

## RESEARCH ARTICLE

# Status of Rice Biochemical Composition under Lodging Treatment

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## Abstract

Extreme weather conditions due to strong wind and high rainfall cause rice lodging. Lodged plants have lower photosynthetic rate and nutrient transport capacity which resulting in lower rice quality. However, physiological process of rice plant under lodging condition is rarely reported. Therefore the objective of this research was to evaluate the biochemical compounds of two rice varieties under artificial lodging treatment. IR64 and HIPA8 varieties were planted at the Experimental Farm in Leuwikopo, Bogor Agricultural University (IPB) in November 2016 to June 2017. At grain filling stage, rice hills were enforced to lodge using manual force until culm break. Analysis of untargeted biochemical compounds was conducted at Research and Development Institute, Laboratory of Regional Health, DKI Jakarta where rice culms from upper and below breaking position were compared. Results showed that rice culms had different biochemical compounds after lodging, especially in fatty acid, terpene, alkane, and steroid group. Lodging culms had a total of 22 to 25 compounds compared to 15 to 17 of the control unlodged plants. It means that lodging stimulated different physiological process in rice plants. A decrease in fatty acid and an increase in the steroid level of lodged plants indicated an increase in oxidative stress of lodging condition. It is possible that low rice grain quality under lodging condition was caused by the changes in the plant physiological processes in response to the environmental stress.

Keywords: extreme weather, metabolomics, oxidative

stress, rice culm, rice lodging

## Introduction

Extreme weather condition, as an impact of climate change, tends to increase in both intensity and frequency (IPPC 2012). Study on extreme weather in reference to high temperatures, drought, and flooding have been widely conducted (Hairmansis et al., 2012; Shaheen et al., 2015; Tenorio et al., 2013; Yamin and Muntono 2005). However, studies on the impacts of strong wind and high precipitation on agricultural crops are limited.

Strong winds and high precipitation can affect rice production through lodging (Subash et al., 2011; Gbegbelegbe et al., 2014; Lesk et al., 2016). Ray et al. (2012) estimated that extreme weather incidents had decreased the area of harvested food crop by more than 30%. In Indonesia, Santosa et al. (2016) estimated that annual rice loss production due to extreme weather is about Rp 3.5 trillion, or around USD \$ 30 million. Therefore, extreme weather could be viewed and treated as abiotic stress to crops.

Plant will respond to stress through morphological, physiological and biochemical mechanisms (Potters et al., 2007). In rice, lodging reduces photosynthetic rate, transport of water and nutrients, yield and ultimately its grain quality (Setter et al., 1997; Duwayri et al., 2000; Kashiwagi et al., 2005; Islam et al., 2007; Sallasi et al., 2013). Therefore, the development of lodging tolerant variety mostly focuses on improving

the morphological characters of culm, including the strength of the culms (Zhang et al., 2014a). The culm strength is affected by the status of soluble sugars, starch, and cellulose within the plants (Zhang et al., 2014b). Kashiwagi et al. (2006) stated that lodging tolerant was determined by the plant's ability to re-accumulate carbohydrate and delay senescence.

Information on biochemical responses on lodging condition has rarely been reported. Biochemical responses can be studied through the biochemical compounds of the stressed crops which can be identified using separation and purification techniques such as gas chromatography and mass spectrometry-GCMS (Maciejewicz, 2001; Horai et al., 2010; Kind et al., 2013 Kusano et al., 2015). The objective of this study was to evaluate the status of biochemical compounds of two rice varieties under simulated lodging treatment. These data could be used to understand the marked decrease of rice quality after lodging events.

## Materials and Methods

### Plant Materials

The field study was conducted in November 2016 to June 2017 at the Leuwikopo Experimental Farm at the Bogor Agricultural University (IPB), Bogor Indonesia. Metabolite compounds were evaluated at the Laboratory of Regional Health DKI Jakarta (LABKESDA). Two rice varieties, i.e., IR 64 and HIPA8, obtained from Indonesian Center for Rice Research Institute (BBPADI) Sukamandi, West Java,

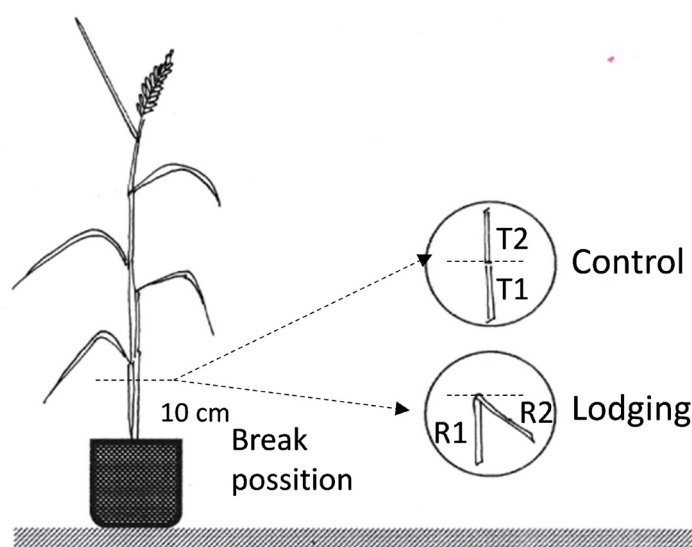
were used in this study.

Fourteen-day-old rice seedlings from the two rice varieties were planted in plastic pots containing about 12 kg media of a mixture of soil top soil and manure (4:1, v/v). Each pot contained one rice seedling. Fertilizer of NPK (15:15:15) at 300 kg ha<sup>-1</sup> and Urea (46% N) at 200 kg ha<sup>-1</sup> were applied three times during the course of the experiment, i.e., 100 kg ha<sup>-1</sup> NPK and 100 kg ha<sup>-1</sup> Urea at planting, 100 kg ha<sup>-1</sup> NPK and 50 kg ha<sup>-1</sup> Urea at 3 weeks after planting, and 100 kg ha<sup>-1</sup> NPK and 50 kg ha<sup>-1</sup> Urea at 6 weeks after planting. Water level was maintained 10 cm in height, and weeding was conducted manually. Pests and diseases were controlled using Furadan 3G<sup>®</sup>, Darmabas 500EC<sup>®</sup> and Antracol 80WP<sup>®</sup> according to manufacturer guidelines.

Lodging treatment was applied at grain filling stage, i.e., 2 weeks after flowering when 85% of the plants have flowered. Rice hill (a group of rice tillers) was pushed manually by using flat wood until the culm break. Breaking position was about 10 cm above soil surface. Harvesting was done when the grain is at the yellow stage and the culm was still green. The culms of 10 cm were sampled at grain harvest by separating below (R1) and above the breaking point (R2) (Figure 1). In the control plants, position of culm sampling was similar to the treated-hills, i.e., below (T1) and above (T2) the breaking point (Figure 1).

### Extraction and Identification of Compounds

An extraction procedure following the method developed by LABKESDA (2017) was used; 10 g



**Figure 1.** Lodging treatment at grain filling stage and sampling position of control and lodged culm. R1 and R2: segments below and above the breaking points of the lodged culm; T1 and T2: segments below and above the breaking points of the control plants.

fresh culm was oven-dried at 80°C for 72 hours and then grinded into fine powder. The powder was then extracted using 100 mL of 95% ethanol for five days. Then, 10 mL of the extracted solution was concentrated using oven at 60°C for about 1 hour.

The extract was analyzed using GC-MS Shimadzu QP2010 and solid phase micro extraction (SPME) injection. A total of 5 µL aliquot was prepared into quartz tubes in a pyrolysis unit, heated in an oxygen-free condition at a temperature of 400°C. The aliquot was injected at a temperature of 250°C and at interface temperature of 280 °C. Analysis used HP Ultra 2 capillary columns, total length of column 30 m, diameter 0.24 mm, and film thickness 0.25 µm. Initial temperature was set at 80°C and was then gradually increased by 3 °C min<sup>-1</sup> to a temperature of 150 °C for 1 min, then gradually increased by 20 °C min<sup>-1</sup> to a temperature of 280 °C for 26 min. Helium as a carrier gas (gas phase) was set at a constant speed of 1.2 µL min<sup>-1</sup>. Mass spectrometry ion source was set at 230°C and power 70eV.

### Data analysis

Biochemical compounds were identified from chromatogram. Biochemical components based on peak size and each retention time was further evaluated using the WILEY7 database and NIST library ver.2.0. Library of biochemical compound is available at National Center for Biotechnology Information, US National Library of Medicine (<https://pubchem.ncbi.nlm.nih.gov>). The data presented have an approximate percent of structural similarity of ≥ 95% according to procedure of WILEY7 and NIST library ver.2.0.

## Results

### Chromatogram

The chromatogram showed an increase in the biochemical components of the two rice varieties under lodging treatment in both below (R1) and above (R2) breaking point. In IR64 variety, R1 and R2 produced

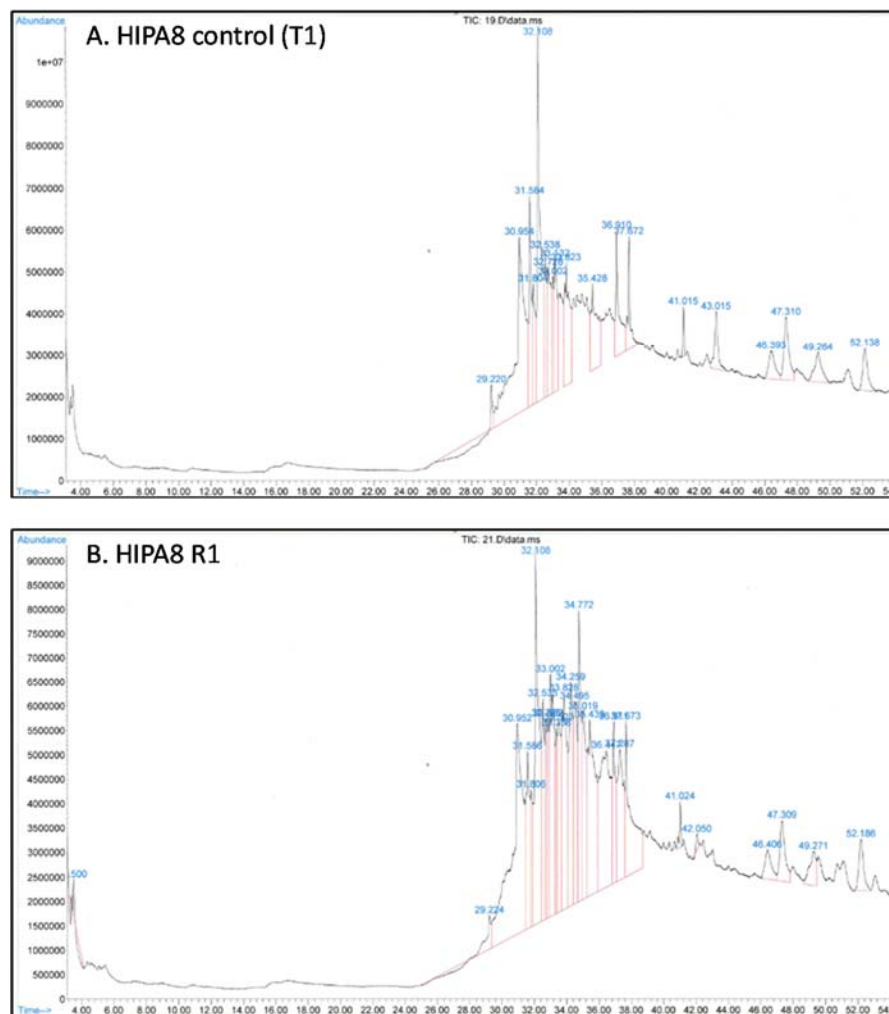


Figure 2. GC-MS chromatogram of HIPA8 culm below breaking point of control (A) and lodged rice plants (B)

25 and 22 biochemical compounds, respectively, at which 40-90% had similarity level on its structure. On the other hand, T1 of normal culm produced 17 compounds and T2 produced 15 components with structural similarity level of 56–90%. In HIPA8, each 24 compounds were detected on both R1 and R2 with a structural similarity level of 25–99% for R1 and 11–99% for R2 (Figure 2). In the control HIPA8, 17 was detected at (T1) with an 81–99% structural similarity and 15 at (T2) with structural similarity of 56–99%.

In lodging condition additional biochemical reactions occurred and different rice variety produced different compounds. As compared to the control, the biochemical compounds under lodging tend to increase in quantity as shown by peak size (Figure 2). It is likely that changing grain quality of rice under lodging as shown by previous study (Duwayri et al., 2000; Kashiwagi et al., 2005; Islam et al., 2007) may have been caused by the plants undergoing different physiological changes under lodging stress. Interestingly, different culm segment in control plants exhibited a slight difference in the amount of identified compounds. It is possible that different parts of the culms experienced different physiological processes, as the carbohydrate content of these parts were also different (Zhang et al., 2014b).

### Changes in the Biochemical Composition of Rice Culm

Based on the criteria of >95% confirmation of the compound on library, R2 of IR64 contained compound groups of fatty acids, terpene, alkanes and steroid (data not shown). Fatty acid group consisted of hexadecanoic acid, 9,12-octadecadienoic acid (Z, Z), 9,12-octadecadienoic acid (Z, Z), 9,12-octadecadienoic acid (Z, Z) and 9,12-octadecadienoic acid (Z, Z). Terpene consisted of squalena and ergost-5-en-3-ol. Alkanes mainly consist of straight-chain hydrocarbon, nonacosane. Steroids consisted of stigmaterol and beta-stigmaterol. In the control T2 (data not shown), fatty acids (hexadecanoic acid; 9,12-octadecadienoic acid [Z, Z]), terpene (squalena; ergost-5-en-3-ol), alkanes (docosane; nonacosane), and steroids (stigmaterol; beta-stigmaterol) were detected.

R1 of IR64 contained fatty acids (hexadecanoic acid; ethyl [9Z, 12Z]-9,12-octadecadienoic acid; 9,12-octadecadienoic acid [Z, Z]; 9,12-octadecadienoic acid [Z, Z]), terpene (squalena; ergost-5-en-3-ol), alkanes (nonacosane) and steroids (stigmaterol; beta-stigmaterol) (Table 1). In the control T1, fatty acids (hexadecanoic acid; 9,12-octadecadienoic acid [Z, Z]; 9,12-octadecadienoic acid [Z, Z]), terpene

Table 1. Main biochemical components of the control and lodged culms of IR64 rice variety

Peak number	Retention time (min)	Composition (%)	Formula	Weight (g mol <sup>-1</sup> )	Name of compound <sup>z</sup>
<i>T1 culm segment</i>					
2	30.98	23.95	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	<i>n-Hexadecanoic Acid</i>
5	32.11	15.06	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	<i>9,12-Octadecadienoic Acid (Z,Z)</i>
6	32.54	4.89	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	<i>9,12-Octadecadienoic Acid (Z,Z)</i>
7	32.73	6.97	C <sub>17</sub> H <sub>34</sub> O	254	<i>14-Methy-8-Hexadecyn-1-Ol</i>
13	36.90	3.88	C <sub>30</sub> H <sub>50</sub>	411	<i>Squalena</i>
14	37.65	3.09	C <sub>29</sub> H <sub>60</sub>	409	<i>Nonacosane</i>
17	47.16	2.18	C <sub>29</sub> H <sub>48</sub> O	413	<i>Stigmaterol</i>
<i>R1 culm segment</i>					
4	30.95	7.07	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	<i>Hexadecanoic Acid</i>
6	31.81	2.61	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	<i>Ethyl (9Z,12Z)-9,12-Octadecadienoic Acid</i>
7	32.12	8.61	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	<i>9,12-Octadecadienoic Acid (Z,Z)</i>
8	32.54	6.72	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	<i>9,12-Octadecadienoic Acid (Z,Z)</i>
18	36.92	4.17	C <sub>30</sub> H <sub>50</sub>	411	<i>Squalena</i>
20	37.67	4.40	C <sub>29</sub> H <sub>60</sub>	409	<i>Nonacosane</i>
23	46.56	1.82	C <sub>28</sub> H <sub>48</sub> O	401	<i>Ergost-5-en-3-ol</i>
24	47.52	4.96	C <sub>29</sub> H <sub>48</sub> O	413	<i>Stigmaterol</i>
25	49.44	2.56	C <sub>29</sub> H <sub>50</sub> O	415	<i>Beta-Sitosterol</i>

<sup>z</sup>The similarity of component ≥ 95%

(squalena), ester (14-methyl-8-hexadecyn-1-ol), alkanes (nonacosane), and steroids (stigmasterol) were detected.

R2 of HIPA8 contained fatty acids (hexadecanoic acid; 9,12-octadecadienoic acid [Z, Z]), terpene (ergost-5-en-3-ol), alkanes (iconase), and steroids (stigmasterol; beta-stigmasterol; 4,22-stigmastadiene-3-one). Below culm segment (R1) contained fatty acids (hexadecanoic acid; 9,12-octadecadienoic acid [Z, Z]), terpene (ergost-5-en-3-ol), alkanes (nonacosane; 1-cloro; 7.11-hexadecadienal), and steroids (stigmasterol; beta-stigmasterol; 4,22-stigmastadiene-3-one) (data not shown).

T2 culm of HIPA8 contained fatty acids (hexadecanoic acid; 9,12-octadecadienoic acid [Z, Z]; 9,12-octadecadienoic acid [Z, Z]; 9,12-octadecadienoic acid [Z, Z]; Z, Z-10,12-hexadecadien-1-ol-acetate), alkanes (nonacosane), and steroids (stigmasterol). T1 culm contained fatty acids (hexadecanoic acid; 9,12-octadecadienoic acid [Z, Z]), terpene (squalena; ergost-5-en-3-ol), vitamins (vitamine E), alkanes (nonacosane) and steroids (stigmasterol; beta-sitosterol; 4,22-stigmastadiene-3-one) (data not shown).

It is obvious that the compound found in the control was not always found in the culm after lodging treatment. Interestingly, the biochemical compositions between the culm of segments R1 and R2 was different while on the other hand T1 and T2 showed similar composition. The three compounds found in both control and the lodged crops of both varieties were 9,12-octadecadienoic acid, hexadecanoic acid and stigmasterol. Ester compound (2-aminoethanethiol hydrogen sulphate), Cholestan-3-one, 4,4-Dimethyl-(5 Alpha), and phytol were found in the lodged culm of both above and below breaking point segments of IR64. In HIPA8, an additional compound was found, i.e., 1-Cinnamyl-3-methylindole-2-carbaldehyde. Based on the total detected compounds in each segment of both varieties, biochemical profile according to the Heatmap cluster of IR64 and HIPA8 varieties is described in Figure 3.

There was a different pattern of biochemical

compounds of lodged culm between IR64 and HIPA8 (Table 2). In R1 of IR64, fatty acid group decreased by 43%, while on R2 actually increased even though only by 1.7%. Steroid compound in R1 increased by 245% while at R2 was by 132% of its control. In lodged HIPA8, total compounds of R1 and R2 were reduced by 36% and 72%, respectively. The number of compounds included in the alkane group increased in both R1 and R2 by 170% and 22%, respectively, which was the opposite to IR64. Moreover, lodged culm (R1) of both IR64 and HIPA8 had 9 biochemical compounds, whereas R2 had 7 compounds in IR64 and 10 in HIPA8.

## Discussion

Lodging affected the composition of the biochemical compounds of on the two rice varieties. The biochemical changes predominantly occurred in the groups of fatty acids, terpenes, alkanes, and steroids in both quantity and the number of the compounds. In both varieties, fatty acids was dominant, i.e., 35% on IR64 and 28% in HIPA8. In IR64, fatty acid content of T1 segment was 49% while R1 was 25%. In HIPA8, 55% was detected in T2 while R1 was 16% of total compound content.

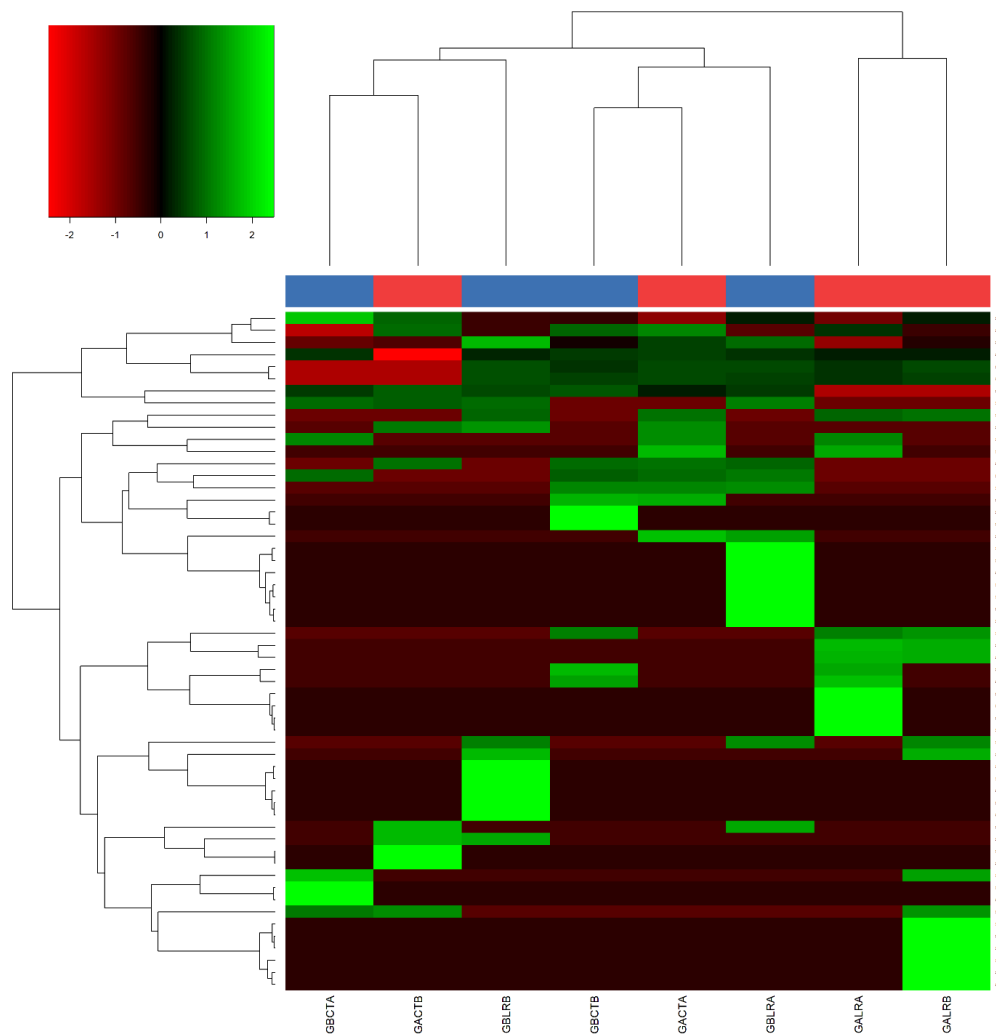
Steroid was the second largest compound group found in the rice culm. The average content in the control IR64 was 6%; lodging increased steroid content of the segment above the breaking point by 11% and by 8% below breaking point. On the other hand, steroid level in HIPA8 seemed independent of treatment, i.e., 12%. However, there was a tendency that proportion of steroid at below culm, irrespective of lodging treatment, was lower than upper culm, e.g., 9%.

The causes of differences in the biochemical composition of lodged and non-lodged culms are still unknown. Lang et al. (2012) stated that protein content from lodged rice grain is 0.021–0.024% higher than non-lodged grains, implying that low grain quality of rice under lodging condition is possibly due to changes in the biochemistry within the plants, however, the hypothesis needs further verification.

**Table 2.** Changes in bioactive compounds identified in lodged rice culms relative to the non-lodged culms

Varieties	Part	Group of biochemical compounds					No. of Compounds <sup>z</sup>	Content (%)
		FC	TER	ALK	EST	STE		
IR64	R1	-	+	-	-	+	9	30.38
	R2	+	-	-	0	+	7	27.64
HIPA 8	R1	-	-	+	0	-	9	42.92
	R2	-	+	+	0	+	10	55.53

Note: <sup>z</sup>) Similarity value  $\geq 95\%$ . , FC: Fatty Acid, TER: Terpene, ALK: Alkane, EST: Esther, STE: Steroid, R1: below breaking point, R2: above breaking point, (+): increases, (-): decreases, (0): has no effect.



**Figure 3** Heat map biochemical compound two varieties (IR64 and HIPA8). S1 to S56 represent biochemical compound; Label below map of GB-C-TA is described as GA-IR64 or GB-HIPA8, C-control or L-lodging, TA-(T2) or TB-(T1) or RA-(R2) or RB-(R1). S1: (2,4,4,4,16-D6) -3 Alpha, 17 Beta-Dihydroxy-5 Beta-Androstane, S2: (2E) -3,7,11,15-Tetramethyl-2-Hexadecen-1-ol, S3: (9E, 12E)-9,12-Hexadecadien-1-ol Acetate, S4: 1,2-15,16-Diepoxyhexadecane, S5: 1,6,6-Trimethyl-8-Oxabicyclo (3.2.1) Octan-2-One, S6: 11,13-Dimethyl-12-Tetradecen-1-ol Acetate, S7: 14-Methylcholest-4-en-3-One, S8: 17-(1,5-Dimethylhexyl)-10, 13-Dimethyl-4-Vinylhexadecahydrocyclopenta (a) Phenanthren-3-ol, S9: 1-Acetyl-3-Methoxy-4-(2-Acetylhydrazino)-5-Acetylaminopyrazole, S10: 1-Cinnamyl-3-Methylindole-2-Carbaldehyde, S11: 1H-Imidazole4-Hexanoic Acid, Delta-Bromo-2, 3-Dihydro-5-Methyl-Epsilon, 2-Dioxo, Ethyl Ester, S12: 2,2-dideutero Octadecanal, S13: 2,5-Furandione, 3-Dedocyl, S14: 2-Aminoethanethiol Hydrogen Sulfate (Esther), S15: 2-Aminoethanethiol Hydrogen Sulfate (Esther), S16: 3,4,5,5-Tetramethyl-1-Phenyl-4,5,6,8-Tetrahydropyrazolo (3, 4-b) (1,4) Diazepine-7 (1H)-One, S17: 3-Cyclopentylpropionic Acid, Tridec-2-ynyl Esther, S18: 4,22-Stigmastadiene-3-One, S19: 4, 4-Dimethylcholestan-3-One, S20: 4,5-Dimethyl-2,6-Bis ((trimethylsilyl) Oxy) Pyrimidine, S21: 7,11-Hexadecadienal, S22: 9,12-Octadecadienoic Acid , S23: 9,17- Octadecadienal, S24: Beta-Sitosterol, S25: Celidonial, Deoxy, S26: Cholestan-3-One, 4,4-Dimethyl- (5 Alpha), S27 : Cyclopentadecanoic Acid, S28: Cyclopropaneoctanal, 2-Octyl, S29: d1-Alpha-tocoperol, S30: Docosane , S31: E-2-Methyl-3-tetradecen-1-ol Acetate, S32: Eicosane, S33: Ergost-5 -en-3-ol, S34: Ethyl (9Z, 12Z) -9, 12-Octadecadienoic Acid, S35: Furane-2-Carboxylic Acid , 5-(3-Methoxyphenoxyethyl), S36: Heptacosane, S37: HexadecanoicAcid, S38 : Hexahydrothunbergol , S39: Icosane, S40: Methyl (5E, 13E) -9,11,15-Tris ((Trimethylsilyl) Oxy) Prosta-5,13-Dien.1-Oate, S41: Neophytadiane , S42: Nonacosane, S43: Nonadecane , 1-Choro , S44: Octaconase, S45: Octadecane, 1-Chloro, S46: Phyrzolo (3,4-b) (1,4) diazepin-7 (1H) -One, 4,5,6,8-Tetrahydro-3,4,5,6-Tetramethyl-1-Phenyl, S47: Phytol, S48: Phytol Isomer, S49: Pyridine , 4- (5- (2-Methoxyphenyl) - (1

The differences in the biochemical compounds could be related to the recovery process from the lodging stress. It is well known that plants will respond to abiotic stress through various modes of action (Potters et al. 2007). Abiotic stress encourages changes in the composition of compound such as fatty acids (Tremolieres et al., 1982; Elenkov et al., 1996; Howlett and Avery 1997; Allakhverdiev et al., 1999; Jemal et al., 2000). Composition changes may occur in both major and minor fatty acids (Patel-Davebdra et al., 2004). Lodging presumably causes damage in plant metabolism and resulting in oxidative stress (Zang et al., 2005); where reactive oxygen species (ROS) in the form of hydrogen peroxide compounds ( $H_2O_2$ ) can be bound with the fatty acids.

Abiotic stresses, including those caused by lodging, could affect membrane stability. According to Dias et al. (2015), abiotic stress greatly affects the plasma membrane. As membrane stability influences physiological activity such as water use efficiency and osmotic pressure (Singh et al., 1992; Azizi-e-Chakherchman et al. 2009), the presence of steroid compound such as stigmasterol and beta-sitosterol are important to protect membrane stability. According to Senthil-Kumar et al. (2013), stigmasterol and beta-sitosterol affect the permeability and the fluidity characteristics of membrane plasma and other membrane organelles. Another role of these steroid compounds is related to the metabolic processes and signaling pathways that affect transcription of gene expression responsible to stress. Therefore, the increase in the stigmasterol and sitosterol compounds under lodging conditions is a recovery mechanism to reduce the impact of oxidative damage (Hassanein et al., 2012).

## Conclusion

Rice crop responses to lodging can be indicated by the changes in biochemical mechanisms, either through the production of new compounds or by increasing the content of the compounds. Lodged and non-lodged IR64 and HIPA8 rice variety produced different biochemical compounds. Breaking point under lodging condition stimulated the changes in the biochemical reactions at both above and below breaking segments of the culm. Biochemical compound groups such as fatty acids, terpenes, alkanes, and steroids were predominantly affected. Segments below the breaking points expressed reduction on *fatty acid* compounds by 40%, but showed an increase on *steroid* compound by > 200% in the IR64. In the HIPA8 the *alkane* compounds increased markedly by 200% below the breaking point.

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