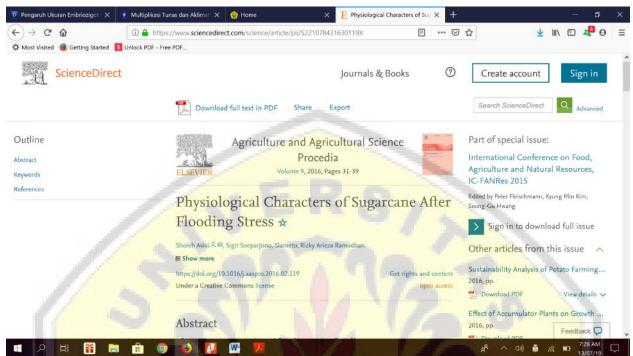
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## Physiological Characters of Sugarcane after Flooding Stress

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#### Abstract

The sugarcane varieties with tolerance to flooding stress are not available in Indonesia. Therefore, this research was aimed to the development of flooding tolerant sugarcane. The research was conducted using Split Plot Design that consists of 2 factors with 3 replications. The main plot was 12 varieties of sugarcane. The sub plot was the level of flooding stress. After flooding treatment, the tolerance sugarcane plant showed a decrease in chlorophyll content, increase stomatal density, enhance brix content, decrease in protein content, and increase of root density. Varieties that tolerant to the flooding stress were PS 881 and PS 862.

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Keywords: chlorophyll, stomatal density, brix, water stress

### 1. Introduction

The sugarcane plantation has some problems such as the excess water which can inhibit growth and production. VanToai et al. (2001) explained that the excess water stress on crops divided into two conditions. The first, the condition is flooding or waterlogging in which only root crops flooded. The second is submergence or complete submergence, where the part of plant or whole plants completely flooded. The excess water stress can be caused by a couple of things such as the rain continuously, that were accompanied by bad conditions of land drainage so that made the land flooded (Islam et al., 2011).

Some destructive conditions that can be experienced by flooding plants is due to the decline of gas interchange between the ground and air, resulting in the decline in the availability of oxygen for roots and decrease the rate of air diffusion. Moreover the flooding stress influence on the physiological process such as respiration, roots

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permeability, the absorption of water and nutrition. Varieties that can grow in the flooding land also adapt to develop some adjustments morphology (Winkel et al., 2014). One of them by increasing the number of a root with the aim increasing the absorbing of water and nutrition elements then distribute to plant leaves. Because of that plant able to run the photosynthesis process (Miro & Ismail, 2013). The flooding stress also stimulate the formation of adventitious roots in the near the surface of the ground.

The decline of plant growth which treat with flooding stress depending on the phases of the growth of plants when plants flooded and length of flooding duration. Flooding stress can decrease the physiology process in susceptible plant, both in vegetative and generative phase (Ezint et al., 2010).

The efforts to be done to overcome the flooding land by conducted a study of physiology character changes that shown by sugarcane varieties after flooding treatment. Next, the verities with good physiology character in flooding condition were selected as sugarcane flooding tolerant. The physiology character which appear in flooding tolerant plant can be used as tool selection of many sugar cane verities in the next research.

### 2. Method

Research was carried out in Agrotechnopark University of Jember, Jubung, Sukorambi, Kabupaten Jember, start in June 2014 - November 2014, in split a plot design factorials consisting of 2 factors with 3 remedial. Main plot were varieties which consists of 12 varieties, namely Bululawang, Kentung, Kidang Kencana, PS 851, PS 862, PS 864, PS 865, PS 881, PS 882, 922 PSJK, VMC 76-16, PSJT 941. While the sub plot was high flooding which used namely 0 cm, - 10cm, - 20cm, -30cm under surface media. The Seeds derived from P3GI in the form of healthy, pure, and vigorous single bud set. Bud sett were planted in polybag measuring  $40 \times 60$  cm with a standing position and shoots facing side and closed by media  $\pm 1$  cm. Composition media consisting of land, sand, and cow manure by comparison 1: 1: 1 (P3GI, 2011). Watering done every day, when moisture media sufficient then watering no need to do.

One month after planted, start given flooding treatment as assigned, that give of water as high as -10 cm, -20 cm, -30 cm from the surface of media, and treatment without water flooding. Variable observation used in this experiment consists of brix content (%) with a hand refractometer, stomata conductivity with leaf porometer. The chlorophyll content using chlorophyll meter (SPAD-502 Minolta), stomata density with a microscope, and the protein total on leaves by using the Bradford method.

#### 3. Result and Discussion

Sugarcane was having different resistance response if it was seen from Physiological character. The resistance responses could be seen in parameters observed as follows.

Stomata conductivity. It is capability of stomata to do gas exchange of CO<sub>2</sub> and H<sub>2</sub>O and other gas derived from the atmosphere and from plants (Samanhudi, 2010). This ability is useful for plants in perform the process photosynthesis, which take CO<sub>2</sub> and H<sub>2</sub>O. Stomata conductivity is also a size of how many H<sub>2</sub>O that is going out or CO<sub>2</sub> coming through stomata. The influence of the use of different varieties to stomata conductivity was presented in the Figure 1. Based on Figure 1 it can be seen that the highest value of stomata conductivity obtained from varieties PS 881 (V8) as 93,58  $\mu$ mol/m<sup>-2</sup> s<sup>-1</sup> and varieties PSJK 922 (V10) as 75,3  $\mu$ mol/m<sup>-2</sup> s<sup>-1</sup>, and the value stomata conductivity the lowest obtained from varieties VMC 76-16 (V11) as 51,52  $\mu$ mol/m<sup>-2</sup> s<sup>-1</sup> and varieties PSJT 941 (V12) as 51,6  $\mu$ mol/m<sup>-2</sup> s<sup>-1</sup>.

At the time of plant experienced flooding stress, plants do response with a means of roots transporting water to a foliar part especially stomata, the process is called transpiration. When performing transpiration, plants tried to bring out excess water that goes to the tissues of plants, then water out of leafs gaseous through the mouth stomata, so that carbon dioxide from the atmosphere be able to enter into stomata without obstructed. The rate of passage of carbon dioxide ( $CO_2$ ) entering stomata is called stomata conductivity.

Stomata conductivity always positively correlate by photosynthesis, because stomata as the site of entry of  $CO_2$ , so that in the condition of flooding stress, the decline in photosynthesis always caused by decrease of stomata conductivity, caused ability stomata to bind  $CO_2$  decreased. The transpiration rate also increase significantly in response flooding stress (Promkhambut et al., 2010). Plant experienced flooding stress shown increase the number of

stomata. In addition the low level of oxygen can be lowered power stomata conductivity because permeability roots obstructed. The decline in photosynthesis could cause by the closing of the stomata, the reduction of chlorophyll content, the senescence of leaves and the space of leaves (Ashraf, 2012).

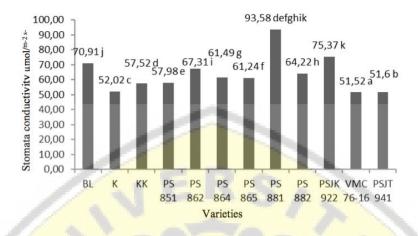


Figure 1. The influence of varieties against stomata conductivity of sugarcane

According to Dewi (2009), although so much water available in a flooding media the ability of plants to absorb water in most species will decline, proven by the decline of osmotic potential. The decline of potential water not caused by transpiration increase, because on flooded condition almost all species showed that the decrease in stomata conductivity (stomata starts to close). At the flooding condition, hypoxia produced by water stress caused a decline in the absorption of water by roots and the closed of stomata (Sibernsen & Mott, 2010). The rate of photosynthesis decreased due to the relative changes in stomata to photosynthesis, an increase in water stress caused reduced chlorophyll resulting in the growth and development plant decreased (Herrera, 2013).

The chlorophyll content. The pigments chlorophyll play an important role in the process of photosynthesis by changing light energy into the chemical energy. Solar energy absorbed by chlorophyll and used to decipher water molecules, forming gaseous oxygen, and reduced molecules NADP to be NADPH. Light energy also used to form molecules ATP, NADP and ATP used to reactions that produce glucose.

The chlorophyll content in flooding treatment tended to be smaller than treatment without flooding (Rachmawati & Retnaningrum, 2013). The influence of varieties to the chlorophyll content was presented on Figure 2.

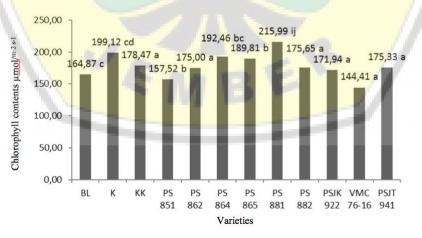


Figure 2. The chlorophyll content on varieties tested

Varieties	Flooding Treatment				
	0 cm	-10 cm	-20 cm	-30 cm	Means
Bululawang	154,56	203,82	188,37	188,54	183,82
Kentung	181,74	214,01	163,06	174,95	183,44
Kidang Kencana	244,59	236,09	217,41	215,68	228,44
PS 851	203,82	193,63	193,63	197,03	197,03
PS 862	236,09	208,92	202,12	237,79	221,23
PS 864	217,41	222,5	207,22	168,15	203,82
PS 865	217,41	225,9	210,74	188,54	210,65
PS 881	227,6	246,29	224,2	186,84	221,23
PS 882	207,22	161,36	188,54	229,3	196,6
PSJK 922	176,65	164,75	180,04	186,84	177,07
VMC 76-16	212,31	207,22	197,03	167,45	196
PSJT 941	174,95	188,8	166,46	166,45	174,17

Table 1. Stomata density after flooding treatment

Based on Figure 2 it can be seen that varieties ps881 (V8) and varieties kentung (V2) and varieties ps864 (V6) having the highest chlorophyll content that is 215,99  $\mu$ mol/m<sup>-2</sup>s<sup>-1</sup> dan 199,12  $\mu$ mol/m<sup>-2</sup>s<sup>-1</sup> serta 192,46  $\mu$ mol/m<sup>-2</sup>s<sup>-1</sup> respectively. While varieties VMC 76-16 (V11) and varieties PS 851 (V4) having the lowest chlorophyll content that is each 144,41  $\mu$ mol/m<sup>-2</sup>s<sup>-1</sup> and 157,52  $\mu$ mol/m<sup>-2</sup>s<sup>-1</sup> respectively.

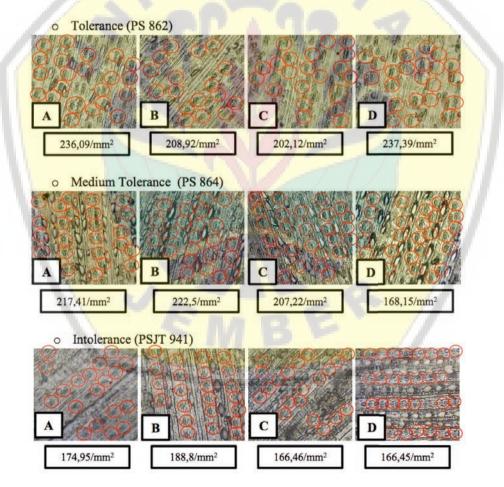


Figure 3. The Stomatal density of several varieties after flooding stress; 1treatment A (0 cm); B (-10 cm); C (-20 cm); D (-30 cm)

The formation of chlorophyll influenced by several factors such is light, by which a condition is surroundings having the excessive sun light detrimental to the chlorophyll pigments so that leaves a will pale. Water shortage can resulted in a decreased the ability leaves to form chlorophyll because leaves will start yellowing, while the excess water caused depreciation size leaves so the formation of chlorophyll will decline.

According to Maryam & Nasreen (2012), the water stress in plants causing a decline in the chlorophyll content followed to the decrease the process photosynthesis. Response has been increased when duration water stress be increased. Large colored leaves become pale. In addition response shown that is the depreciation a size leaves in plants. The provision of water stress lasting several months causes deficiency oxygen in plants. In defense of condition flooded, of paddy for example have to do escape as an effort to defense from water stress, conducts e.g. lengthening cells. Rice plants can increase the height of plant about 25 cm per day. Lengthening cells rapid it allows leaf tips to growing out the surface of water and so that the can do photosynthesis efficient and gas exchange for respiration (Nishiuchi et al., 2012).

The water stress treatment significantly could reduce chlorophyll content on leaves because during the roots experienced water stress, ability to absorb the roots in riot gear especially nitrogen declined, and roots pore used to perform the process transpiration and water be found at about rooting can be expelled. In addition, the water stress cause nitrogen used in the formation of chlorophyll being dissolved and settles on one side of media planting so they could not reach of rooting.

The decline in the womb chlorophyll that occur is not cause a big change to plant cane who have been given water stress for eight weeks, expected physiological state plants cane start to change and adapt to environmental conditions. Will in the formation of chlorophyll being dissolved and settles on one side of media planting so they could not reach of rooting. The growth of plants cane still had continued although in the seized condition, that means the photosynthesis is still taking place although not perfect as on sugarcane, which not be water stressed treatment.

Stomata Density. Based on the data that has been obtained be seen that there has been increasing density stomata on some varieties due to water stress, shown in Table 1. Varieties Kidang Kencana, PS 862, and PS 881 have a value of the density stomata most compared with others varieties, that is in each varieties 228,44 mm<sup>2</sup> (Kidang Kencana), 221,23 mm<sup>2</sup> (ps 862 and PS 881). While, varieties of PSJT 941 with the density stomata most lower than the other varieties 174,17 mm<sup>2</sup>. Density stomata on some varieties due to water stress, presented in Table 1.

According to Zhang et al. (2014) the mostly water in plants diffused through stomata, so that stomata plays an important role in keep status hydration on the leaves. The size and density of stomata affect high low conductivity of stomata. An increase in density stomata gives the impact of an increase in the rate of photosynthesis. In general, leaves that experienced the rate of gas exchange high stomata having the measure of a smaller.

Varieties	Flooding Level				
	0 cm	-10 cm	-20 cm	-30 cm	
BL	17,21	17,55	18,57	17,17	
К	15,83	18,91	18,71	18,49	
КК	15,58	17,93	18,31	18,52	
PS 851	15,83	17,80	18,31	17,38	
PS 862	18,32	18,85	19,15	18,98	
PS 864	15,55	17,90	18,61	17,12	
PS 865	16,32	18,23	17,82	17,30	
PS 881	15,85	18,16	18,54	18,88	
PS 882	14,33	18,38	16,92	17,01	
PSJK 922	14,70	18,02	17,65	19,00	
VMC 76-16	18,06	18,51	17,68	17,29	
PSJT 941	17,10	17,34	18,67	18,31	

Table 2. The content of brix every varieties after flooding treatment.

*The content of brix.* Sugar is the result of plant-processed sugarcane. Before done purification nira to sugar, the quality of nira of plant cane is necessary testing in roomy first to see the suitability cane ready to harvest. One way to analyze the quality of nira cane is through testing the brix content. Brix is a solid substance dissolved every 100 grams a solution is found in a solution of nira sugar cane, where a solid substance it can be in the form of sucrose or salt. For example the value of brix an aqueous solution of nira = 16, that means that of 100 grams nira, 16 grams of a solid substance is dissolved and 84 grams is water (Filianty et al., 2006).

Based on the study of Islam et al. (2013), show that plants the given treatment water stress for a few days produce brix content high namely 20.8 % so that the sugar cane the it can be said still tolerant of water stress given. The influence of high water stress against the womb brix can be presented in the charts on Table 2. The value of high brix content on treatment T1 (-10 cm) and T2 (and 20 cm) was supposedly associated with the density stoma that tends to be high too (see Table 1). The high density of stomata causing the entry of more carbon dioxide into the plant that the rate of photosynthesis will increase that resulting in deposits of the brix will be increased as well. Based on table 1 seen that varieties of PS 862 is one of the variety that has high density of stomata, then supported from the result of the observation brix content in Table 2 that varieties of PS 862 has any brix highest namely 18, 82 % in effect on all high water stress.

Sugarcane varieties that having high brix content enough although in the condition of undergoing flooding stress, alleged to have resistance to water stress because on high water stress - 20 cm under the surface of the ground, plants cane was not difficult in producing sucrose. But, to know yield of varieties of the tolerant cane was necessary grinding at the harvest time to see the difference in value yield crystalline sugar and value brix in roomy, if they do not differ much so that varieties has been meet yield desired.

The Plant cane that experienced water stress has high brix content enough because when plants to be inundated by water causing organic element that was found in a media cropping of being dissolved and not wasted from the media so that the planting more easily absorb organic element then used of plants in conducting the process of photosynthesis, compared with a plant sugarcane was not provided with treatment water stress, organic element in which it is dissolved in water can not be absorbed in an optimum manner because some wasted with water that splashed into the media cropping.

Besides the plane cane's age was young where be in vegetative phase, so that production sucrose produced is still high and will transport into an organ plants that is growing up, but the excess sucrose will be stored on the part of the rod of a plant cane.

*Total protein content.* Protein in the tissues of plants had some functions that were carrying electrons for photosynthesis and respiration. Protein formed from an element nutrition absorbed by plants especially nitrogen. Nitrogen is element building blocks of chlorophyll, protein and other amino acid that are useful for plant (Parman, 2007).



Figure 4. The effect of variety to leaf protein content of sugarcane varieties.

Based on the charts on Figure 4 it can be seen that v12 (PSJT 941) with water stress treatment t1 (-10 cm) and v5 (PS 862) with high water treatment t1 (-10 cm) had content that was protein the highest number, namely mg / g for (PSJT 941) and 1.98 mg/g for (PS 862). While v9 (PS 882) with water stress treatment t1 (-10 cm) had the lowest protein content namely 0,37 mg/g.

The high protein content on water stress treatment T1 (-10 cm) than water stress treatment T0 (0 cm), predicted that Bululawang varieties, PS 851, PS 864, PS 865 which not be water stress treatment having condition of being scant good caused by the genetic for example the condition of seeds unhealthy or environmental factors for example preventing disturbances pest and disease.

Observation in the roomy shows that variety PS 865 having a growth delays and size plants a dwarf, then to variety of Bululawang, PS 851, and PS 864 the possibility of the process of transport nutrient goes more slowly compared many other varieties so that the formation of protein is held up. The variety that has low total protein content believed to be due to the plans ability in absorbing element nitrogen decreased caused by water stress that interferes with roots in the process of the absorption of nitrogen.

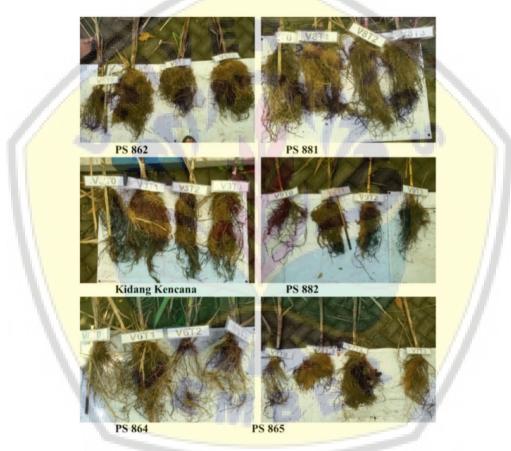


Figure 5. Rooting plants cane after the provision of water stress.

Pores roots that experienced water stress filled by the water in excess capacity, plants respond by conducting transpiration, nitrogen of ions soluble in water not capable of being absorbed by a plant so the process of forming protein and chlorophyll is held up. in plants that experienced water stress, the yellowing leaves because a decrease in nitrogen absorbed by plants, fixation N and production substance poisonous like nitrites and sulfides which moves from in the ground to the root to leaves if carried away in large quantities (Kumar et al., 2013). Roots cane after flooding. Based on observation at land, after plants given water stress treatment for 12 weeks, dismantling to look at the rooting of a plant cane, be seen that all varieties changed morphology.

Many adventitious roots appear, as the effect of water stress because those plants avoid of condition hypoxia (deficiency of oxygen) so that its adventitious roots appear to be expected to absorb oxygen optimally where oxygen used of plants to do the process of respiration. On varieties ps 862 (see Figure 5) note that high water stress-10 cm, -20 centimeters and -30 cm having rooting more than plants that do not experience. Rooting many signifying plants respond to the water stress by forming adventitious roots much aimed at to keep oxygen intake and nutrient could still absorbed by plants.

In Table 3 it can be seen that variety PS 862 having density stomata, brix and high protein, high protein content cause root adventitious formed more. On The Variety of PS 881 (see Figure 5) known that all high water stress rooting having a more bushy and long, which means the plants capable of enduring water stress by forming adventitious roots in large numbers. Rooting that many plants respond to signify the presence of water stress by forming adventitious roots otherwise, which aims to keep oxygen intake and disturbances, fixed element can be absorbed by plants. In table 3 can be seen that PS 881 varieties having conductivity stomata, chlorophyll content, density stomata, brix high enough.

On variety Kidang Kencana (see Figure 5) note that a seen most prominent at high water stress treatment -10 cm, rooting plant thicker than other treatment, on rooting 0 cm treatment, -20 cm, -30 cm also quite thick. The number of its adventitious roots formed in addition to avoid the condition due to lack of oxygen caused by flooding, but also to prop up the upper part of a plant where variety Kidang Kencana having the measure of the stems which are large enough so that the sugar cane was not collapsed.

On variety PS 882 (see Figure 5) note that high water stress treatment -10 centimeters and -20 cm exert rooting more than controlling treatment of rooting that tends to have fewer. It was because puddle causing a plant to set up more adventitious roots as a response from the stress. But from the result of observation be seen that variety has brix content more lowly when compared to PS 881 variety. On variety PS 864 (see Figure 5) out that rooting plants cane on all water stress treatment, its adventitious roots formed just a little. It was because the varieties having roots that is not bushy so as to water stress treatment led to the formation of adventitious roots not too much, but that variety has the womb chlorophyll and protein in a high enough.

On variety PS 865 (see Figure 5) note that water stress treatment -10 cm, -20 cm give a fairly significant effect on rooting plant cane compared to controlling treatment which rooting tend to be slightly. Many adventitious roots formed because nutrient intake more easily absorbed applied to the process of the growth of plants. The variety has chlorophyll, density stomata and protein which is high enough.

#### Conclusions

Based on the observation has been done, so we can take some conclusions, there were: Varieties that tolerant to the flooding stress were PS 881 and PS 862, whereas Bululawang and PS 882 showed medium tolerant. Moreover, PS 851 and VMC 76-16 were identified as the flooding stress sensitive varieties.

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