Fourier Transform Mid-Infrared (FTIR) Spectroscopy to Identify Tannin Compounds in The Panicle of Sorghum Mutant Lines

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Fourier Transform Mid-Infrared (FTIR) Spectroscopy to Identify Tannin Compounds in The Panicle of Sorghum Mutant Lines

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Abstract. G5 and G8 are two sorghum mutant lines projected as sorghum varieties for ruminant's feed. However, most of the sorghum panicles contains phenolic compounds, especially in the form of condensed tannins. The objective of this research was to investigate the phenolic and tannin compounds from the panicles of two sorghum mutant lines. The second objective was to identify tannin content by Fourier Transform Mid-Infrared (FTIR) Spectroscopy in the sorghum panicles. The two mutant lines were compared with Pahat variety. This research was arranged into a randomized complete block design with two factors. The first factor was the sorghum variety/mutant lines, whereas the second factor was the generative phase of harvesting time (flowering, soft dough and hard dough). The results showed that the panicles of G5 sorghum contained the lowest total phenol compounds ($P<0.01$). The average mean of G5 and G8 mutant lines produced lower total tannin content (27.3 and 18.2%, respectively) than Pahat variety ($P<0.01$). Generally, total phenol and total tannin in all variety/mutant lines increased with the increasing maturity stage ($P<0.01$). All the spectra of the sorghum panicle samples in different harvesting age investigated shared certain special similarities. A band due to alkane medium (C-H) stretch was observed at 2937-2854 cm^{-1}. At 1521-1514 and 1446-1408 cm^{-1}, a band due to the C-C aromatic compounds was observed. Based on those findings, it can be concluded that G5 and G8 mutant lines produced less total phenol and total tannin contents than Pahat variety. Meanwhile, FTIR could be used to describe the profile of tannin compounds qualitatively.
Keywords: FTIR, Mutant lines, Sorghum, Tannin

1. Introduction

Sorghum is a cereal crop that has potential use as forage in ruminant [1]. The mixture of stem, leaves and panicle (all edible part) was usually utilized as forage. A nutritional constraint of sorghum grain in the panicle is the interaction of nutrient content especially protein with polyphenols [2]. Tannins are also present in sorghum grain, which are the antinutrient compounds [3]. Tannins may interact with and form complexes with proteins, which may lead to precipitation due to the large size of tannins [4]. Furthermore, sorghum tannins are known to inhibit enzymes such as amylases [2]. Jayanegara and

Sofyan [4] also reported that tannins are secondary metabolites that are synthesized by plants and could form complex compounds with other macromolecules. Tannin contents are also influenced by differences in varieties/lines of plants. Therefore, it is necessary to investigate the compounds of tannins in different variety/lines of sorghum. The National Nuclear Energy Agency of Indonesia (BATAN) has developed two mutant lines projected as sorghum varieties for ruminant's feed, namely G5 and G8. The two mutant lines were mutation products from Pahat variety as grain sorghum. Based on agronomic characteristics of leaf midrib color, Pahat variety is white midrib (WMR) sorghum type. The G5 and G8 are brown midrib (BMR) and green midrib (GMR) type, respectively. There is no information from previous studies around the different tannin compounds associated with the types of sorghum.

Among the techniques nowadays, Fourier Transform Mid-Infrared (FTIR) is widely used in the characterization of powder samples of plant origin. Several studies related to tannin measured by FTIR were done to identify tannin compounds in *Acacia mangium* Bark [5], maritime pine (*Pinus pinaster*) [6], pure oak extract [7] and quebracho [8]. From the above information, there has been no information about tannin compounds in the panicle of sorghum, that measured qualitatively by FTIR. This study aimed to investigate the phenolic and tannin compounds from the panicles of two sorghum mutant lines. The second objective was to identify tannin content qualitatively by FTIR in the sorghum panicles.

2. Experimental Procedures

2.1. Sample Preparation

A collection of Pahat variety, G5 and G8 mutant lines sorghum seeds were obtained from agricultural division CIRA BATAN and grown in field station laboratory CIRA BATAN (6º17'38.9" S; 106º46'28.8" E, elevation 38 m) from February to May 2018. The average temperature conditions were 28ºC. Harvesting was done during flowering (70 days after sowing/das), soft dough (95 das) and hard dough (115 das) phases. Panicles were placed into individual paper bags and dried for 48 h at 60ºC (n=3 replicates in 3 block treatments). Samples were then grinded at 1 mm and prepared for FTIR measurement and chemical analyses.

2.2. FTIR Measurement

Preparation sample was made from ± 2 mg sample and 200 mg K Br, mixed quickly homogeneously using mortar. Furthermore, sample measured using IRPrestige-21 Fourier Transform Mid-Infrared (FTIR) Shimadzu® Japan within the wave range of 4000-500 cm^{-1} in ± 60 seconds. Shimadzu IR solution 1.50 (Shimadzu Corporation) was used to determine peak positions. Tannic acid (Sigma-Aldrich) was also measured as tannin spectra standard.

2.3. Total Phenol, Total Tannin and Condensed Tannin Determination

Determination of total phenol in samples used Folin-Ciocalteu method [9]. Each sample was dissolved in 10 ml of pure acetone for 20 minutes at room temperature using sonicator. The sample of 0.5 ml was put in 10 ml volumetric tube containing 0.5 ml Folin-Ciocalteau reagent, 5 ml distilled water, 1,5 ml Na_2CO_3 solution and filled the volume using distilled water. The sample was left for two hours and then observed using spectrophotometer at 765 nm wavelength. Results were compared with standard curves obtained from gallic acid solution (50, 100, 150, 250 and 500 mg/l).

Determination of total tannin employed Makkar et al. [10] method. An amount of 100 mg of polyvinylpolipyroledone (PVPP) was filled in vial tube. As much as 1 ml of destilled water was added along with 1 ml tannin extract (100 mg PVPP was enough to bind 2 mg total phenol) and then homogenized. The vial tube containing solution was incubated for 15 minutes in 20ºC and then centrifuged at 3000 rpm for ten minutes. As much as 1 ml supernatant was filled into vial tube and added with 0.5 ml folin reagent and 2.5 ml Na_2CO_3. All reagent was homogenized and allowed for 40 minutes at room temperature. Absorbance reading was observed using spectrophotometer at 725 nm wavelength.

Results were compared with standard curves. The total tannin compound is the difference between total phenol and total phenol non tannin.

Condensed tannin measurements using Makkar et al [10] (2003) method. As much as 0.5 ml of samples extract added with 3 ml buthanol-HCl reagent and 0.1 ml ferric reagent, and then homogenized. All samples were incubated in 97-100ºC temperature for 60 minutes. The samples were cooled and observed using spectrophotometer at 550 nm wavelength. Condensed tannins were calculated as: absorbance × 78.26 × dilution factor / %DM.

2.4. Data Analysis

Data of total phenol, total tannin and condensed tannin were analyzed using a randomized complete block design with two factors. The first factor was the sorghum variety/mutant lines, whereas the second factor was the generative phase of harvesting time (flowering, soft dough and hard dough). Data were analyzed using analysis of variance (ANOVA) and tested by Duncan Multiple Range Test [11].

3. Results and Discussion

3.1. Fourier Transform Mid-Infrared (FTIR) Spectroscopy

FTIR spectra for tannic acid and sorghum panicles at different ages are shown in Fig. 1 and 2, respectively. FTIR measurements were carried out to determine spectrum suspected to be related to phenol or tannin content. Figure 1 shows that the spectrum of tannic acid where it can find a strong absorbtion around 3600-3000 cm^{-1} with a wide and strong band centered at 3275 cm^{-1}. This band is assigned to the hydroxyl groups (O-H) H-bonded broad and strong and C-H (aromatic medium) (Table 1). At 2833 cm^{-1}, a sharp peak due to the alkane medium (C-H) is observed.

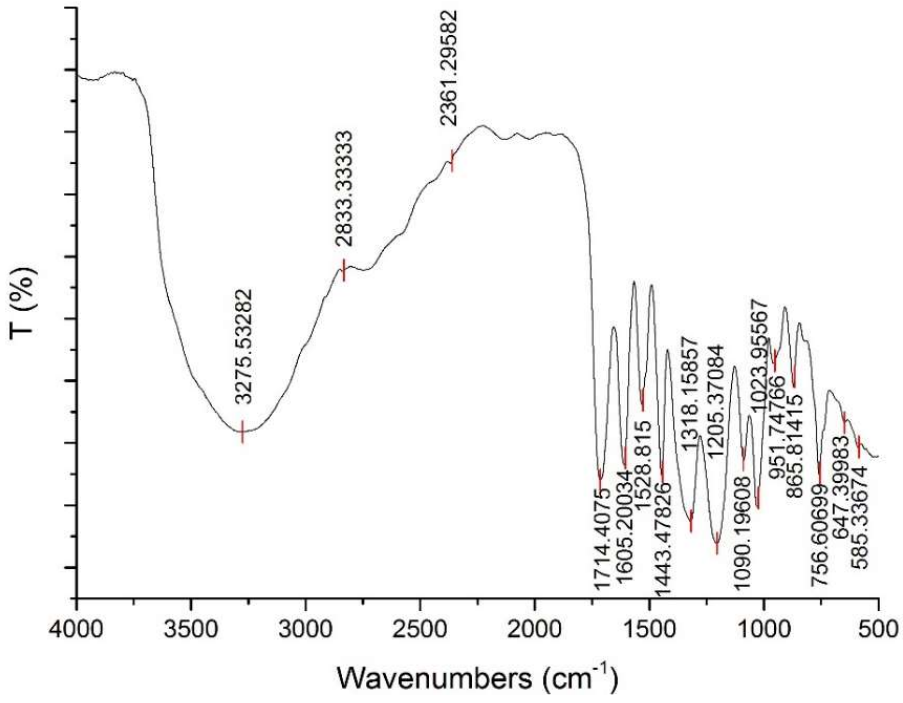

Figure 1. FTIR spectra of tannic acid.

Sharp peak at 2927-2860 cm^{-1} associated with the symmetric and antisymmetric -C-H- stretching vibrations of CH_2 and CH_3 groups respectively [12]. Tannic acid contains some aromatic esters due to the signal characteristics of carbonyl groups C=O stretching (1714 cm^{-1}) and C-O (1205 cm^{-1}). Pantoja-

Castro and Gonzales-Rodriguez [12] stated that C=O stretching and C-O vibration observed near 1730-1705 cm^{-1} and 1300-1100 cm^{-1} range respectively.

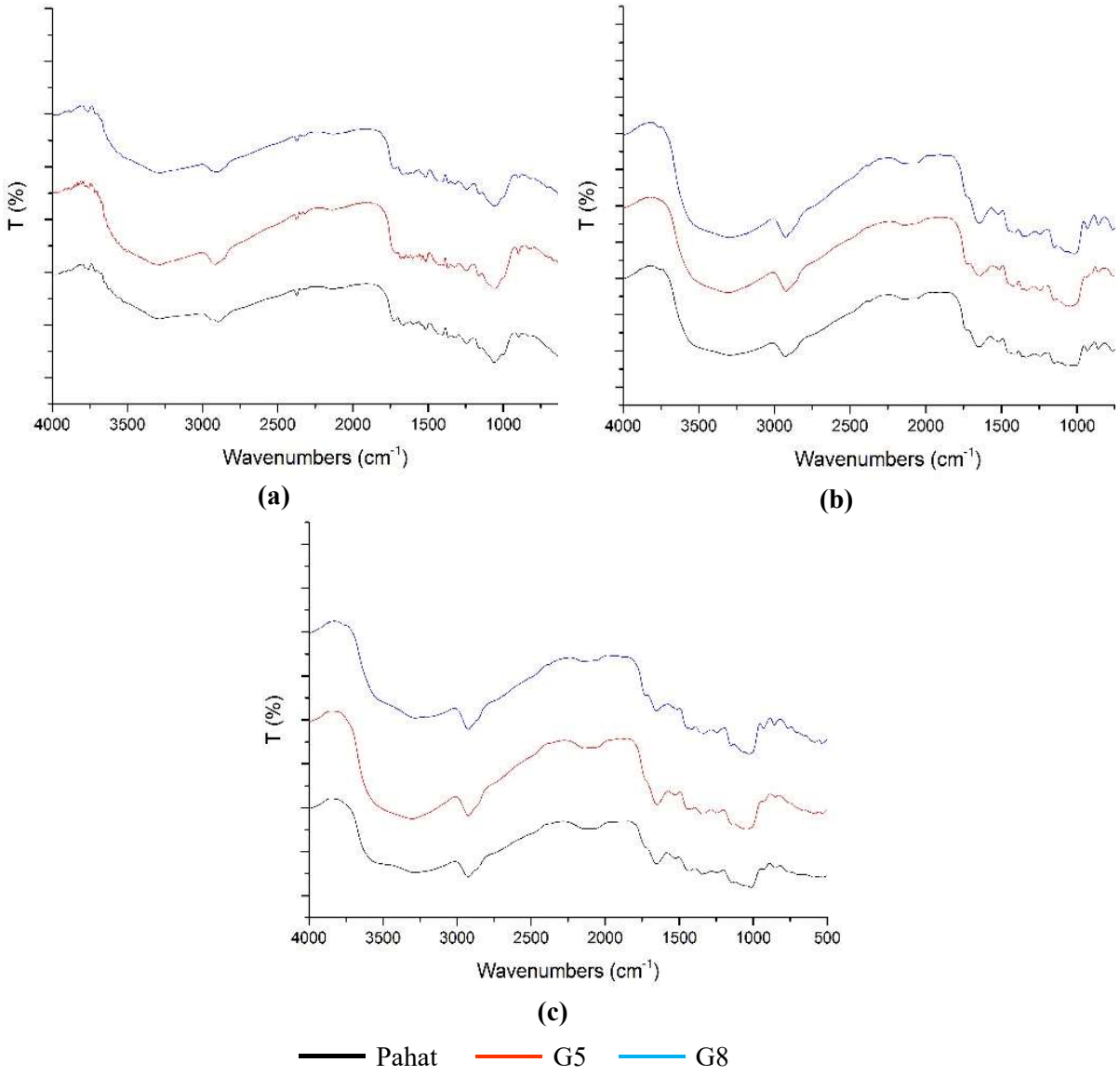

Figure 2. FTIR Spectra of sorghum panicles at flowering (a), soft dough (b) and hard dough (c) phase.

All the spectra of the sorghum panicle samples in different harvesting age investigated shared certain special similarities (Figure 2). A broad band due to the OH stretch is observed near 2211-3277. It was similar area with tannin acid at 3275 cm^{-1} wavenumbers (Figure 1). A band due to alkane medium (C-H) stretch are observed at 2937-2854 cm^{-1}. At 1521-1514 and 1446-1408 cm^{-1}, a band due to the C-C aromatic compounds are observed. A weak signal at 1571 cm^{-1} is related to carbonyl groups [5]. Peaks determining during 1600-1400 cm^{-1} are characteristics of aromatic compounds [5, 13]. Interesting things were obtained in the spectrum that showing C=O stretching vibration (near 1714 in tannic acid), where there were no peaks formed from the three sorghum panicles at hard dough phase. The influence of this observe might illustrate the quantitative differences of tannin content with Folin-Ciocalteu [9] and Makkar et al. [10] measurement. Various peaks in the 1300-600 cm^{-1} associated with substituted benzene ring [5, 13]. In recent study, the differences in vibration obtained at 1300-600 cm^{-1} wavenumbers from

different samples and harvesting phase. This is clearly obtained in smooth vibrations from all samples at flowering phase. This could affect in tannin compounds from quantitative measurement. Total phenol, total tannin and condensed tannin measurements were then carried out to quantify their characteristics.

Table 1. Characteristic of peak bands of FTIR-spectra [14].

Group	Wavenumber (cm^{-1})	Characteristics
O-H	3600-3200	H- bonded broad and strong
C-H	3100-3000	Aromatic medium
C-H	3000-2850	Alkane medium, sharp (stretch)
C-C	1600-1400	Aromatic medium-weak, series of sharp bands
C-O	1820-1670	Ester and carbonyl generally strong, conjugated lower
C-O	1300-1000	Alcohol and ether strong, ester two bands or more
1,2-disubstituted	1200-900	Benzene ring, three peaks, two medium, one strong
1,3-disubstituted	1100-700	Benzene ring, four peaks, two medium, two strong

3.2. Total Phenol, Total Tannin and Condensed Tannin Contents

Table 2 shows the quantitative measurement results of total phenol, total tannin and condensed tannin compounds. There were a significant interaction on the total phenol, total tannin and condensed tannin compounds between variety/mutant line and generative phase ($P<0.01$). The panicles of G5 sorghum produced the lowest total phenol compounds ($P<0.01$). The average mean of G5 and G8 mutant lines produced lower total tannin content (27.3 and 18.2%, respectively) than Pahat variety ($P<0.01$). The panicles of G5 and G8 also had lower condensed tannin compounds than Pahat ($P<0.01$). Generally, total phenol and total tannin in all variety/mutant line increased with the increased maturity stage ($P<0.01$).

Table 2. Total phenol, total tannin and condensed tannin content of sorghum panicle.

Generative Phase	Pahat	G5	G8	Mean
Total Phenol (mg GAE/g)				
Flowering	0.87±0.15	1.09±0.11	0.91±0.12	0.95±0.19^{c}
Soft dough	1.33±0.14	1.05±0.07	1.10±0.31	1.12±0.35^{b}
Hard dough	1.58±0.06	1.06±0.11	1.47±0.37	1.14±0.30^{a}
Mean	1.26±0.28^{a}	1.06±0.09^{b}	1.16±0.33^{ab}	
Total Tannin (mg TAE/g)				
Flowering	0.80±0.13	0.56±0.08	0.41±0.09	0.59±0.31^{c}
Soft dough	1.16±0.09	0.95±0.06	0.87±0.04	0.99±0.23^{b}
Hard dough	1.33±0.06	1.04±0.09	1.39±0.35	1.24±0.28^{a}
Mean	1.09±0.36^{a}	0.84±0.21^{b}	0.89±0.46^{b}	
Condensed Tannin (mg CE/g)				
Flowering	0.14±0.09	0.32±0.02	0.16±0.08	0.21±0.04^{b}
Soft dough	0.21±0.04	0.34±0.15	0.23±0.06	0.26±0.12^{b}
Hard dough	1.22±0.05	0.39±0.07	0.67±0.05	0.76±0.08^{a}
Mean	0.52±0.06^{a}	0.35±0.11^{b}	0.35±0.07^{b}	

gallic acid equivalent (GAE); tannic acid equivalent (TAE); catechin equivalent (CE); ^{abc}different superscripts within row or coloumn in same parameters were different.

Dykes et al. [15] showed that genetics affect phenol content and antioxidant activity in sorghums. The highest phenol and tannin compounds were produced by Pahat variety. This due to the high productivity factor of Pahat sorghum as grain variety. Wahyono et al [1] reported that Pahat variety is food/grain type of sorghum which has large stem diameter and high grain production characteristics. G5 is the type of BMR which associated with low lignin and high non fiber carbohydrate (NFC) content in stem. This will affect seed production, including the components of secondary metabolites. However, this needs further investigation. The tannin content increased with the increased generative stage due to the natural mechanism of plants to increase the synthesis of secondary metabolite compounds to protect the maturation process of grain. Tannins are phenolic compounds that protect the sorghum grain agains insect, birds and fungal attack [2]. The different result was reported by previous study. Sriagtula [3] stated that the highest tannin content was produced at soft dough stage. This differences due to the different in sorghum type, variety and climate condition during cultivation. The lowest value of tannin at flowering phase (in all samples) was also related to the results from FTIR measurements, which shows that there is no peak in 765 cm^{-1} wavenumbers (1,3-disubstituted, benzene ring) (Figure. 2).

The range of tannin content in sorghum panicles according to Sriagtula [3] is around 0.12-1.04% dry matter (DM). Duodu et al. [2] reported that high tannin sorghum cultivars contain 2-4%, enough to bind all protein content in grain. In present study, the range of tannin content is around 0.41-1.39 mg TAE/g. The part observed in this study is panicle, therefore there are some differences in the range of tannin content. The measurement unit in this study also using equivalent standard by Dykes et al. [15], While in previous study using % DM. The three types of sorghum were used in this study were white grain types of sorghum. White grain sorghum contains lower total phenol and condensed tannin compounds than red and brown grains [15].

4. Conclusion

G5 and G8 mutant lines produced less total phenol and total tannin contents than Pahat variety. Meanwhile, FTIR could be used to describe the profile of tannin compounds qualitatively. Total phenol, total tannin and condensed tannin were also increased in all variety/mutant line with the increased maturity stage.

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