



Evaluation of drought-tolerance in some tropical wheat genotypes (*Triticum aestivum* L.) at different osmotic-stress level

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ABSTRACT

Abiotic factors, such as temperature and drought, are the main factors limiting the cultivation under the tropical condition. Two-stage experiments were conducted to examine the drought-tolerant potential of some wheat genotypes against the osmotic stress under the tropical condition at the Laboratory and Greenhouse of Hasanuddin University and Indonesian Cereal Research Institute. The experiments were arranged in a randomized block design with the split-plot pattern and respectively provided with four and three replications. The main plot was potential osmotic stress (0, -0.33, and -0.67 MPa) and the sub-plot was selected wheat genotypes (17 genotypes). The results indicate that based on the germination percentage, shoot/root ratio, proline content, stomatal behavior, and relative water content, the wheat lines of O/HP-78-A22-3-7, WBLL*2KURUKU, O/HP-6-A8-2-10, and O/HP-22-A27-1-10 are identified to have better drought-tolerance than the other genotypes based on the analysis of responses to parameters observed. The positively adaptive response of some tropical wheat genotypes to drought stress may be used as a potential donor for further development of drought-tolerant wheat varieties under the tropical climate in Indonesia.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one sub-tropical crop that has become the most important food commodity in the world followed by rice and maize. Wheat plays an important role to support the world food security as approximately consumed by 36% of the world total population. The recent yield rate increase is still too low to fulfill the world grain requirements as it is estimated that the wheat demand in 2050 will increase by 70% (International Maize and Wheat Improvement Center, 2014). It is necessary to increase wheat productivity to meet the increasing wheat demand. Wheat productivity is greatly influenced by both biotic and abiotic factors

like environmental problems, such as drought. Drought is abiotic stress that has become an important environmental problem limiting the agricultural system worldwide. Nowadays, cultivating plants has been directed to overcome the biotic and abiotic stress problems (Mir et al., 2012). Drought or water deficit or water scarcity, which has become a main abiotic stress problem, has a negative effect on the development of crop growth, differentiation, and productivity (Zlatev and Lidon, 2012). Moreover, water stress problem is related to osmotic stress or salinity, which disturbs the relationship between the mineral-nutrient intake through their available effects and the plants' nutrient transport. Due to some case studies in the tropical area, Indonesia has

recently become the world second largest wheat importer after Egypt, reaching 11.5 million tons. According to the US Wheat association, it is reported that the wheat consumption in Indonesia was continuously increasing from time to time starting from 2011 until 2015 in which the wheat consumption for food was still in the range of 6.25 million tons in 2011–2012, and it increased by 11.2% in 2012–2013, 3% in 2013–2014, 2.8% in 2014–2015 and 8% in 2015–2016, reaching 7.95 million tons (Rittgers et al., 2011).

The varieties of wheat cultivated in Indonesia are the introduced varieties. After passing through the adaptation testing stages in several appropriate environments and having the ability to yield in some experimented lands, the wheat lines are released becoming the new national wheat varieties that generally have specific adaptation to highland yet not adaptive to drought. The researches on the adaptation of wheat varieties in Indonesia have been conducted by several researchers (Nur et al., 2014). Unfortunately, there is no recommended drought-tolerant wheat genotypes or varieties to encounter the temperature and drought stress factor as the main obstacles in Indonesia.

Drought-tolerant selection is a method of reducing the impact of water deficit on the crop yield. Faghani et al. (2014) have suggested that drought stress is one major environmental problem inhibiting the plants growth and productivity throughout the world. Meanwhile, the drought effects on plants include water content loss, reduced leaf water content, and the stomatal opening and closing mechanism. The first plants response to stress is that there are some stomatal-opening obstacles. The mechanism of plant leaf's stomatal opening is controlled by the water changes' potential. The plants' physiological and metabolic processes require the adaptation to the environmental stress. The plants may adapt to drought stress by accumulating some organic materials, such as stable amino acids (proline). Proline is formed and accumulated in large quantity by the tolerant (mutant) plants when they are subjected to stress (Zegaoui et al., 2017).

The function of proline is to maintain the balance of water between vacuoles, cytoplasm, and their environment. Proline accumulation due to the plants response to the environmental stress is generally known and varied depending on age and the variety of plants. The drought effects may result in proline

biosynthetic pathways and the relationship between proline accumulations and changes in some physiological properties (Relative Water Content and Fluorescence Parameters) in which proline may have a different positive effect on drought-resistance of the tested genotypes (Bandurska et al., 2017). The research was conducted by evaluating the tolerance of some wheat varieties to water stress, and the result showed there were different responses of each wheat line, such as higher Relative Water Content (RWC), proline content, and stomatal behavior, hence these traits may become drought-tolerant indicators (Khakwani et al., 2012).

The other investigation on relative water content and proline content in *Citrullus amarus*-landrace revealed that the measured RWC was better to characterize the water deficits. The plant landrace from the arid (dry) areas may retain higher RWC during the dry seasons when compared to that from the moderate temperate climates. In both landraces, the preliminary genetic biosynthesis is regulated by drought stress (Zegaoui et al., 2017). Furthermore, Swapna and Shylaraj (2017) have revealed that the positive and adaptive responses to the drought stress may be used in the genetic development program to improve the drought-resistant plants.

Developing genotype candidates at the target growing environments and drought conditions, as well as minimizing confounding effects of other stresses are performed to enhance the selection of drought-tolerant plants. Moreover, the significant progress may be achieved when breeders and other interdisciplinary experts work together with a common goal to produce drought-tolerant and high-yield wheat timely (Mwadzingeni et al., 2016). Therefore, the objectives of this research were to evaluate some wheat lines in the tropical condition that have a high tolerance to drought stress and to figure out the best drought-resistant wheat lines for the wheat development programs in the future.

MATERIALS AND METHODS

Two stage experiments were conducted to evaluate the responses of some selected tropical wheat lines to drought stress under tropical condition in 2018 at the laboratory and Greenhouse of Hasanuddin University Makassar, Laboratories of Faculty of Agriculture, Universitas Gadjah Mada Yogyakarta

and Laboratory of Indonesia Cereal Research Institute (ICeRI), Maros, Indonesia. Both experiments were arranged in a randomized complete block design with split-plot pattern and provided with respectively four and three replications. The osmotic-stress treatments were given by using different concentrations of Polyethylene Glycol-6000 (PEG-6000) Solution in Water, consisting of control (0 MPa), -0.33MPa, and -0.67 MPa, considered as the main plot. Meanwhile, the sub-plot was the use of 17 wheat genotypes consisting of 16 lines and one control variety (Table 1).

Germination and seedling

The good seeds were selected after soaked in the distilled water, in which the normal seeds were chosen and then sterilized with Sodium hypochlorite (NaOCl) 5% for five minutes to prevent fungus infection. In each treatment, a hundred seeds were placed on the Whatman filter paper softened with 10 mL distilled water and PEGs solution inside a Petri-dish with a diameter of 90 mm. The treatments consisted of control treatment, which was 10 mL distilled water (0% PEG) with an osmotic potential that was equal to 0 MPa, PEG solution of 121 g.L⁻¹ with an osmotic potential of 12.1 % that was equal to -0.33 MPa, and

PEG solution 200 g.L⁻¹ with an osmotic potential of 20% that was equal to -0.67 MPa. The seeds were kept at the room temperature of 25–27°C under normal light.

The germination percentage (G%) was calculated based on the total number of the germinated seeds divided by the total number of the seeds used. The germination observation and calculation were daily performed for seven days after sowing the seeds (International Seed Testing Association, 2008).

Proline content Assay was analyzed according to Bates et al. (1973). To determine the seedling leaf proline content, 0.5 g leaf sample was crushed in pestle using a mortar with sulfosalicylic aqueous acid solution 3% (w/v), and the homogenate was filtered using Whatman No.2 filter paper, then two mL filtered extract was mixed with two ml ninhydrins acid and 2 mL glacial acetic acid. The solution mixture was incubated at 100°C for one hour and then let on ice for 15–20 minutes. Subsequently, four mL toluene was added to the mixture reaction. After being centrifuged, the organic phase was extracted into a quartz cuvette, and then the absorbance was measured at 520 nm Spectronic 21-D (UV visible spectrophotometer) against toluene as blank.

Table 1. The selected tropical wheat lines in this study

No	Lines	Abbreviation	Origin*
1	FUNDACEP 30	Fundacep	CIMMYT
2	QUIAU	Quiau	CIMMYT
3	WBLL*2KURUKU	Wbll	CIMMYT
4	PRL/2*PASTOR	Pastor	CIMMYT
5	KIRITATI/4/2/*SERI.1B*2	Kiritati	CIMMYT
6	TRCH*2/3/C80.I/3	Trch	CIMMYT
7	SAAR/2/*WAXWING	Waxwing	CIMMYT
8	O/HP-82-A-15-1-4	HP-82-4	IceRI
9	O/HP-12-A1-1-9	HP-12-A1	IceRI
10	O/HP-78-A22-3-7	HP-78-7	IceRI
11	O/HP-6-A8-2-10	HP-6-A8	IceRI
12	O/HP-22-A27-1-10	HP-22-27	IceRI
13	O/HP-92-A1-1-3	HP-92-A1	IceRI
14	O/HP-12-A5-4-5	HP-12-A5	IceRI
15	O/HP-78-A2-5-2	HP-78-2	IceRI
16	O/HP-82-A15-2-3	HP-82-3	IceRI
17	Var. GURI-3 (Control)	Guri 3	IceRI

Remark: *CIMMYT: The International Center for Maize and Wheat Improvement; ICeRI: Indonesia Cereal Research Institute

Hydroponic Culture

Seven-day-old normal seedlings were selected for each treatment and then planted on Rockwool and Hydroponic media with Nutrient Film Technique (NFT) system. Hydroponic cultures were made at the greenhouse with a controlled temperature of approximately 28°C during the daylight to 20°C at night light. The nutrient solution of Hydroponic media was AB-mix nutrition formula A and B. Formula A contained (in g.L⁻¹): Ca(NO₃)₂ 1176; KNO₃ 616; Fe-EDTA 38, while formula B contained (in g.L⁻¹) KH₂PO₄ 335; (NH₄)₂SO₄ 122; K₂SO₄ 36; MgSO₄ 790; CuSO₄ 0.4; ZnSO₄ 1.5; H₃BO₃ 4; MnSO₄ 8; and MoO₄ 0.1. Every 200 Liters hydroponic solution was mixed with one Liter of formula A and B. The electrical conductivity of the nutrient solution was 2.5–3.2 dS.m⁻¹, while the Total Dissolved Solids (TDS) was 1250–1600 ppm with pH 5.5–6. TDS, EC, and pH meter was used to record data for TDS, electrical conductivity, and pH of the nutrient solution, respectively. Three hydroponic constructions were used as the main plot, and each consisted of three layers as replications. Each hydroponic construction used 100 Liters hydroponic solution. The media volume should be daily controlled and kept at 100 Liters. The media flowing rate was set close to 0.75 L.min⁻¹. The hydroponic media was treated with water solution and PEG concentration as described above. The hydroponic experiments were set up in a randomized complete block design with split-plot pattern with three replications. Either main plots or sub-plots were the same as described above. Hydroponic experiments were performed until the plants entered the generative phase (85–90 days after planting).

Seedling root and shoot attributes were observed and measured. The shoot length (cm) was measured from the shoot tip to the collar region. Meanwhile, the root length (cm) was measured due to its seminal root that the ratio of shoot/root length might be simply calculated.

The stomatal behaviors including the stomatal density and stomatal index were recorded. The collection of stomatal observation was made when the wheat plants were at the generative phase by removing the lower leaf epidermal incision and then placing it on an object glass under microscope (Zeiss Primostar P95-C Microscope with Axio Cam Erc5s connected to a Personal Computer (PC) with

Axio-Vision Rel.4.8.2 software) at 40×10 (400) magnifications. Three fields of view (280×210 μm or 0.0588 mm² per field of view) per leaf were determined to count the number of stomata and other epidermal cells. Stomatal density was calculated from the number of stomata in a field of view, while the Stomatal Index (SI) was estimated as the eq.1., where S is number of stomata and E is number of epidermal cells (Ogaya et al., 2011).

$$SI = [(S \times 100)/(E + S)] \dots \dots \dots (1)$$

Relative Water Content (RWC) was determined based on the plant leaves which flags were used to measure each sample's fresh weight (FW). The samples were then soaked in the distilled water for 16–20 hours. The leaf sample was then wiped with tissue paper, and the soaked sample was weighed and measured as Soaked weight (SW). The sample was then dried at 80°C in an oven to determine the dry weight of each sample (DW), and the RWC (%) was calculated using eq.2.

$$RWC = [(FW - DW)/(SW - DW)] \times 100 \dots \dots \dots (2)$$

All collected data were then examined and analyzed using analysis of variance (ANOVA) with STAR (Statistical Tool for Agricultural Research) Software Version 2.0.1 @Copyright International Rice Research Institute (IRRI) 2013–2020. The means of the treatments were then compared using LSD (Least Significant Difference at p<0.05) test to evaluate and calculate the considerable difference between treatments, and the graphics were set using Microsoft Excel software.

RESULTS AND DISCUSSIONS

Based on the analysis result, there were significant differences in the germination behavior, shoot and root growth, shoot/root ratio, proline content, and RWC between the wheat genotypes tested. Stomatal behavior based on the stomatal density, epidermal cell density, and stomatal index of each genotype observed may be different in response to the osmotic stress.

Germination Percentage (G%)

The plant strategy to face the drought starts from the germination phase and vegetative growth to form the vegetative organs and deep root system. The germination percentage of wheat

genotypes was significantly affected by the osmotic stress level (Figure 1). All wheat genotypes showed that the decreasing germination percentage due to the osmotic stress level. The decreasing value of germination percentage as response to the osmotic stress treatment was different between each wheat genotype. The genotype of Guri-3 showed a decreasing germination value that was relatively lower in the osmotic stress of -0.33 MPa, while in the osmotic stress of -0.67 MPa, only Wbill, Kiritati, HP-12-A1, HP-78-7, HP-6-A8, HP-78-2, HP-78-2 and Guri-3 showing the decreasing germination that was lower than the other wheat genotypes (Figure 2).

The seed germination response of some tropical wheat genotypes under different drought stress was observed using the treatment of different osmotic stress levels, which showed that the increasing osmotic stress level led to a decreasing

germination percentage. Some wheat genotypes showed highly decreasing germination percentage as the osmotic stress level increased. It means some genotypes are intolerant to the drought stress. Identifying genotypic tolerance to the drought stress during germination using PEG on wheat crop has been conducted by several researchers. Several studies have reported that PEG concentrations might be used to select the wheat crops' germination stage between 15–25% (Baloch et al., 2012). This result showed that the optimum PEG concentration for the drought stress tolerance screening was highly dependent on wheat genotype.

The increasing osmotic stress from 0 to -0.33 and -0.67 MPa might lower the decrease germination percentage of HP-78-2, Wbill, HP-78-7, HP-6-A8, Kiritati, HP-22-7, HP-82-3, HP-12-A1, Waxwing lines and the tested variety of Guri-3. Among the observed genotypes, HP-78-2, Wbill, HP-78-7, HP-6-A8,

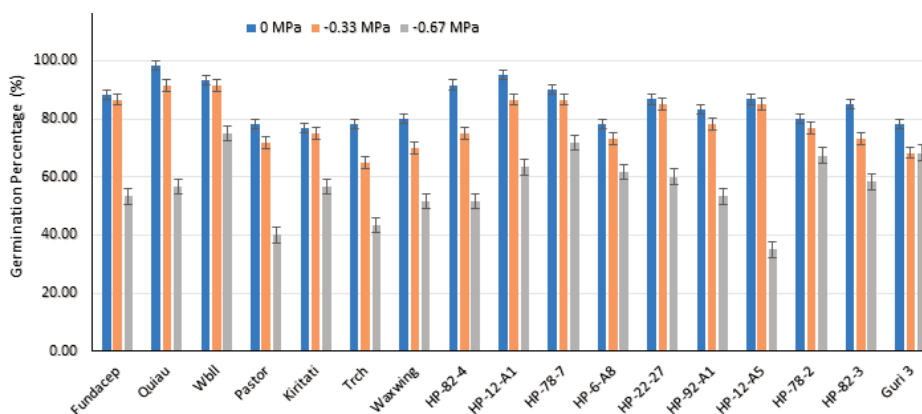


Figure 1. Germination percentage of 17 tropical wheat genotypes under osmotic stress of 0 MPa, -0.33 MPa, and -0.67 MPa. The Means Standard Errors were shown by the vertical bars.

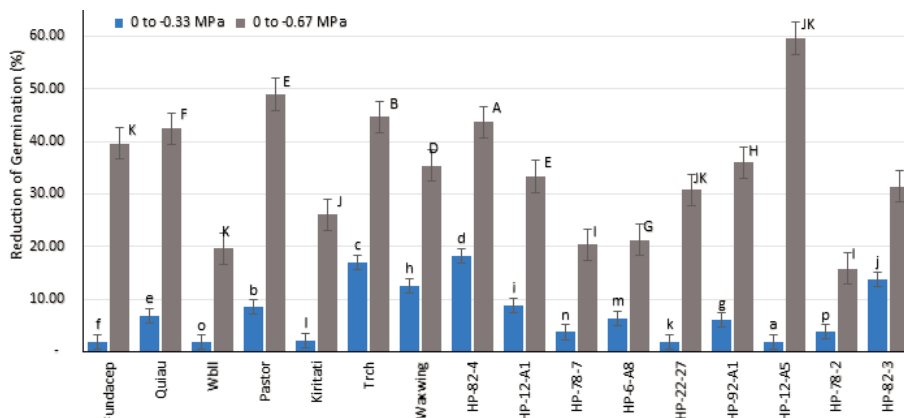


Figure 2. Decreasing germination percentage under osmotic stress. The top vertical bars followed by different letters were significantly different according to LSD test at $p < 0.05$.

Kiritati, and HP-22-7 showed a lower decrease of germination percentage than the others in both osmotic stress levels. This finding is supported by the previous studies reporting that the decreasing seed germination percentage was linearly increased by PEG concentration, including wheat and sorghum (Rajendran et al., 2011). The osmotic stress caused by PEG concentration induced the plant seed's drought stress. The osmotic stress relatively increases to the decreasing osmotic potential. This potential osmotic decrease may influence the seed ability to germinate and become less vigorous due to the seed's decreasing water absorption in which there is an ability selection of each different line in response to the osmotic stress to germinate and grow. Drought is a multifaceted pressure condition inhibiting the crops' growth. Seed germination is a plant's sensitive and critical stage which process is inhibited or even entirely prevented by the drought (Liu et al., 2016). The increasing PEG concentration on media might cause the decreasing osmotic potential that influences the seed germination ability. Meher et al. (2018) have reported that the most sensitive and critical stage in

the plants' life cycle is due to the seed germination phase. Drought condition may inhibit the seed germination phase and metabolism that only those with higher drought-tolerance are able to germinate.

Shoot, Root, Proline Content, and RWC

The decreasing or increasing shoot and root length, as well as the shoot/root ratio, of all wheat genotypes was significantly influenced by the osmotic stress (Table 2). Guri-3 showed the highest value of shoot length in all osmotic stress levels and followed by Kiritati. The root length of all tested genotypes were relatively similar to control. Meanwhile, the control variety did not become the best genotype when compared to all tested genotypes, even in the osmotic stress of -0.67, the wheat lines of HP-6-A8, HP-78-7, and HP-12-A1 showed the longest roots in response to the stress. The highest value of shoot/root ratio was observed in HP-82-3, Pastor, and Guri-3.

The Proline content in plants leaf at the highest osmotic stress level (-0.67 MPa) ranged from the level of 3.075 μmol.mg⁻¹ (Quiiau) to 13.505 μmol.mg⁻¹ (HP-12-A5). Proline content may significantly increase

Table 2. Effects of osmotic stress on the shoot and root growth parameters of 17 tropical wheat genotypes

Genotypes	Shoot length (cm)			Root length (cm)			Shoot/root ratio		
	0 Mpa	-0.33 Mpa	-0.67 Mpa	0 Mpa	-0.33 Mpa	-0.67 Mpa	0 Mpa	-0.33 Mpa	-0.67 Mpa
Fundacep	15.8 bcd	15.8 bcd	5.5 i	10.7 fg	12.5 de	5.3 i	1.47 abc	1.27 abc	1.03 fg
Quiiau	13.0 g	15.4 bcde	13.5 de	10.5 g	15.0 ab	10.5 cd	1.24 hi	1.03 ef	1.29 de
Wbll	15.5 cdef	14.5 def	13.3 de	11.0 efg	12.0 e	10.3 cde	1.41 bcdef	1.21 bcd	1.30 de
Pastor	17.0 abc	16.8 ab	13.7 cde	12.0 bcd	15.3 ab	8.8 fg	1.42 bcdef	1.09 def	1.56 b
Kiritati	17.5 ab	15.0 cdef	13.5 de	13.43 a	14.2 bc	9.4 efg	1.29 defgh	1.06 def	1.44 bcd
Trch	16.0 bcde	15.8 bcd	15.3 b	11.0 efg	12.1 de	11.2 bc	1.45 abcde	1.31 ab	1.37 cd
Waxwing	16.3 bcd	16.5 abc	10.2 gh	11.8 cde	12.5 de	6.8 h	1.40 cdefg	1.32 ab	1.51 bc
HP-82-4	16.3 bcd	16.5 abc	11.4 fg	12.0 bcd	14.5 abc	8.5 g	1.36 cdefgh	1.14 cde	1.35 cde
HP-12-A1	14.3 fg	14.0 ef	10.2 gh	12.8 ab	14.8 abc	10.8 c	1.13 i	0.95 f	0.93 g
HP-78-7	14.5 efg	13.5 f	12.8 ef	9.3 h	10.5 f	11.8 b	1.57 ab	1.29 abc	1.08 fg
HP-6-A8	16.5 bcd	16.5 abc	14.5 bcd	11.5 def	13.0 d	14.7 a	1.44 abcde	1.27 abc	0.99 g
HP-22-27	15.5 cdef	13.5 f	15.2 bc	12.3 bcd	10.8 f	11.0 bc	1.27 fghi	1.26 abc	1.39 cd
HP-92-A1	15.5 cdef	16.2 bc	9.5 h	12.5 abc	12.3 de	9.8 de	1.24 ghi	1.32 ab	0.98 g
HP-12-A5	15.5 cdef	14.2 ef	6.8 i	11.5 def	10.8 f	5.7 i	1.30 efg	1.32 ab	1.19 ef
HP-78-2	15.5 cdef	14.5 def	13.5 de	11.5 def	12.8 de	9.5 ef	1.31 defgh	1.13 cde	1.43 bcd
HP-82-3	15.3 def	15.0 cdef	13.5 de	10.5 g	14.0 c	7.0 h	1.46 abcd	1.07 def	1.94 a
Guri 3 (C)	18.5 a	18.0 a	17.0 a	11.7 cdef	12.8 de	9.3 efg	1.59 a	1.41 a	1.83 a

Remarks: Means followed by different letters in the same column were significantly different according to LSD Test at α = 5%.

or decrease in response to the osmotic stress level (Figure 3). The effect of osmotic stress level, genotypes, and their interaction was significant at $p < 0.05$. The effects comparison of non-osmotic stress (Control) and both osmotic stress levels (-0.33 and -0.67 MPa) indicated that all wheat genotypes might increase the proline content at the osmotic stress condition, except Fundacep, Pastor, and Trch. Meanwhile, Waxwing, HP-12-A1, HP-78-7, HP-6-A8, HP-92-A1, HP-12-A5, HP-82-3, Kiritati, and Guri-3 resulted in high increasing proline content. It indicated that plant tolerance was the main mechanism to the drought stress, which was characterized by the increasing proline accumulation in all genotypes. Relative water content (RWC) significantly decreased in response to the osmotic stress in all genotypes (Figure 4). The wheat genotypes of HP-6-A8, HP-78-2, Wbll, HP-92-A1, Quiaiu, HP-22-7, HP-78-7, and Guri-3 showed the highest RWC under osmotic stress condition. The RWC's value of Quiaiu, Wbll, HP-6-A8, and HP-78-2 tended to be higher than the control genotype

(Guri-3) when the osmotic stress was striking.

The growing shoot showed that some lines were very susceptible to osmotic stress as the drought stress inhibited the plant growth. Fundacep, HP-12-A5, HP-92-A1, Waxwing, and HP-12-A1 showed a very slow growth rate at the osmotic stress level of -0.67 MPa. Silva et al. (2013) suggested that plants grown in the environments with drought pressure were generally shorter than those under optimum conditions. Meanwhile, the roots' response to osmotic pressure was by elongating the plant roots. The osmotic stress-induced drought conditions on the growing media stimulated the root growth to grow longer. Drought conditions inhibits the plant leaf growth, while photosynthetic results are allocated more to the roots, thereby decreasing shoot/root ratio, which is preferable for plants to increase their root system capacity to absorb more water. Some wheat genotypes showed a significant decrease in shoot/root ratio value, yet some others showed that the shoot/root ratio was more sustainable (Table 2). The

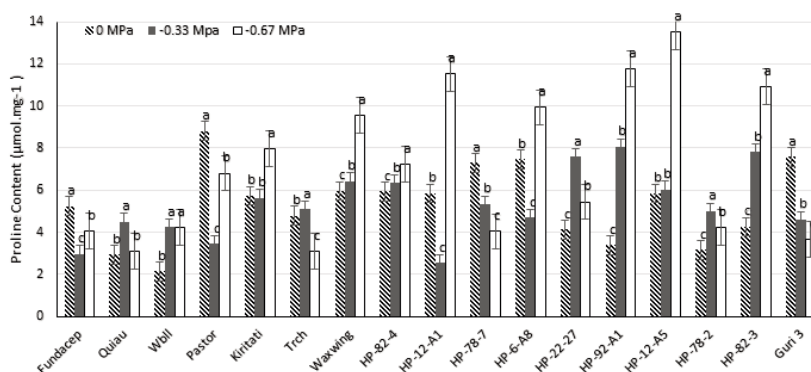


Figure 3. The influence of osmotic stress on proline content (every three vertical bars were followed by different letters which were significantly different at $p < 0.05$ by the LSD Test)

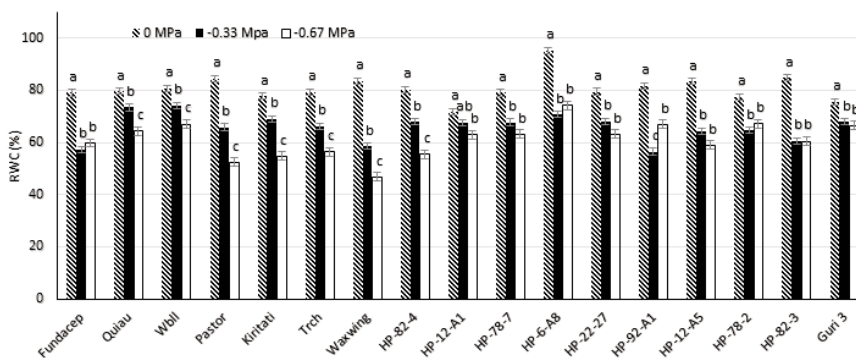


Figure 4. The influence of osmotic pressure on relative water content (RWC) (every three vertical bars were followed by different letters were significantly different at $p < 0.05$ by the LSD Test)

genotypes that could maintain the ratio value indicated that they were more tolerant to drought stress.

The degree of response indicated how each plant performs the drought tolerance mechanism. Genotypes of HP-6-A8, HP-78-7, Trch, HP-22-7, HP-12-A1, Quiau, Wbll, HP-92-A1, and HP-78-2 showed that they were more responsive to the osmotic stress than the others. The genotypes of HP-6-A8 and HP-78-7 showed the longest root length, which was approximately 14.67 and 11.83 cm, respectively. Meanwhile, Waxwing, HP-12-A5, and Fundacep had a lower ability to the drought-resistant, indicated by their shorter root length of respectively only 6.77, 5.67, and 5.33 cm. This finding is consistent with previous studies conducted by Shukla et al. (2015), revealing that the plant ability index to the drought stress was resistant to the screening tolerance characterized by the increased root growth. In this research, drought stress simulated by the osmotic stress stimulated the increasing proline accumulation in most tested wheat genotypes. Shukla et al. (2015) reported that wheat lines (*T. aestivum* and *T. harzianum*) had already improved their tolerance to drought by mediating the developed synthesis and proline accumulation due to the drought-stress tolerance. Abdallah et al. (2015) found that the higher contents of free proline in the plant might increase the plant's tolerance to drought stress. The genotypes

that had more than 80% increasing proline content levels were HP-92-A1 (247.85%), HP-82-3 (156.80%), HP-12-A5 (131.09%), Guri-3 (106.28%), Wbll (97.19%), and HP-87-7 (80.82%). The significant increase in proline content indicates that proline content is the main parameter for drought stress tolerant plant screening. The proline accumulation in the plant leaves is the response to the drought stress and considered as one plant's resistance mechanism to the drought stress. Faghani et al. (2014) explained that proline protected the metabolic processes in response to the unprofitable conditions by replacing water to stabilize those important cellular structures. Drought stress increases the accumulation of free proline in plant leaves. The leaves containing proline could maintain the water potential of their tissues through the mechanism called osmotic regulation. Bates et al. (1973) reported that the increasing proportional proline of plant under drought stress could be used as the evaluating parameter in selecting the drought-resistant varieties.

Relative water content (RWC) is one of the important parameters to figure out the status of plant leaves water deficiency. According to Meher et al. (2018), PEG had already induced significant water stress, chlorophyll content, and parameter to select the high yielding genotypes to maintain the cellular decrease under water stress environment

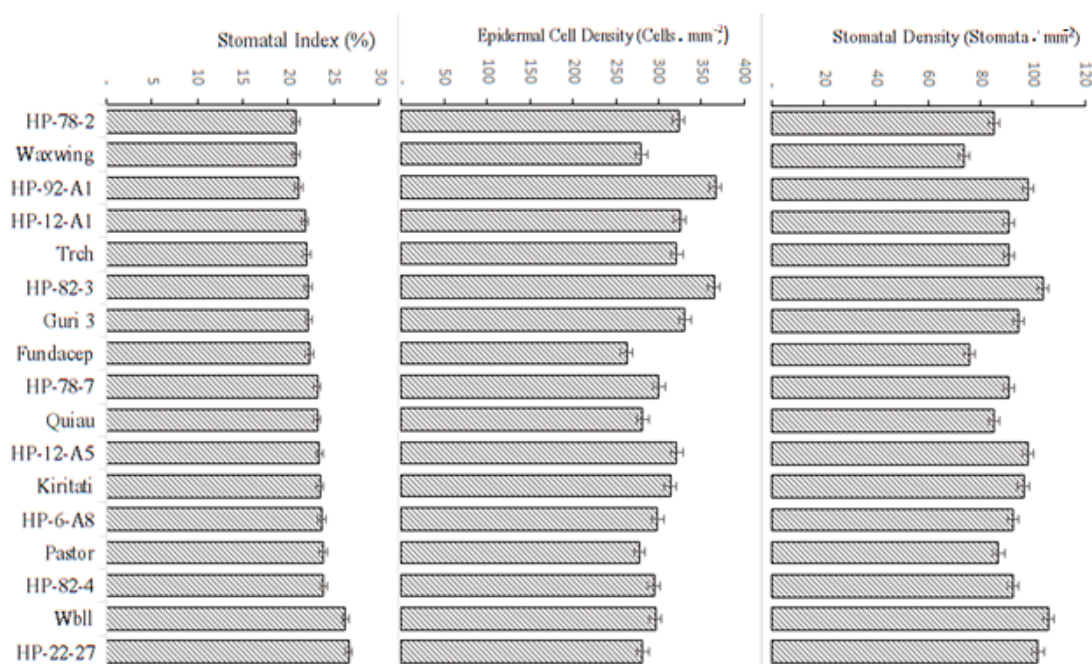


Figure 5. Mean values of stomatal density, epidermal cellular density, and stomatal index at 0, -0.33, and -0.67 MPa. The Means Standard Errors were shown by the vertical bars

to be more resistant to the drought stress and considered stable. Information related to the plant leaves' water deficiency and unfavorable conditions is due to the heat and drought provided by the RWC content. Chaturvedi et al. (2012) state that the plant has the ability to evolve its resistance mechanism under water stress, developed by the natural mechanism to reduce the plant leaves' loading energy. Sikuku et al. (2012) found that more resistant variety could maintain the high relative water content (RWC) than the susceptible ones. Furthermore, the highly resistant varieties to RWC that can maintain the protoplast hydration under the drought stress conditions in a longer period may ensure the plants sustainable productivity. The genotypes of HP-6-A8, HP-78-2, Wbll, HP-92-A1, Quiau, HP-22-7, HP-78-7, and Guri-3 showed that they had higher relative water content (RWC) under the highest osmotic stress (-0.67 MPa).

Stomatal Behavior

Stomatal density ranged between 73.7-105.82 stomata.mm⁻² in both Waxwing and Wbll. HP-92-A1 and HP-82-3 showed higher number of epidermal cells compared to the other genotypes (Figure 5). Meanwhile, HP-22-27 and Wbll showed the highest value of stomatal index.

The observed wheat genotypes showed that there was no significant change in their epidermal cell density, stomatal density, or index in response to the osmotic stress condition. The high value of stomatal density ranged from 90.7–105.82 stomata.mm⁻², while the low value of stomatal density ranged from 73.7–86.9 stomata.mm⁻². The epidermal cellular density ranged from 296.67–366.59 cell.mm⁻² (high epidermal cell density), while the low epidermal cellular density ranged from 262.66–281.56 cell.mm⁻². Meanwhile, the stomatal index ranged from 20.83–26.6%. The previous research reported that plants adaptation to drought might take place due to the increasing stomatal density and decreasing cellular size under the drought conditions. The study reported that water stress might increase the stomatal density and index and decrease water potential under normal conditions. Conversely, the stomatal density might increase or decrease under severe drought pressure. Meanwhile, the plant leaves had a flexibility level in response to the environmental changes (Xu and Zhou, 2008).

CONCLUSIONS

The tropical wheat genotypes of O/HP-78-A22-3-7, WBL*2KURUKU, O/HP-6-A8-2-10, and O/HP-22-A27-1-10 were found to be more tolerant to the drought stress than the others genotypes. Four genotypes were found to have a more positive response to drought stress condition, indicated by their responses to the osmotic stress level. Also, the proline content and Relative water content (RWC) improved the wheat genotypes' tolerance level under the drought stress when compared to Guri-3 as the tested variety.

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REFERENCES

- Abdallah, E. F., A. Hashem, A. A. Alqarawi, and H. A. Alwathnani. 2015. Alleviation of adverse impact of cadmium stress in Sunflower (*Helianthus annuus* L.) by Arbuscular mycorrhizal fungi. *Pakistan Journal of Botany*, 47: 785–795.
- Bates, L. S., R. P. Waldren, and Teare. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 205–207.
- Baloch, M. J., J. Dunwell, N. U. Khan, A. A. Khakwani, M. Dennett, and W.A. Jatoui. 2012. Profiling dehydrin gene sequence and physiological parameters in drought tolerant and susceptible spring wheat cultivars. *Pakistan Journal of Botany*, 44: 801–806.
- Bandurska, H., J. Niedziela, M. Pietrowska-Borek, K. Nuc, T. Chadzinikolau, and D. Radzikowska. 2017. Regulation of proline biosynthesis and resistance to drought stress in two barley (*Hordeum vulgare* L.) genotypes of different origin. *Plant Physiology and Biochemistry*, 118: 427–437.
- Chaturvedi, G. S., A. Singh, and R. Bahadur. 2012. Screening techniques for evaluating crop germplasm for drought tolerance. *Plant Archives*, 12: 11–18.

- Faghani, E., J. Gharechahi, K. Setsuko, M. Mehdi, K. R. Ali, N. Farzaneh, F.L. Karimi and S.G. Hosseini. 2014. Comparative physiology and proteomic analysis of two wheat genotypes contrasting in drought tolerance. *Journal of Proteomics*, 114: 1–15.
- International Maize and Wheat Improvement Center. 2014. Annual report turning research into impact. <http://www.cimmyt.org/annual-reports>.
- International Seed Testing Association. 2008. International rules for seed testing CH-8303. <http://www.seedtest.org/>
- Khakwani, A. A., M. D. Dennett and M. Abid. 2012. Growth and yield response of wheat varieties to water stress at booting and anthesis stages of development. *Pakistan Journal of Botany*, 44: 879–886.
- Liu, Y., H. Xu, X. X. Wen and Y. C. Liao. 2016. Effect of polyamine on seed germination of wheat under drought stress is related to changes in hormones and carbohydrates. *Journal of Integrative Agriculture*, 15: 2759–2774.
- Meher, P. Shivakrishna, R. K. Ashok and R. D. Manohar. 2018. Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. *Saudi Journal of Biological Science*, 25: 285–289.
- Mir, R. R., A. M. Zaman, N. Sreenivasulu, R. Trethowan and R.K. Varshney. 2012. Integrated genomics, physiology, and breeding approaches for improving drought tolerance in crops. *Theory Applied Genetic*, 125: 625–645.
- Mwadzigeni, L., H. Shimelis, E. Dube, D.L. Mark and J.T. Toi. 2016. Breeding wheat for drought tolerance: Progress and technologies. *Journal of Integrative Agriculture*, 15: 935–943.
- Nur, A., M. Azrai, H. Subagio, Soeranto, Ragapadmi, Sustiprajitno, and Trikoesoemaningtyas. 2014. Perkembangan pemuliaan gandum di Indonesia. *Iptek Tanaman Pangan*, 8: 97–105.
- Ogaya, R., L. Llorens and J. Penuelas. 2011. Density and length of stomatal and epidermal cells in living fossil trees grown under elevated CO₂ and a polar light regime. *Acta Oecologica*, 37: 381–385.
- Rajendran, R. A., A. R. Muthiah, A. Manickam, P. Shanmugasundaram, and A. J. Joel. 2011. Indices of drought tolerance in sorghum (*Sorghum bicolor* L. Moench) genotypes at early stages of plant growth. *Research Journal of Agriculture and Biological Science*, 7: 42–46.
- Rittgers, C., G. McDonald, S. Meylinah. 2019. Indonesia Grain and feed annual 2009. [https://gain.fas.usda.gov/Recent GAIN Publications / Grain and Feed Annual_Jakarta_Indonesia_3-26-2019.pdf](https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual_Jakarta_Indonesia_3-26-2019.pdf)
- Shukla, N., R. P. Awasthi, L. Rawat, and J. Kumar. 2015. Seed biopriming with drought-tolerant isolates of *Trichoderma harzianum* promote growth and drought-tolerance in *Triticum aestivum* L. *Annals Applied Biology*, 166: 171–182.
- Sikuku, P.A., M.P. Onyango and G.W. Netondo. 2012. Physiological and biochemical responses of five nerica rice varieties (*Oryza sativa* L.) to water deficit at vegetative and reproductive stage. *Agriculture and Biological Journal of North America*, 3: 93–104.
- Silva, E.C., M.B. Albuquerque, A.D.A. Neto, C.D.S. Junior. 2013. *Drought and its consequences to plants from individual to ecosystem*, p. 17-47. In: S.Akinci (Ed.). *Response of Organisms to Water Stress*. Croatia: Intech.
- Swapna, S., K.S. Shylaraj. 2017. Screening for osmotic stress responses in rice varieties under drought condition. *Rice Science*, 24: 253–263.
- Xu, Z.Z. and G.S. Zhou. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany*, 59: 3317–3325.
- Zegaoui, Z., S. Planchais, C. Cabassa, D. Reda, B.O. Abrous and C. Pierre. 2017. Variation in relative water content, proline accumulation and stress gene expression in two cowpea landraces under drought. *Journal of Plant Physiology*, 218: 26-34.
- Zlatev, Z. and F. C. Lidon. 2012. An overview on drought-induced changes in plant growth, water relations and photosynthesis. *Emirates Journal of Food and Agriculture*, 24: 57–72