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Antioxidant properties of sorghum ratoon (*Sorghum Bicolor)* of local Bima Indonesia

Fitrahtunnisa[1*], Ermin Widjaja[2] and Ulyatu Fitrotin[1]
[1]The Assessment Institutes for Agricultural Technology of West Nusa Tenggara, Jl. Raya Peninjauan Narmada, Mataram West Lombok, Indonesia
[2]Indonesian Agency for Agricultural Research and Development, Jl. Tentara Pelajar No. 10 Bogor 16114 – West Java, Indonesia
fit_biotek@yahoo.co.id

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Abstract

Antioxidant activity, total phenolic content and chemical composition were studied in three local Bima-Indonesian sorghums, including onenon-pigmented (Gando Bura) and two pigmented pericarp varieties (Gando Keta and Latu Kala). The antioxidant activities were evaluated by the DPPH Radical Scavenging Activity (RSA) method and the folin ciocalteu reagent was used to determine the total phenolic content. The results showed that pigmented varieties have DPPH RSA, phenolic content, tannin, vitamin E and protein higher than non-pigmented variety. Gando Keta has DPPH RSA 91.59% higher than Latu Kala (80.63%) and Gando Bura (42.13%). In the second harvesting (first ratoon), the DPPH Radical Scavenging Activity of Gando Keta decreased (14.35%), Latu Kala (52.6%) and Gando Bura (52.49%). The total phenolic content of Gando Keta decreased 20.42%, Latu Kala (9.58%) and Gando Bura (41.19%). Thus, this study informs that Gando Keta has the best antioxidant properties compared to other varieties among local Bima-Indonesian sorghum.

Keywords: Local, Bima, sorghum, ratoon and antioxidant.

Introduction

Indonesia has a great potential in developing diversified food products in the form of cereals and tubers. However, Indonesian people's dependence on wheat still cannot be replaced by local products. The high consumption of wheat flour in Indonesia can be reduced by substituting of local commodities. So that it can move the wheels of the Indonesian people's economy, especially the agricultural sector, one of which is sorghum[1].

The use of sorghumin Indonesia is still less popular and not optimal. So far, sorghum has only been used as animal feed, whereas sorghum is very suitable to be used as an agroindustrial commodity because of its high resistance to dry commodities, adaptability to high land, and low production costs.

West Nusa Tenggara is one of the provinces that has a fairly large amount of dry land and has great potential as a sorghum development area. There are at least six local sorghum varieties that have been registered as genetic resources of West Nusa Tenggara Province and will be developed as one of the strategic commodities to substitute wheat flour.After the first harvest, the sorghum crop was left alone by farmers without fertilizing. Even though these plants are still producing and have the opportunity to be cultivated better.

Conservation and Characterization of sorghum biodiversity has become an attention of researchers in the last several years. The main focus is the collected accession with high adaptation to limited environmental conditions. This will support food security in local agriculture, rural development and environmental sustainability[2].

Some source of natural antioxidants always be looked for and developed because of small effect on health[3,4]. A range of flavonoids, hydroxycinnamic acids, and hydroxybenzoic acids have been identified as polyphenols in sorghum[5]. The pigmented pericarp sorghum (red and black) contains tannin higher than the white pericarp sorghum[6]. Measurements and determination of phenolic compounds have been conducted in pigmented and six varieties of non-pigmented pericarp sorghum[7]. Classification of sorghum in order to grain, forage nutritive value and forage yield[8]. Lignin content and composition of 30 Indonesian sorghum accessions has been studied, without Local Bima-Indonesian sorghum[9]. Moreover, antioxidant properties of sorghum ratoon of Local Bima-Indonesian has not been studied. Therefore, the objectives of this study were to examine antioxidant activity, total phenolic content and chemical composition of three local Bima-Indonesian sorghum ratoon. This information contributes in creating functional grains with maximum health benefits through sorghum genotype selection.

Materials and methods

Sorghum plants were obtained from visitors land plot of the Assessment Institutes for Agricultural Technology (AIAT) of West Nusa Tenggara. Three local Bima-Indonesian sorghum were Gando Bura (white pericarp with tannin), Gando Keta and Latu Kala (red to black pericarps with tannin). This local sorghum was AIAT collection and cultivation in land plot of AIAT West Nusa Tenggara. Sorghum plants were grown in the same environment and harvested at the same time. Sorghum is planted in beds; each variety is grown in a different bed with three sorghum seeds per planting hole. At the beginning of planting until the early vegetative phase, the plants are watered manually every day. Sorghum plants were fertilized with Standard Operational Procedure of sorghum fertilizer[10]. Sorghum is harvested when the sorghum is ripe on a scale with the seeds at the base of the panicle starting to harden when it collects with nails.

The sorghum panicles are then dried in the sun and knocked out manually by hand and stored in the AIAT Source Seed Management Unit storage warehouse at -20. During the first harvest, the stems of the sorghum plant are cut and left 10cm from the ground with the aim of being as a ratoon.

Quantification of Condensed tannin: Condensed tannins were determined by Feregrino-Pérez[11] methods. Results were calculated and expressed as mg catechin equivalent (CE)/g sample, on dry basis (db).

Proximat Composition: Composition of proximate, protein, ash and carbohydrate contents were analyzed in triplicate, according to AOAC[12]. The lipid content followed the AOCS[13] protocol.

Preparation of crude extract of three lokal Bima-indonesian sorghum: Crude extract of three lokal Bima-Indonesian sorghum were obtained by Wu, G. et al.[5] method. Sorghum seed was powdered and sieved. One gram of sorghum powder was transferred into flasks and added with 5mL methanol solution. The flasks were then located in shaker (SIBATA, SU-2TH) (120 rpm) for 1h in room temperature, followed by maceration at 4°C for 24h. The crude extracts were obtained by centrifugation (eppendorf centrifuge 5417 R Hamburg German) at 4000g 4°C for 10 min, filtration through a Whatman paper no 42. This treatment was done twice, the first supernatantand second supernatant were collected and was stored at -4°C, until analysis for DPPH radical scavenging activity and total phenolic content determination.

DPPH antioxidant assay: Radical Scavenging Activity DPPH of three local Bima-Indonesian sorghum varieties antioxidant activity was determined by Wang, J. et al.[14] method. 1mL of sorghum extract was added with 3mL of DPPH 0.375mM, and incubated for 30 min in the dark at room temperature. The absorbance was measured at 522nm by a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). This activity is given as percent DPPH radical scavenging which is calculated with following equation.

$$\text{Scavenging activity \%} = \left[\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}}\right] X\ 100\%$$

The control contained 3mL of 0.06mM DPPH solution and 1 mL of methanol. Ascorbic acid was used as positive controls. Data were reported as means ± SD for three replications.

Analysis of total phenolic content: The total phenolic content in the crude extract of sorghum grains was analyzed base on the Folin-Ciocalteu methods[15]. 2mL of sorghum extract was added with 1mL Folin-Ciocalteu reagents, mixed, and stand for 1 min. Then 4mL of 4% of sodium carbonate (Na_2CO_3) solution were added, mixed, and located in a dark room for 2h at room temperature. Absorbance of the resulting blue complex was measured at 760nm using a Shimadzu UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). As a standard using gallic acid and methanol as the blank. The results were expressed as mg gallic acid equivalents (GAE)/g of sorghum grains. Data were reported as means ± SD from 3 replication.

Statistical analysis: Completely randomized design in triplicate was used in this experiment. Results are presented as means value ± standard deviation. One-way ANOVA; pair-comparison of treatment means was achieved by Duncan's procedure at $P<0.05$ using statistical software SPSS 17 for Window was used for statistical analysis in this study.

Results and discussion

Local Bima-Indonesian sorghum varieties and chemical composition: Gando Bura, Gando Keta and Latu kala are local varieties obtained from Bima, Sumbawa Island, West Nusa Tenggara province, Indonesia. Classification of sorghums according to their genetics and chemical composition are categorized into three groups: first type: white sorghum that non pigmented pericarps without tannin, second type: pigmented pericarps (usually red colored) have testa without tannins; and third type: pigmented testa with condensed tannin and bird resistant[16]. Based on sorghum classification above, the physical characteristics of sorghum Gando Bura is categorized into first type, white pericarp with tanin, while Gando Keta is categorized in second type, pigmented pericarp with tannin, and Latu Kala is categorized in third type, pigmented with tannin and bird resistant. Figure-1 shows the physical characteristics of the sorghum Gando Bura, Gando Keta and Latu kala.

The color of pericarp sorghum is influenced by the amount of anthocyanin content in each variety. White, red, brown, and black are usually sorghum pericarp color. The previous experiment has resulted that the expression of B1 and B2 genes controlled the pericarp colour and the tannins require both the genes to be expressed. Tannins are high-molecular weight polyphenols in sorghum which are known as proanthocyanidins[16].

A structure located of tannin between the pericarp and the endosperm of grain of some sorghum varieties. Gando Keta and Latu Kala have composition of protein, vitamin E, and tannin higher than Gando Bura (Table-1).

Table-1: Chemical composition of local Bima-Indonesian sorghum.

Parameter	Varieties		
	Gando Bura	Gando Keta	Latu Kala
Ash (%)	1,44	1,56	3,68
Total Fat (%)	3,24	3,46	2,91
Protein (%)	7,73	8,59	8,9
Carbohydrate (%)	73,97	74,13	71,99
Vitamin E (mg/100gr)	0,897	3,396	1,107
Tannin (mg/100gr)	90,68	485,26	485,86
Ca (mg/100gr)	27,885	28,011	27,765
P (mg/100gr)	83,001	89,137	74,661

Antioxidant activity of sorghum ratoon of local Bima Indonesian: The three varieties of sorghum observed exhibited significant variation in DPPH Radical Scavenging Activity (RSA) (Figure-2) at $p<0.05$. Variety Gando Keta had the highest RSA at 91.59% followed by Latu Kala (80.64%), and Gando Bura (42.13%). The white and color pigmented pericarps show a significantly different in DPPH RSA. The color pigmented had higher significantly different in DPPH RSA ability than the white pericarps. This result in line with other researcher that have described higher DPPH free RSA in pigmented red pericarp sorghum varieties than the white pericarp[5,7]. These results are due to the tannin content (Table-1) and total phenolic content in each variety (Figure-3). Gando Keta has tannin concentration higher than Latu Kala and Gando Bura (Table-1). Tannin has ability to scavenge free radicals. Sorghum genotypes do not contain tannins have lower antioxidant capacity than sorghum containing tannins[4]. The other researcher resulted that the high antioxidant activity in sorghum is due to high of total phenolic content, total proanthocyanidin content, and variation of phenolic compound type in sorghum[7]. Flavonoidshy droxycinnamic acids and hydroxybenzoic acids were detected in sorghum. The concentration and composition of phenolic compound in sorghum is different across production environments and sorghum genotypes[5].

The % DPPH Radical Scavenging activity in this study higher than the previous result on the same method, it is due to the concentration of DPPH used in this study lower concentration. It assumed that Gando Keta has lower than black pericarp (Shawaya variety=material sorghum was used in previous research) in % DPPH radical Scavenging Activity[5].

In the first ratoon, % DPPH RSA of three local varieties sorghum decreased. It was due to the decrease of total phenolic content of three Local Bimasorghum. Gando Keta decreased smaller than the others. It was influenced by genetic of Gando Keta and showed that Gando Keta is more tolerant in critical condition than other lokal Bima varieties.

Phenolic content of sorghum ratoon of local Bima Indonesia: The three varieties of sorghum observed exhibited significant variation in total phenolic content (TPC) (Figure-3) at $p<0.05$. Variety Gando Keta had the highest TPC 10.8.2 mg/g GAE followed by Latu Kala (2.316 mg/g GAE), and Gando Bura (1.984 mg/g GAE). This study used methanol solvent to extract phenolic compounds in the crude extract of local Bima-Indonesian sorghum which is same with[5]. Using this method the phenolic compound was detected in red to black sorghum were Hydroxycinnamic acids, 3-deoxyanthocyanidins, flavones, dihidroflavonol and flavanone in some varieties. Gando Keta and Latu Kala has red pericarp contains Hydroxycinnamic acids (caffeic acid and ferrulic acid in lower concentration than black pericarp), 3-deoxyanthocyanidins (luteolinidin in low concentration than black pericarp), Flavones (luteolin in low concentration, and apigenin is not detected), dihidroflavonol (taxifolin is in higher concentration than black pericarp), and Flavanone (naringenin in high concentration). Gando Keta has darker red color than Latu Kala is due to the concentration of total phenolic content of Gando Keta more than Latu Kala (Figure-3). Furthermore, the black pericarp contain more phenolic compound such as Hydroxycinnamic acids (caffeic acid and ferrulic acid in high concentration), 3-deoxyanthocyanidins (luteolinidin in low concentration and apigenidin in high concentration), Flavones (luteolin in low concentration, and apigenin in high concentration), dihidroflavonol (taxivolin not detected), and Flavanon (naringenin in high concentration). Therefore, the black pericarp higher in % DPPH RSA than red pericarp. In white pericarp of Gando Bura sorghum is not detected Hydroxycinnamic acids, 3-deoxyanthocyanidins, Flavones, dihidroflavonol and Flavanon. It is hypothesized that the phenolic compound scavenge free radical in white Gando Bura just tannin in methanol solvent.

Furthermore the other researcher used different extraction solvent (70% acetone extraction solvent at ambient temperature) resulted that black pericarp contained catechin, Pentahydroxyflavanone-(3->4)-catechin-7-O-glucoside and catechin isomeras the most active compound in scavenging for free radicals followed by 1-O-Caffeoylglycerol-O-glucoside[7]. Red pericarp contained caffeic acid as the most abundant of phenolic compound, ferulic acid, pyrano-naringenin-(3- > 4)-catechin-7- O-glucoside isomer, and n'.n'-dicafferoylspermidine, and white pericarp contained caffeic acid and n'.n'-dicafferoylspermidine. This difference of solvent conclude that methanol solvent detected anthocyanin and aceton solvent proanthocyanidin.

The content of phenolic compounds depends on genotype. The study of sorghum flavonoid pathway to control genetic with homology in other species, like Arabidopsis and maize has reported[17]. These flavonoid genetics included regulatory proteins, biosynthetic enzymes, and transporters. The gene Tannin1, along with two non-functional alleles of Tannin1: tan1-a and tan1-b regulated the accumulation of proanthocyanidin in sorghum grain. The gene Yellow seed1, which could encode a MYB protein, orthologous to the maize 3-deoxyanthocyanidin regulator P1 controlled the biosynthesis of 3-deoxyanthocyanidins in the sorghum pericarp[18]. In white sorghum QL12 was not detected individual flavonoids, so that the two genes, Yellow seed1 and Tannin1, might not be expressed in this sorghum genotype[5].

The phenolic content decreased in first ratoon. It was hypothesized that in this period sorghum plant cannot grow optimally, so that the formation of phenolic compound is not optimal. Paddy ratoon will decrease the yield. It was due to that time period of paddy ratoon was short. So, vegetative phase of paddy was not optimal.

The new shoots appear as the result of truncation of growing rice plants previously so that cell regeneration is inhibited due to rootstock organs and old roots[19].

Varieties	Leaves and steams	Panicles
Gando Bura		
Gando Keta		
Latu kala		

Figure-1: Physical characteristics of local Bima-Indonesian sorghum.

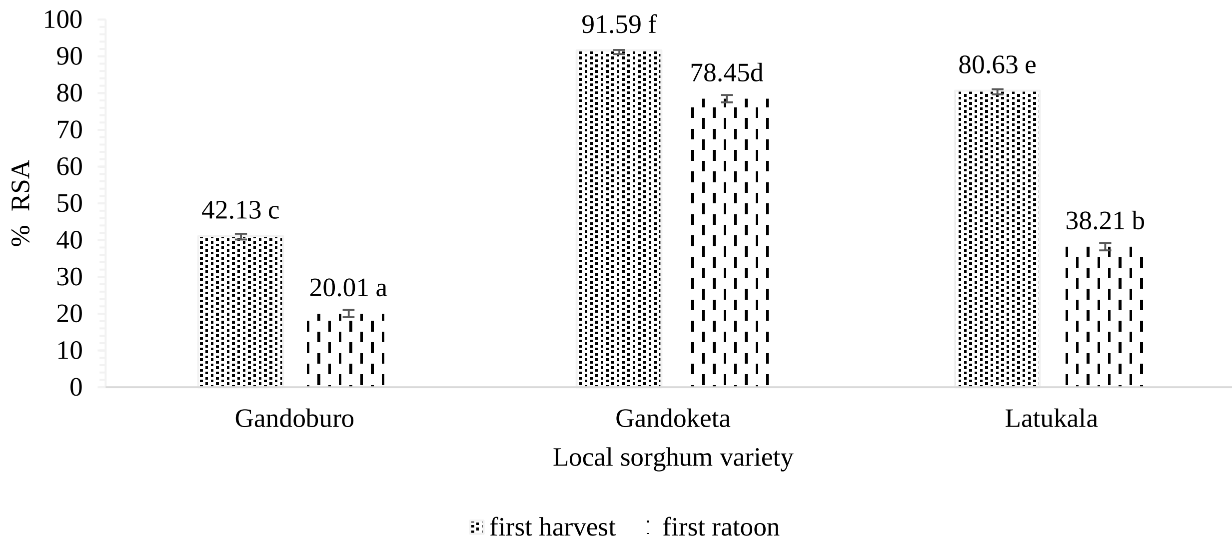

Figure-2: % DPPH-RSA of sorghum ratoon of lokal Bima Indonesian sorghum. Values with different superscripts are significantly different (P<0.05).

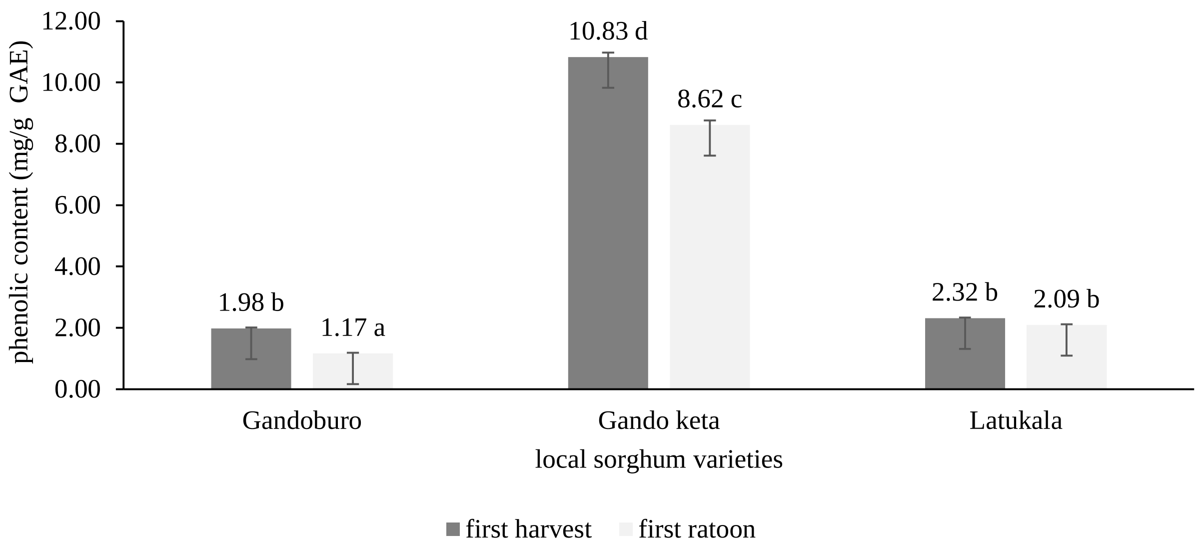

Figure-3: Total phenolic content of sorghum ratoon of local Bima-Indonesian sorghum. Values with different superscripts are significantly different (P<0.05).

Conclusion

Gando Keta and Latu Kala has antioxidant activity higher than Gando Bura. The higher of phenolic content and tannin in Gando Keta and latu Kala as cause of the higher antioxidant activity. The antioxidant activity of all local variety decreased in first ratoon and Gando Keta is the most tolerant.

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References

1. Kinanti P.S.K, B.S Amanto and W. Atmaka (2014). Kajian Karakteristik Fisik dan Kimia Tepung Sorghum (*Sorghum Bicolor* L) Varietas Mandau Termodifikasi Yang Dihasilkan Dengan Variasi Konsentrasi dan Lama Perendaman Asam Laktat. *Jurnal Teknosains Pangan*, 3(1), 135-144.

2. Manzelli, M., Stefano, B. and Vincenzo, V. (2005). Agricultural Biodiversity In Northwest Somalia – An Assessment among Selected Somali Sorghum (*Sorghum bicolor* (L.) Moench) Germplasm. *Biodiversity and Conservation*, 14, 3381–3392.

3. Durga, K.V., Sheela D. N. and Nair, C. (2017). In Vitro Determination of Anti-Cancer, Anti-Inflammatory and Anti-Diabetic Potential among Selected Species of Spilanthes Jacq. *Int. Res. J. Biological Sci.*, 6(6), 26-29.

4. Fitrotin, U., Utami, T. Hastuti, P. and Santoso, U. (2015). Antioxidant Properties of Fermented Sesame Milk using *Lactobacillus plantarum* Dad 13. *Int. Res. J. Biological Sci*., 4(6), 50-55.

5. Wu, G., Stuart, K.J., Janet F., Bornman, B., Sarita, J. B. and Zhongxiang, F. (2017). Changes in Whole Grain Polyphenols and Antioxidant Activity of Six Sorghum Genotypes under Different Irrigation Treatments. *Food Chemistry*, 214, 199-207 (2017).

6. Awika, J. M. and Rooney, L. W. (2004). Sorghum Phytochemicals and Their Potential Impact On Human Health. *Phytochemistry*, 65, 1199–1221.

7. Rao, S., Abishek, B., Santhakumara, B., Kenneth, A., Chinkwoa, B., Gangcheng, W., Stuart, K. J. and Christopher, L. B. (2018). Characterization of Phenolic Compounds and Antioxidant Activity in Sorghum Grains. *J. Cereal Sciences*, 84, 103-111.

8. Beana, R.L., Baumhardt, F.T. McCollum and McCuistiona (2013). Comparison of Sorghum Classes for Grain and Forage Yield and Forage Nutritive Value, 142, 20-26.

9. Wahyunia, Y., Miyamotob, Hartatia, T., Widjayantie, Windiastria, N.V. Sulistyowatia, Rachmata, A., Ragamustarib, S.K. and Tobimatsub, Y. K. (2019). Variation in Lignocellulose Characteristics of 30 Indonesian Sorghum (*Sorghum bicolor*) Accessions. *Industrial Crops and product*, 142, 1-10.

10. Nandang, S and Nana, S. (2013). Petunjuk Teknis Usaha Tani Sorghum. BPTP Jawa Barat.

11. Feregrino-Pérez, A. A., Berumen, L. C., García-Alcocer, G., Guevara-Gonzalez, R. G., Ramos-Gomez, M., Reynoso-Camacho, R. and Loarca-Piña, G. (2008). Composition And Chemopreventive Effect of Polysaccharides from Common Beans (*Phaseolus vulgaris* L.) on Azoxymethane-Induced Colon Cancer. *Journal of Agricultural and Food Chemistry*, 56(18), 8737–8744.

12. AOAC (2005). Association of Official Analytical Chemists Official Methods Association of Official Analytical Chemists Maryland: AOAC International 17.

13. AOCS (2009). Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction. Method Am 5-04. Official methods and recommended practices of the American oilchemists' society Official Methods and Recommended Practices of the American Oil Chemistrs Society Champaign.

14. Wang, J., Yuan, X., Jin, Z., Tian, Y. and Song, H. (2007). Free Radical and Reactive Oxygen Species Scavenging Activities of Peanut Skin Extract. *Journal of Food Chemistry*, 104(1), 242-250.

15. Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolibdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158.

16. Dykes, L., Rooney, L. W., Waniska, R. D., & Rooney, W. L. (2005). Phenolic Compounds and Antioxidant Activity of Sorghum Grains of Varying Genotypes. *J. of Agricultural and Food Chemistry*, 53(17), 6813–6818.

17. Rhodes, D. H., Hoffmann, L., Jr, Rooney, W. L., Ramu, P., Morris, G. P., & Kresovich, S. (2014). Genome-Wide Association Study of Grain Polyphenol Concentrations in Global Sorghum [*Sorghum bicolor* (L.) Moench] Germplasm. *J. of Agricultural and Food Chemistry*, 62(45), 10916–10927.

18. Ibraheem, F., Gaffoor, I. and Chopra, S. (2010). Flavonoid Phytoalexin-Dependent Resistance to Anthracnose Leaf Blight Requires A Functional Yellow Seed in *Sorghum bicolor*. *Genetics*, 184(4), 915–926.

19. Susilawati and Purwoko (2012). Pengujian Varietas Dan Dosis Pupuk Untuk Meningkatkan Pertumbuhan Dan Hasil Ratun-Padi Di Sawah Pasang Surut. Prosiding Seminar Nasional Lahan Suboptimal, ISBN (979-587-659-7)