

## PROLINE AND SPECIFIC ROOT LENGTH AS RESPONSE TO DROUGHT OF WHEAT LINES (*Triticum aestivum* L.)

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### ABSTRACT

The national wheat imported reaches approximately 7 million ton per year, recently. The wheat plantation in Indonesia is strongly affected by the high temperature which triggers the stunned roots. Thus the plan wheat growth and production reduced. The experiment to investigate of selected-local wheat lines which were already established in Indonesia and resistant to drought effect. The level of drought influence on several growth parameters of vegetative plant and thus affected to seeds production as well as on total yield. The results show that the introduced-variety of wheat line, SO3 shows the significant tolerant to drought and able to maintain the SRL (specific root length) and increase the proline contents to with stand the drought condition compare to other lines (M7 and M8). The proline as amino acid climbed extremely effected by drought, in contrast the root growth in particular their length reduced. Those plant conditions influence the plant vegetative growth and generative phases, especially for seed production. Additionally, the M8 and SO3 selected-line presents the stability on yield production compare to other varieties, since it able maintain the content of proline and ratio of root length. Therefore, those selected lines are appropriate to grow in Indonesia which produces at approximately 3.5 t ha<sup>-1</sup>.

Keywords: drought; high temperature; proline; specific root length; wheat

### INTRODUCTION

Regards to national wheat consumption for food industry causes Indonesia placed the second country import wheat for industry during 2012-2014 (Wright & Meylinah, 2014), hence the government regularly imported from Australia as the main wheat country production. Therefore, it is

important to develop an adaptive wheat that can adapt to tropical climate in Indonesia. In addition, the common factor which limits production in tropical regions is the availability of water. Gardner, Pearce, & Mitchell (1985) reported that water is an essential component for metabolism processes of plant and has different roles as follows: act as (i) solvents and various chemical medium; (ii) medium to transport organic and inorganic solutes; (iii) factor in the regulation of the plant cell turgor, which is associated with cell enlargement, cell structure and placement of the leaves; and play important roles in (iv) hydration and neutralization of charge on colloidal molecules; (v) the process of photosynthesis, water acts as the main raw material; and (vi) cooling the surface of the plant (evaporation). In addition, some researches on cereal crops showed that drought stress conditions resulted in decreased growth rate and weight of grain on yield. Those results were caused by a decrease of precipitation and increase in temperature (Saradadevi, Bramley, Siddique, Edwards, & Palta, 2014). This makes the water an important factor in determining the success of the cultivation of wheat.

Drought stress can have effect for both plant growth and yield. As plant morphological is known that, during the vegetative phase, reduction in water availability led to a decrease in leaf area, and its remobilization of nutrition in sink (Ma, Huang, Yang, & Chai, 2013), in number of leaves per plant, and also in leaf age until autumn (Anjum, Xie, & Wang, 2011). It is caused by reducing production due to delays in the process of photosynthesis assimilate because of the lack of water, so these impact on the reduction of the volume and size of organs in plants. Drought stress conditions are also known decreasing the fresh weight and dry weight of plant. Limiting water also led to a reduction of CO<sub>2</sub> gas exchange at the

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leaf surface, which is a major component of the process of photosynthesis. It is then not only limit the size of the source, but also the results of plant section. During drought conditions, the process of filling in phloem, assimilation and biomass also disrupted (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009; Anjum, Xie, & Wang, 2011). It was reported clearly that plants also respond to drought condition shortening the life cycle. The performance of plant is shown mostly with days to flowering and harvest much faster, that condition also known to trigger production of a number of hormones, such as GA (Gibberellins), which triggers the formation of generative organs structure, although the plants are young (Taiz & Zeiger, 2010). Increased grain filling rate occurs during drought stress conditions. However, it resulted a decrease in crop yields and caused disruption of assimilate into the grain filling process (Ma, Huang, Yang, & Chai, 2013). However, in order to adapt to the gripped condition, the plant is known to have a physiological response by producing certain compounds to reduce the impact of non-availability of water, such as a decrease in the content of proline. This study aimed to obtain a candidate lines and determine the level of proline of plant resistance to drought stress.

## MATERIALS AND METHODS

The research was conducted during the growing season from January to May 2015. The study was carried out at experimental screen house that was located in Junrejo, Batu, East Java which has a height of 700 meters above sea level and the soil type of Inceptisol. The daily temperature ranging between 22.7°C - 25.1°C with an average humidity of about 76%.

The tools used in this experiment were: digital analytical-balance, digital cameras Minolta, thermos hygrometer, LAM (Leaf Area Meter) Type Li-Cor 3100C, Spectrophotometer (Thermo Scientific) and pH soil meter. Meanwhile the material that is used in this study include: wheat lines M7, M8 and SO3, 10 kg treated-soil, types Inceptisol, NPK compound fertilizer (N 16: P<sub>2</sub>O<sub>5</sub> 16: K<sub>2</sub>O 16). This study used methods of Split Plot Design where main plots consisting of four levels of soil water content (100%, 75%, 50% and 25% of field capacity) and promising lines of wheat (M7: Maros 7; M8: Maros 8; and SO3: Republic 3) were used as subplots, on four replications.

## Media Preparation and Planting

This experiment used Inceptisol soil as a growing medium. The water content in soil was reduced by drying the soils at room temperature about 3 days. The dried soil were placed to polybag 20 x 20 x 40 cm in which each bag consist of 10 kg of dried soils and placed at the distance of 25 cm x 25 cm. The Furadan 3GR was used as insecticide and applied at a dose of 0.1 g per plant as anticipate to pests during early growth phase. Before planting, the moisture of plant was measured and then irrigated up to 100% field capacity in all treatment combinations after that the water content was given regarding to the treatment.

## Water Content Capacity Preparation

### Moisture Treatment

Treatment of water stress started on 5 weeks after planting (WAP). Based on the soil analysis, it was known that the water content at field capacity (2.5 pF) was 0.326 g g<sup>-1</sup> of soil while the moisture conditions of permanent wilting point for plants (4.2 pF) was 0.150 g g<sup>-1</sup> of soil.

Therefore, the amount of water (mL) applies to the growing media in each treatment with the weight of the polybag 10 kg each (W), follow the following equation:

Water content (WC) 100% field capacity:

- a) WC 100% = ("pF 2,5 pF 4.2") "10,000 x g  
Water should be added in order to achieve a 100% field capacity was 1,760 g or 1,760 mL. The total weight of the dried soil at 100% field capacity was 11 760 g.
- b) WC 75% = 100% x 75%  
Water should be added in order to reach 75% of field capacity is 1,320 g or 1,320 mL. The total weight of the dried soil at 75% of field capacity was 11 320 g.
- c) WC 50% = 100% x 50%  
Water should be added in order to reach 50% of field capacity was 880 g or 880 mL. The total weight of the dried soil at 50% field capacity was 10 880 g.
- d) WC 25% = 100% x 25%  
Water should be added in order to reach 25% of field capacity is 440 g or 440 mL. The total weight of the dried soil at 25% field capacity was 10,440 g.

Observation are involved leaf area, specific root length, number of spikelets per spike, number of spikes per plant, total content of nitrogen in

grains, proline content in leaves, and harvest index which investigated at 6, 8, and 10 weeks after planting.

The data of experiment were analyzed by the F test at 5%, and continued by the HSD test ( $\alpha = 5\%$ ).

## RESULTS AND DISCUSSION

Decreased levels of water availability affect various components of selected wheat varieties were observed. Several previous studies showed that there was a reducing of vegetative growth and a producing organs assimilate (Anjum, Xie, & Wang, 2011). The decrease of photosynthesis rate due to the decline of  $\text{CO}_2$  exchange the surface of leave, as the consequence for reducing of assimilate production (Budak, Kantar, & Yucebilgili Kurtoglu, 2013). Thus, the results of the experiment indicates that each line of wheat show different morphological changes in term of vegetative growth and yield as well.

The vegetative stages such sources organ declined on size as well as the capacity (Taiz & Zeiger, 2010). The leaf area observed show that different results due to the level of water soil field capacity. On the Figure 1 present that on 6 weeks after planting, in general there was no different in the response on leaf area caused by water soil

field capacity. This indicates that on 6 weeks after planting the number of leaves of all selected lines remain stable. Moreover, on 8 weeks after planting, while the leaves number show two fold rise on 100% and 75% Water Soil Field Capacity (WSFC). By contrast at 50% and 25% WSFC, the leave area decrease on all line of wheat, in particular, at 10 weeks after planting. It assumed that this condition affected eventually on the remobilization of nutrition into leaves as the photosynthesis machinery (Inoue, Inanaga, Sugimoto, An, & Eneji, 2004; Plaut, Butow, Blumenthal, & Wrigley, 2004).

In general, all lines have decrease in all components of growth and yield. The results show that the observation on leaf area does not influenced by different soil water content on 6 weeks after planting. However, on 8 and 10 weeks after planting the leaves area show significant differences as a result of soil moisture level. It might be affected by the limited hormones such as auxin as a result of the drought effect. The stressed cell continued under limited water and at the age of 8 and 10 WAP observations have no significantly different of M7, M8 and SO3 lines provided mean value of leaf area. However, the dramatically dropped of leaf area provide under WSFC 50% and 25%.

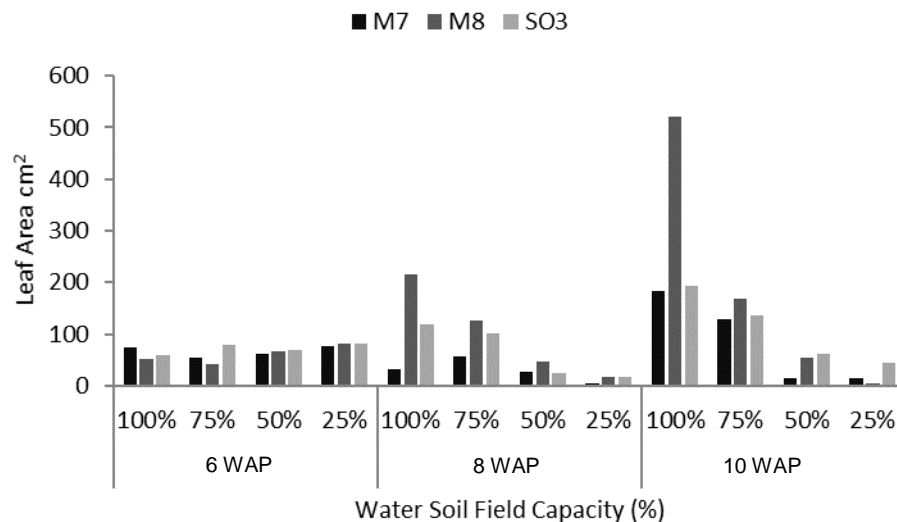


Figure 1. Differences of leaf area per plant on 6, 8 and 10 weeks after planting

Table 1. Average of specific root length to each treatment soil moisture and wheat lines on various age observation

Treatment	Specific root length (SRL)		
	6 WAP	8 WAP	10 WAP
<b>Water content:</b>			
A100	98.47	34.33 a	59.04
A75	99.36	33.88 a	63.60
A50	101.16	44.12 a	56.02
A25	119.33	60.24 b	55.77
HSD 5%	ns	17.33	ns
CV (%)	37.87	28.28	51.34
<b>Lines:</b>			
M7	90.57	36.62 a	50.99
M8	104.68	61.11 ab	46.05
SO3	97.49	46.70 a	41.35
HSD 5%	ns	ns	ns
CV (%)	32.32	27.54	27.24

Remarks: A number followed by the same letters in the same column are not significantly different based on HSD test at 5%; ns = no significant; WAP = weeks after planting differently to drought stress levels.

There was no interaction on specific root length (SRL) observation between soil moisture level and wheat lines on 6 and 10 week after planting, exception on 8 week after planting (Table 1). Meanwhile, the value of SRL was particularly triggered by different level of water which treated on soil. Those performances of roots of plant presented at 8 weeks after planting of 50% and 25% water soil field capacity. In general, due to a decrease in soil water content reached 50% led to increases the value of SRL even on 25% WSFC. It was assumed that the lower water content gave the thin roots development much longer and was expected to be able to reach water deeper in the soil surface. The morphological adapted to environment for cell growth in long term under limited condition, such as the modification of size and shape of organ of plant (Anjum, Xie, & Wang, 2011). Beside, this is clear that morphological roots affected by the water availability in the soil, hence the root development could support the plant growth.

The results show that in general there were decline on the average yield components due to decreased levels of groundwater. On the parameter number of spikelet per plant, there was no interaction between soil moisture and lines of wheat. The treatment of soil water content gives a decrease in the number of spikelet per panicle (Table 2). Meanwhile, treatment of groundwater levels to give effect to lines on the average number

of spikelets per plant. Generally, in all lines tested, decline in the number of panicles per plant were observed. However, each line tested shows decline in the average value of the index of harvest due to decreased soil moisture content particularly on 25% of water soil capacity level. The consequence level of drought is presented as well as clearly on the number of spike per plant (Figure 2).

Table 2. Average number of spikelet per spike for each treatment soil moisture and wheat lines

Treatment	Average of spikelet per spike
<b>Water content:</b>	
A100	17.08 c
A75	16.50 bc
A50	15.25 b
A25	12.37 a
HSD 5%	2.08
CV (%)	10.66
<b>Lines:</b>	
M7	14.67
M8	15.87
SO3	15.36
HSD 5%	ns
CV (%)	10,21

Remarks: Number followed by the same letters in the same column are not significantly different shows based on HSD test at 5%; ns = not significant; WAP = weeks after planting.

Normal water (WC) 100% produced the number of spike per plant on all lines were high around at 5.5 spikelets per plant. However reducing the water content on 75%, 50% only about 2.5 spikes (Figure 2).

Nitrogen content of each line tested decrease sharply started on 75% to 25% water soil capacity on level of water to give an effect on decreased levels of total N per yield per plant which was investigated in grain (Figure 3). Based on the main photosynthesis materials, the nutrient acquisition reduced due to the inhibited-roots development. Although the total nitrogen

decreases in the grains of all lines, the proline content was increased significantly in leaves. It was assumed that the decreasing of nitrogen content in grain was caused by the nitrogen retrans location from grain to leaves in the drought condition (Figure 4). On the other hand, all of the wheat lines produced the proline as the main amino acid when plants are under the stressed condition such as drought. Figure 4 shows that the proline content in leaves, increase two fold on 25% water soil field capacity compare to the total proline content in leaves on normal water soil field capacity (100%).

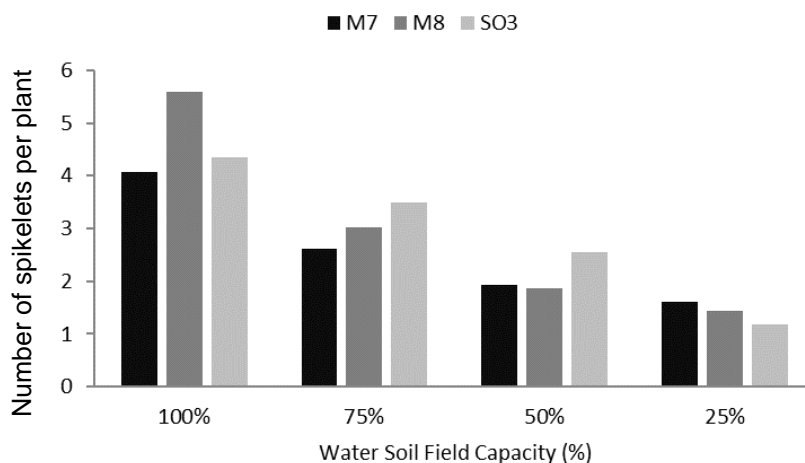


Figure 2. Number of spikelets per plant

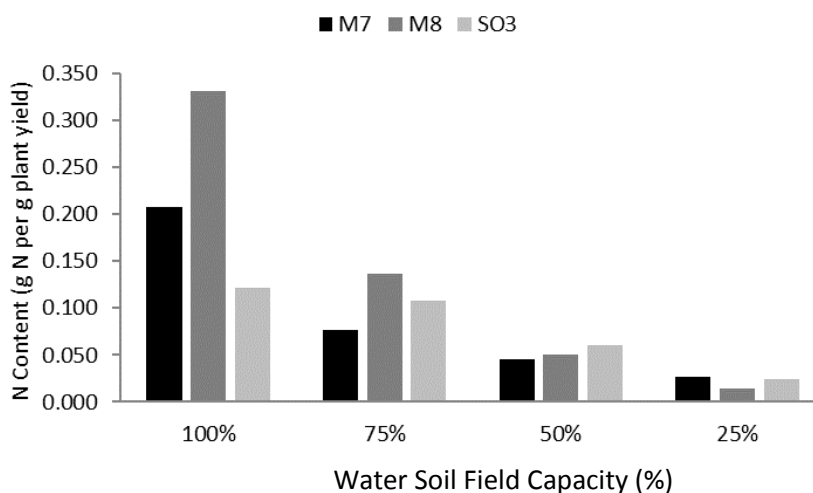


Figure 3. Total nitrogen content in seeds

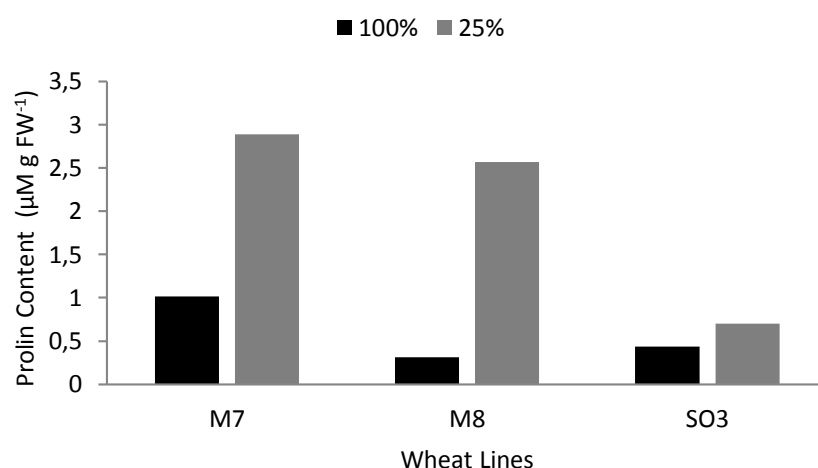


Figure 4. Total proline content in leaves

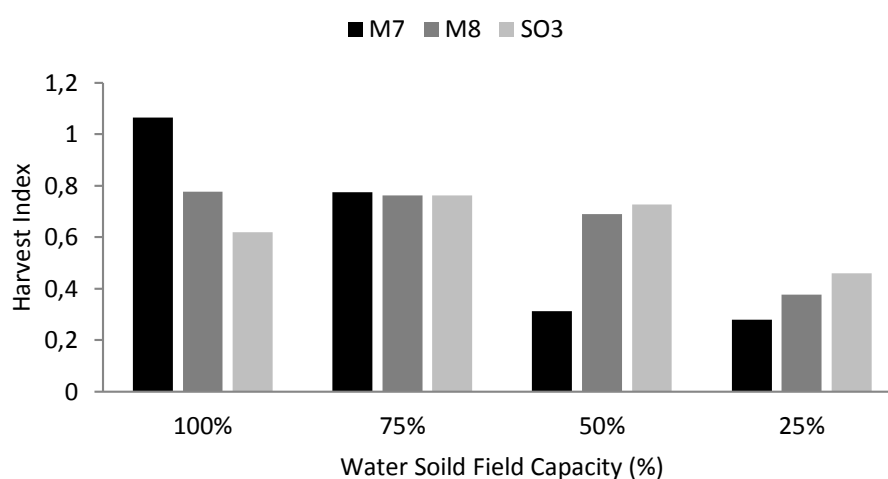


Figure 5. Harvest index on different water soil field capacity

The decline of water soil field capacity due to the drought condition (25%) led to decrease of the harvest index on line M7, M8 and SO3 of wheat. Moreover, the decreasing water soil field capacity until 50% consequentially dropped the total nitrogen content in grain per plant. There is no positive correlation between decreasing of total nitrogen content in plant with climbed of proline content, but in that case, the observation of all lines are clearly that the lack of the water in soil generated the decline of harvest index reached 50% compare to normal soil condition (Figure 5). The proline synthesis might be allocated on particular hormone synthesis as response of plant

to drought and influence the other adapted processes (Choudhary, Sairam, & Tyagi, 2005). However, all the line of wheat in this experiment shows that the total content of proline increases two folds under limited water soil or drought on M7 and M8 lines, meanwhile it increases slightly on SO3 line.

## CONCLUSION AND SUGGESTION

Depleting of water content generally in three wheat lines, trigger inhabitation of nutrition absorption, thus the plant does not growth normally such present on several parameter

observed are leaf area, number of spikelets, root specific length, harvest index, total of nitrogen and proline contents. Those observations occur clearly started at 8 weeks after planting, vegetative stages, indeed under 50% and 25% water soil field capacity. The next experiment will be investigated the hormones accumulation and cell differentiation of all local wheat lines.

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