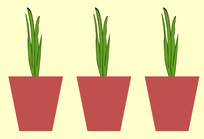
To investigate the morphology and the expression ratio of genes involved in cellulose (*CesA5* and *CesA7*) and lignin (*PAL*) synthesis in Napier grasses

Materials and Methods

Plant materials : two-month olds stem cuttings (3 replicates)



Pakchong 1 (PC1)



Giant King Grass (KG)

Morphology and anatomy study

Gene expression analysis by semi-quantitative RT-PCR

Results

The morphology of PC1 and KG was not significantly different (Table1,2).

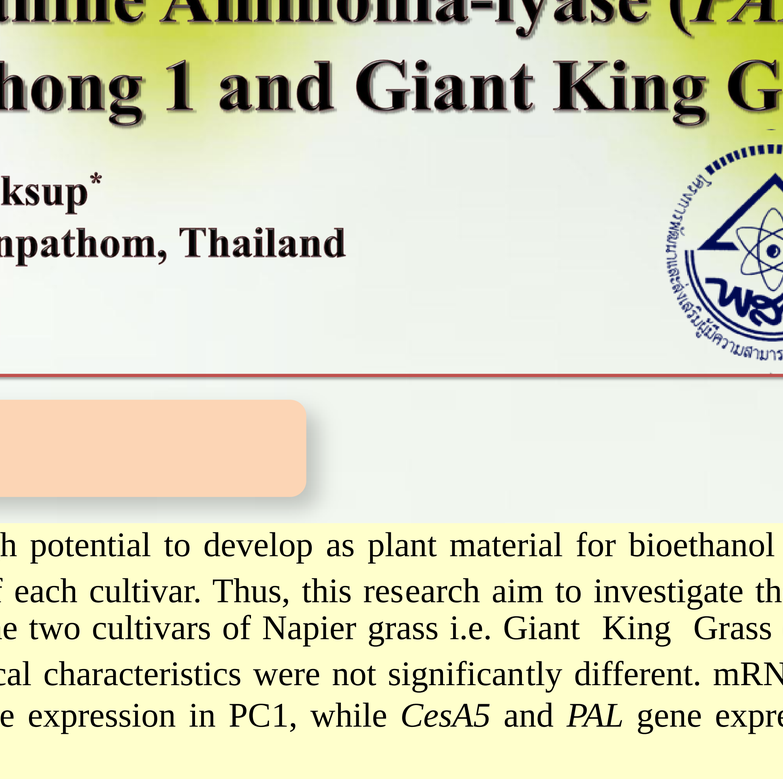
Table 1. Leaf morphological characteristics

Cultivar	Leaf no.	Leaf width (cm)	Leaf length (cm)	Leaf width : leaf length ratio
PC1	6 ± 1.33	1.76 ± 0.44	50.88 ± 3.13	0.036 ± 0.00
KG	5 ± 0.58	1.64 ± 0.22	40.74 ± 7.30	0.044 ± 0.01

Table 2. Stem morphological characteristics

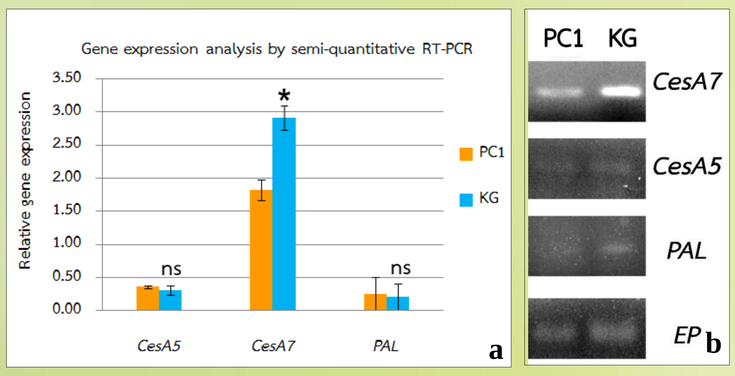
Cultivar	Stem circumference (cm)	Internode no.	Internode length (cm)	Stem height (cm)
PC1	2.51 ± 0.28	4.00 ± 0.00	8.63 ± 0.90	98.93 ± 12.04
KG	2.84 ± 0.41	2.33 ± 1.20	4.03 ± 2.46	61.20 ± 18.65

Results



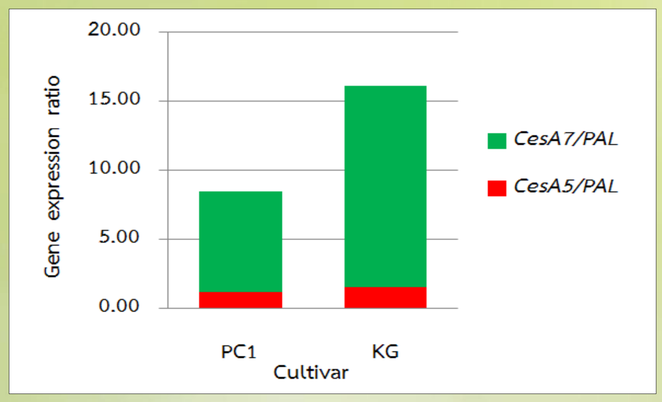
The anatomy of leaf is similar while the leaf margin is different (Fig1).

Figure 1. Cross section of leaf blade, leaf vein section and leaf margin.



The relative gene expression of *CesA7* gene in KG was significantly higher than the expression in PC1 (Fig2).

Figure 2. Relative expression of *CesA5*, *CesA7* and *PAL* genes (a). Expression levels of *CesA7*, *CesA5, PAL* and reference (*EP*) genes from PC1 and KG (b).



The expression ratio of *CesA5/PAL* and *CesA7/PAL* from KG is higher than from PC1 (Fig3).

Figure 3. Expression of ratio of *CesA5*/*PAL* and *CesA7*/*PAL* genes.

Discussion and Conclusion

Leaf margin of PC1 and KG showed different patterns as doubly serrate with short and long teeth, respectively (Fig1e, Fig1f). However, the morphology and the anatomy were not significantly difference. It has been reported that *CesA* and *PAL* genes showed positively related to the cellulose and lignin contents [2,3]. In this study, the expression ratio of *CesA5/PAL* and *CesA7/PAL* from KG is higher than from PC1 (Fig3). Thus, the ratio of cellulose to lignin content might greater in KG. Therefore, KG will be selected for further study and validation as a potential lignocellulosic materials.

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Reference

1. Kumar, R., Singh, S., & Singh, O. (2008). Bioconversion of lignocellulosic biomass : biochemical and molecular perspectives. *The Journal of Industrial Microbiology and Biotechnology*, *35*(5), 377–391.
2. Rohde, A., Morreel, K., Ralph, J., Goeminne, G., Hostyn, V., Rycke, R. D., Kushnir, S., Doorsselaere, J. V., Joseleau, J. P., et al. (2004). Molecular phenotyping of the *PAL1* and *PAL2* mutants of *Arabidopsis thaliana* reveals far-reaching consequences on phenylpropanoid, amino acid, and carbohydrate metabolism, *The Plant Cell*, *16*, 2749–2771.
3. Turner, S.R., & Somerville, C.R., (1997).Collapsed xylem phenotype of Arabidopsis identifies mutants deficient in cellulose deposition in the secondary cell wall. *Plant Cell*, *9*, 689–701.