Chlorophyll fluorescence and stomatal conductance of ten sugarcane varieties under waterlogging and fluctuation light intensity

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INTRODUCTION

Waterlogging in the field is a major problem in agriculture across the world. Moreover, the IPCC (Intergovernmental Panel for Climate Change) in 2014 has reported that climate change might affect the spatial distribution of rainfall. In some places heavy rainfall may occur frequently, however, at the other places rainfall occurs sporadic. The presence of excessive water due to heavy rainfall accompanied by poor soil drainage system can cause plant stress due to present of hypoxia (Araki et al., 2012), moreover oxygen diffusion into soil decreases up to 320,000 times when soil pores are filled by water than by gas (Armstrong and Drew, 2002; Colmer and Flowers, 2008).

Crop performance under waterlogging stress decreases in vegetative organ, tiller number and yield of wheat (Kozlowski, 1984; Davies & Hillman, 1988; Huang et al., 1994; Dickin & Wright, 2008), sugarcane (Singels et al., 2010; Silva et al., 2014) and even decreases physiological performance i.e. photosynthesis, respiration, transpiration and translocation (Else et al., 2001). Besides water stress, crops are always subjected to other abiotic stresses such as light fluctuation from low to high light that may aggravate crop stress. Excess light may decrease maximum quantum yield of photosystem II ($f_v/f_m$) leading to photo-inhibition of crop (Ksas et al., 2015).

Light fluctuates due to passing clouds, canopy cover and change in leaf angle, this light fluctuation decreases photosynthetic induction response leading to reduce cumulative $CO_2$ fixation (Soleh et al., 2017; Slattery et al., 2018). Non-steady state photosynthesis may be accompanied by differences in the $f_v/f_m$, moreover, it is little to know of dynamic $f_v/f_m$ response under fluctuation light intensity and stressful condition which may affect photosynthesis of non steady state. Light photon is absorbed by chlorophyll then re-emitted partly as fluorescence (Maxwell & Johnson, 2000), so that the fluorescence could describe plant health particularly in plants exposed to stressful condition. Besides gas exchange measurement, chlorophyll fluorescence induction is one of
powerful tools for measuring plant stresses (Lazár 1999, 2006) and indirectly it could provide useful information of leaf photosynthetic performance (Baker and Rosenqvist, 2004). Nowadays, crop physiological performance of crop e.g. photosynthesis and fluorescence are mostly examined under steady state, whereas, crop are always facing dynamic abiotic factors such as light and water.

Another response that is correlated with abiotic stress such as waterlogging is rate of stomatal conductance. Stomatal closure reported had declined $f_v/f_m$ (Lawlor & Cornic, 2002), there was reported decreasing stomatal conductance of seashore mallow to 27% under waterlogging (Zhou et al., 2012). Nevertheless, stomatal closures did not correlate to $f_v/f_m$ under mild drought conditions (Baker and Rosenqvist, 2004). While, fluctuation light intensity will be affected on rapid stomatal opening leading to affected on photosynthesis, there was 10-15% limitation of photosynthesis across several C3 and C4 species were due to lag time to reach steady state from low to high light (McAusland et al., 2016). Furthermore, the study of growth and physiological traits of sugarcane varieties subjected to light intensity and waterlogging using cluster analysis to reveal correlation among traits under stress condition is one useful method for breeders and agronomist to better understand physiological changes during growth and development stage of sugarcane varieties grown on stress condition. This method reported was successfully differentiated the ability of rice in salinity condition into 4 groups (Chunthaburee et al., 2016) and in Arabidopsis shows distinctive under multiple stress condition (Sawelam et al., 2014).

This study was to evaluate dynamic $f_v/f_m$ of ten varieties of sugarcane that were grown under waterlogging stress with exposure to light fluctuation from low to highlight. Furthermore, evaluation of crop performance under dynamic environmental condition i.e. fluctuation light is still needed to be explored. It is very rare information of plant performance evaluated under combined stressful condition of waterlogging and fluctuation light intensity.

**MATERIALS AND METHODS**

**Plant materials**

Ten sugarcane varieties derived from various origin were chosen: one local variety: Kidang Kencana (KK), seven derived from Indonesian Sugar Research Institute: PS881 (V1), PS862 (V2), PS864 (V3), PS882 (V4), PSJK922 (V5), PSJT941 (V6), and PS921 (V7), and two derived from private sugar company: GMP1 (V8) and GMP2 (V9).

**Plant growth place**

The ten varieties were grown in the plastic pot with size of 25 x 25 x 50 cm and grown one seedling per pot. Row spacing between pots were 70 x 70 cm. Unsterilized field soil (Fluventic Eutrupepts) was used for growing medium, and NPK fertilizer was added 20 g per pot. Pots of WW (well watered) and WL (waterlogging) were placed in the field of experimental station of Faculty of Agriculture, Padjadjaran University on April to October 2017 (6°55’13”S 107°46’24”E, 740 m altitude). Waterlogging (WL) treatment pots were placed into small pond to keep plants watered during the experiment. There were 6 pots for each variety under WL treatment.

**Chlorophyll fluorescence measurement ($f_v/f_m$)**

The $f_v/f_m$ represents maximum quantum yield of photosystem II (PSII) where the variable fluorescence ($f_v$) is the difference between the maximum ($f_m$) and minimum ($f_o$) fluorescence emission in dark-adapted leaves (Kitajima & Butler 1975). Fluorescence induction was measured by using Handy PEA fluorometer (Hansatech Instruments Ltd) when plants were subjected to waterlogging condition for 2, 4, and 7 DAT (days after treatment) at the vegetative stage (1.5 month old). The measurements were made on 2nd leaf from the uppermost expanded for each genotype. During the measurement, leaves were subjected to dark adaptation for 5-10 min then subjected to highlight of 1500 µmol m$^{-2}$ s$^{-1}$ in normal measurement, while measurement of fluctuation light intensity leaves were subjected to various light intensity from low to highlight of 50, 100, 200, 800, and 1600 µmol m$^{-2}$ s$^{-1}$ for 1 min respectively then subjected to 3000 µmol m$^{-2}$ s$^{-1}$ at 28 DAT. For further analyses of $f_v/f_m$, we made regression analysis of $f_v/f_m$ and $g_{c}$ at 4 DAT of waterlogging treatments.

**Stomatal conductance measurement**

Stomatal conductance was measured by using a Leaf-Porometer (Decagon Devices Inc., USA); three to four plants for each genotype were measured. The measurement was conducted at 2, 4, and 7 DAT or at the vegetative stage (1.5 month old), conducted before noon at the sunny day using the same leaf as $f_v/f_m$ measurement.

**Root weight and volume**

At the 85 DAT, all varieties were sampled destructively to evaluate root weight and volume. Plants were separated by shoot and root destructively, then root weight and volume measured by using laboratory balance and measuring cylinder respectively (Harrington et al., 1994).

**Relationship between $f_v/f_m$ and $g_{c}$ and Dendogram**

Regression analyses of $g_{c}$ and $f_v/f_m$ were made to show relationship of both parameters. In addition dendrogram analyses was made at 7 DAT across $g_{c}$, and at 85 DAT across panicle number (data not shown) and root volume of tens varieties by ward’s method.
Statistical analyses
The experimental design was a randomized block design, the measurements were conducted for 3–4 plants for each genotype and then averaged, followed by LSD-Tukey tests. All data were analysed using JMP program (SAS Institute, 2000).

RESULTS

Before treatment of waterlogging (0 DAT), the $f_v/f_m$ of ten sugarcane varieties ranged from 0.8 in V3 to 0.9 in V9 (Fig. 1A), while $f_v/f_m$ after the treatment at 4 DAT ranged from 0.6 in KK to 0.8 in V1, $f_v/f_m$ value of KK was significantly lower compared to other varieties except to V2 and V3 (Fig. 1B). Chlorophyll fluorescence measurement ($f_v/f_m$) at 7 DAT generally had slightly increased again compared to 4 DAT. The value of $f_v/f_m$ ranged from 0.6 in V3 to 0.8 in V1 and showed significantly different in both varieties of V3 and V1 (Fig. 1C). While, $f_v/f_m$ of plants under fluctuation light intensity of dark adaptation from low of 50 µmol m$^{-2}$ s$^{-1}$ to high light of 1600 µmol m$^{-2}$ s$^{-1}$ were difference across ten sugarcane varieties at 25 DAT of waterlogging, V5 was the highest of $f_v/f_m$ at the end of illumination of 1600 µmol m$^{-2}$ s$^{-1}$. The trend of decreasing the value of $f_v/f_m$ was displayed in Figure 2, in which all of genotype showed decreasing value of $f_v/f_m$ along with addition of illumination/light. Even though, we found that V5 showed increased $f_v/f_m$ value and it was starting at between 800 to 1600 values of light.

At the 0 DAT value of stomatal conductance ($g_s$) ranged from 544 in V2 to 953 mmol H$_2$O m$^{-2}$ s$^{-1}$ in V6, $g_s$ of V6 had significantly higher than the varieties of V1, V2, V7, and V9 (Fig. 3A), while at the 4 DAT $g_s$ ranged from 255 in V5 to 407 mmol H$_2$O m$^{-2}$ s$^{-1}$ in V9 even if there were no significantly difference among the varieties (Fig. 3B), at the 7 DAT $g_s$ ranged from 240 in KK to 516 mmol H$_2$O m$^{-2}$ s$^{-1}$ in V7, $g_s$ of KK was significantly lower than V7 and V9 (Fig. 3C).

Regression analyses between $f_v/f_m$ and $g_s$ showed negative correlation even it was significance at the 4 DAT (Fig. 4A and B). This result indicated that increasing value of $f_v/f_m$ had low value of $g_s$ under WL. At the end of experiment i.e. 85 DAT, root weight of all varieties were evaluated, it ranged from 130 g in V1 to 305 g in V7 under WL and it ranged from 77 g in KK to 133 g in V6 under WW. While root volume ranged from 283 ml in KK to 407 ml in V2 under WL, and it ranged from 77 ml in V3 to 173 ml in V4 under WW. In general, root volumes of WL were significantly higher than WW (Fig. 5). In addition, using Ward’s method showed that the varieties that used in this study differentiated to two groups by using stomatal conductance at 7 DAT, panicle number and root volume under treatment of WL data’s at 85 DAT (Fig. 6). The first group involved the three out of ten varieties (V1, V3 and KK) as the sensitive group to waterlogging and it was showed by low values on growth and physiological traits. While, seven out ten varieties classified as the second group that showed waterlogging tolerance to resistance.

Fig 1. Chlorophyll fluorescence ($f_v/f_m$) of tens sugarcane varieties (V1-V9, KK), the measurements were conducted under light saturated of 3000 µmol·m$^{-2}$·s$^{-1}$ at 0 DAT or before the treatment of waterlogging (A), 4 DAT (B), and 7 DAT (C). Vertical bars indicate SE of five plants. The differences marked with lower case letters are significant at $P < 0.05$ (Tukey’s means comparison test).
DISCUSSION

Under sub-optimal conditions, plants often show suboptimum response due to environmental limitation. The present of waterlogging and fluctuation light intensity in the field may affect aggravated stress of plants. Once soil pores are filled of water fully, gas diffusion will reduce leading to present hypoxia and/or anoxia, increase of stomatal resistant, reduce of photosynthesis and the other responses. These conditions will greatly affect on capacity of plant survive (Parent et al., 2008). The study hypothesized there is a variation of $f_v/f_m$ and $g_s$ response among sugarcane varieties grown under WL and fluctuation light. The response may affect crop performance under such condition. Besides photosynthesis response, $f_v/f_m$ could be used as a tool to evaluate plant response (Lazár 1999, 2006) particularly under stresses condition.

A clear genetic difference in the response of $f_v/f_m$ was found in sugarcane genotype of KK compared to other varieties except in V1 and V3 at the 4 DAT, while, V3 was lower compared to V1 and V6-V9 at the 7 DAT, KK is local variety which does not improved, so that physiological traits might not be improved compared to the others varieties. Moreover, the clear differences in $f_v/f_m$ were also identified under fluctuation light intensity among varieties of V3, V5 and V6 compared to other varieties at the illumination of 1600 µmol·m$^{-2}$·s$^{-1}$. The variation of

Fig 2. Chlorophyll fluorescence ($f_v/f_m$) of tens sugarcane varieties (V1-V9, KK), the measurements were conducted continuously to two leaves for each varieties under fluctuation light intensity of 0, 50, 100, 200, 800, and 1600 µmol·m$^{-2}$·s$^{-1}$ at 28 DAT.

Fig 3. Stomatal conductance ($g_s$) of tens sugarcane varieties (V1-V9, KK), the measurements were conducted at 0 DAT or before the treatment of waterlogging (A), 4 DAT (B), and 7 DAT (C). Vertical bars indicate SE of five plants. The differences marked with lower case letters are significant at $P < 0.05$ (Tukey’s means comparison test).

Fig 4. Relationship between $f_v/f_m$ and $g_s$ of tens sugarcane varieties (V1-V9, KK), at 0 DAT or before the treatment of waterlogging (A), and 4 DAT (B). the measurement were conducted to four leaves for each variety.
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f_v/f_m under constant and fluctuation light confirmed that some sugarcane varieties had a different characteristic in both condition. These differences in f_v/f_m were also
difference in g_s; however, the correlation between f_v/f_m and 
g_s was negative. These stomatal closures will not associate
directly to decreased f_v/f_m or photosynthetic efficiency, it will depend on internal CO_2 in the leaf (Lawson et al.
2002; Baker & Rosenqvist, 2004). It is well known that g_s and photosynthesis under constant light have a positive 
correlation where high photosynthetic value is always followed by high in g_s (Wong et al. 1979; Farquhar & 
Sharkey 1982). Characteristic of the g_s under constant light is similar to that of variety of V7 that showed significantly
higher in g_s and f_v/f_m at 7 DAT, while the f_v/f_m of V7 was 
not significant higher under fluctuation light intensity.
It might be difference mechanism of f_v/f_m under both 
condition of light. Like g_s response under fluctuation 
light was not limiting factor of photosynthesis or may less 
(Soleh et al., 2017; Knapp & Smith, 1989) or under mild 
drought stresses such demonstrated in Apple (Massacci & 
Jones, 1990).

Stomatal closure is also related to other external signal 
such as CO_2 concentration, humidity, temperature and 
absisic acid (Lawson, 2009). It is our best knowledge 
that this is another study reporting natural variation of 
f_v/f_m within single species under abiotic stress, apart 
from natural variation of photosynthetic induction 
response of soybeans (Soleh et al., 2016). Abiotic stresses 
such as waterlogging and light fluctuation had shown 
the difference in f_v/f_m within varieties. It seems to be 
possible that difference in f_v/f_m and g_s within varieties 
due to external signal such as absisic acid that might be 
affected on stomatal closure due to lack of O_2 then it 
led to decreased in internal CO_2. Another mechanism 
of plants to survive under waterlogging stress is by 
growing more of adventitious roots and developing 
more aerenchyma tissue to fix O_2 (Gomathi et al., 2015; 
Nishiuchi et al., 2012). In this study, all varieties of 
sugarcane grown under WL had shown higher in root 
weight and volume than those varieties grown under WW 
(Fig. 5). In the same figure, variety that had higher in f_v/f_m 
was higher in root volume as well (i.e variety of V7), it 
is possible to develop improved waterlogging tolerance 
is by improving better in f_v/f_m responses beside another 
traits. The differentiation among varieties in adaptive 
traits was showed by dendogram. The result indicated 
that one group was more sensitive to treatment and 
this treatment could be used for screening in breeding 
program of sugarcane. The genotype V1, V3 and KK 
were the sensitive varieties (Fig 6). The dendogram could 
be used in crops differentiation of adaptive traits such in 
rice (Muhamad et al., 2016).

In conclusion, we reported genetic difference of f_v/f_m 
on some sugarcane varieties grown under waterlogging 
and fluctuation light intensity. The difference is might 
be modulated by difference response of g_s particularly 
under constant light condition, while under fluctuation 
light, there might be modulated by another response such 
as carboxylase capacity (Soleh et al., 2016). Improving 
sugarcane traits under abiotic stress i.e. waterlogging 
should be considered to improve chlorophyll fluorescence 
response and stomatal conductance for improving dried 
matter production.
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