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Research Article

Effective Screening of Tropical Wheat Mutant Lines under Hydroponically Induced Drought Stress Using Multivariate Analysis Approach

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Abstract

Background and Objective: In developing wheat varieties adaptive to lowlands and drought stress, one of the screening methods are by hydroponic culture. Specific development related to the character screened and selection environment is required for the selection process to be more stable and accurate. The study aimed to identify the character and environment of hydroponic screening selection and to select the mutant strains of tropical wheat that are adaptive to drought stress. **Materials and Methods:** The study consisted of two experiments, namely screening of 21 wheat mutant genotypes and 4 comparison varieties on hydroponic culture and its validation on soil potting media for the yielding character. Four concentrations of Polyethylene Glycol (PEG) (0, 5, 10 and 15%) and two levels of water status, 80 and 60% of field capacity were used in the hydroponic culture screening test and on soil media, respectively. The data obtained were analyzed using analysis of variance and multivariate analysis. **Results:** Results showed that a concentration of 10% PEG was the best PEG concentration in selecting wheat genotypes adaptive to drought stress in hydroponic cultures. The weight character of 100 seeds and the number of grain per panicle are secondary characters that support productivity. The selected selection index was able to select seven genotypes that are adaptive to drought stress. **Conclusion:** These results concluded that the screening of drought on wheat through hydroponic culture was considered to be able to replace the screening of wheat drought on the soil in pots.

Key words: Genotypes, hydroponic, PEG, screening, wheat mutant, wheat drought

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Wheat is one of the main cereal foods in the world, including in Indonesia. However, an inappropriate climate type makes it difficult for wheat plants to develop in Indonesia¹. On the other hand, demand for wheat continues to increase every year following population growth². This makes the Indonesian government is highly dependent on wheat imports, which is a short-term solution to overcome this problem³. According to Zebua *et al.*⁴, based on trend calculation, Indonesia has an additional import volume of approximately as 11, 793 t every year. High imports in Indonesia cause the country's foreign exchange to decrease and food sovereignty to be highly dependent on other countries^{5,1}. Therefore, the development of wheat that can adapt in Indonesia becomes important research in order to maintain food sustainability in Indonesia.

One solution in developing wheat in Indonesia is the development of wheat varieties that are adaptive to the tropical environment. In general, wheat varieties that are adaptive to tropical environments are very few and adapt well above 800 m above sea level. Competition with horticultural products that have high economic value makes the variety difficult to develop⁶. Therefore, mutation breeding is one alternative to induce wheat diversity that is adaptive to the lowlands. Mutation breeding of tropical wheat has been carried out in previous studies, such as Nur *et al.*¹, Farid *et al.*³ and Nasaruddin *et al.*⁶. These studies are expected to continue until the release of the varieties.

Global warming becomes a crucial issue in the broad development of agriculture. The increase in high temperatures and changes in climate patterns and their intensity cause some growth environments to be categorized as marginal areas⁷. Marginal environments can reduce the potential for plant growth and death which correlates with decreasing productivity⁸. One of the main stresses in Indonesia is drought stress⁵. This stress is caused by an unbalanced between absorbed water and water that comes out through evapotranspiration. Lack of water can inhibit nutrient absorption, cell division and enlargement, cell physiology metabolism and fertilization which results in decreased plant growth and productivity⁹. Therefore, the development of wheat varieties in Indonesia should also include the nature of tolerance and adaptability to drought stress.

Screening for adaptability and tolerance to drought stress should pay attention to the critical phase and screening method. Hydroponic culture is considered to have an easily controlled environment, especially in the induction of drought, compared to soil media. Soil texture greatly

influences the degree of drought from soil media¹⁰, therefore, there is no guarantee that the entire selection environment for each genotype is the same.

The success of selection through hydroponic culture is inseparable from the environment and the character of the selection¹¹. Character selection is expected to be able to distinguish between genotype tolerance traits¹². However, the main character that is polygenic should also be supported by several other characters, so that selection in a stressful environment becomes more stable and reflects its adaptability^{13,14}. A good selection environment is a selection environment that can show a wide variance of tolerance traits, so that the selection environment is sought not to be too strict and weak^{11,15}. Therefore, the development of drought screening through hydroponic culture needs to be carried out in depth to select tropical mutant wheat lines that are adaptive to drought stress. The purpose of this study was to identify the character and environment of hydroponic screening selection and to select the mutant strains of tropical wheat that are adaptive to drought stress.

MATERIALS AND METHODS

Study area: This research was conducted in the Hasanuddin University teaching farm from May-August, 2017. The study consisted of two experiments, namely screening on hydroponic culture and its validation on soil media in pots with two levels of water availability.

Drought screening with hydroponic culture: The experiment used a split plot design with a randomized complete group design as the environmental design. The main plot in this experiment was the concentration of Polyethylene Glycol (PEG) consisting of four levels, namely 0, 5, 10 and 15%. Meanwhile, the subplots in this experiment were wheat genotypes consisting of 21 M4 mutant lines and four comparative varieties (Dewata, Selayar, Nias and Munal) which were repeated three times, resulted in the total of 300 experimental units.

Prior to the implementation of the trial, a hydroponic installation and a plastic house were set. Then the wheat seeds were first germinated in Rockwool media on a plastic gutter. Seeds that have germinated with a height of 10 cm are transferred to the husk+coco peat charcoal media on a net pot with the axis of a flannel fabric in a hydroponic installation. Hydroponic media used AB Mix with a concentration of one pack of AB Mix per 25 L of water+Hyponex 2 g L⁻¹. PEG treatment was given two weeks after planting with concentrations of 0, 5, 10 and 15%. The pH of the solution was

maintained at 5.5-6.5 by adding NaOH when it is lower than 5.5 and HCl when it is higher than 6.5. The pH measurement was carried out using a pH meter. Water volume for one treatment plant is 150 L. Nutrient solution was added daily according to the amount of water decreased from 150 L to maintain the PEG concentration in the media during the treatment. Observations made consisted of plant height, number of tillers, number of productive tillers, seed filling time, harvest age, flowering age, panicle length, number of spikelet's per panicle, percentage of empty florets, number of grain per panicle, the weight of seed per panicle, weight of 100 seeds, proline, stomata density and yield.

Validation of hydroponic index selection models on potting soil media:

Validation was carried out on 12 genotypes which included 6 tolerant M5 lines (positive selection index), 4 less tolerant lines (negative selection index) and 2 comparative varieties (Nias and Munal). The selection environment on the soil media used consisted of two water levels, 80 and 60% of the field capacity, repeated three times. The implementation of the experiment began with the selection of the index and the preparation of planting media. The media used were soil: compost: sand = 1: 1: 1 on a 25×35 cm poly bag that is not hollow with weight media of 3 kg. The prepared media was calculated first in the field capacity to determine the treatment level of water availability 60 and 80% the volume of water that is given daily. Prior to planting, seeds were treated in the insecticide carbaryl (sevin), while after planting seeds were treated with carbofuran (furan) to prevent ant pests and other pests. The plant was fertilized with a dose of 150 kg ha⁻¹ Urea, 200 kg ha⁻¹ SP36 and KCl 100 kg ha⁻¹ at 10 Days After Planting (DAP) and second fertilization with Urea dose of 150 kg ha⁻¹ at 30 DAP. To maintain the level of water availability, the media were weighed every day to determine the amount of evapotranspiration occurred. Based on the reduction of media weight every day, a volume of water was added according to the magnitude of decrease in media weight. Furthermore, maintenance was carried out until the crops are harvested. The observations made were focused on the character of yield.

Data analysis: The collected data were analysed first using analysis of variance. The results of the analysis of variance focused on the effect of the interaction of PEG concentrations and genotypes, specifically on the character of yield. The interaction of significant concentrations of PEG and varieties was followed by an analysis of the relative reduction in the character of yield at each PEG concentration. Concentrations

of PEG approaching 50% become drought selection environment candidates.

Identification of selection characters was carried out using in-depth analysis of the selection environment candidates. The analysis was conducted by changing the character's response into a tolerance index value, Stress Tolerance Index (STI). All STI values were then correlated with Pearson correlation using Rstudio through the corrplot package. Significantly STI correlated characters were followed by analysis of path analysis with Rstudio through the Agricolae package. A rational path analysis model was used as the basis for selecting the best selection environment. Character selection in the best selection environment was used as a foundation in the formation of the selection index. Selection indexes were formed using the concept of weighting indexes. The weighting value was formed through principal component analysis using the STAR 2.0.1 program. The selection index formed was used to select 25 wheat genotypes. Adaptive wheat is characterized by a positive index value, while genotypes of less adaptive wheat are marked by a negative value. This is also consistent with the research of Peternelli *et al.*¹⁶ in sugarcane and Anshori *et al.*¹⁴ in rice. The index results were validated by regression analysis of two water availability statuses, 80 and 60% of field capacity. The genotypes used in the regression analysis consisted of 12 selected genotypes which were analyzed using Minitab 17 software.

RESULTS

Effect of Polyethylene Glycol (PEG) concentration and wheat mutant genotype:

In the Table 1, Polyethylene Glycol (PEG) Concentration(C), Varieties (V) and its interaction between CxV have been significantly affected to almost characters. Number of spikelet per panicle, number of grains per panicle, percentage of empty floret, proline, flowering age, grain weight per panicle, weight of 100 grains and yield were significantly influenced by the effect of C, V and CxV. Plant height, panicle length, number of tillers, number of productive tillers and seed filling time yield were significantly influenced by the effect of C and V. Harvest age was significantly influenced by the effect of C. As for stomatal density was significantly influenced by the effect of V. Based on this result, data analysis of the mutant lines screening under drought stress in hydroponic culture could be carried out.

Selection environment for drought stress: Based on Table 2, the increasing of PEG concentration induced a high relative

Table 1: Analysis of variance of all characters in the screening of wheat drought through hydroponic culture

Source	PH	NSP	SD	PL	NT	NGP	PEF	Proline
Concentration (C)	240.56**	383.41**	0.05 ^{ns}	191.11**	28.50**	2320.38**	0.12**	8021.94**
Error (Ec)	14.35	1.38	0.01	0.33	0.76	6.20	0.00	1.52
Varieties (V)	248.30**	47.08**	0.07**	12.75**	48.47**	1241.04**	0.01**	540.94**
c×v	0.38 ^{ns}	1.13**	0.01 ^{ns}	0.35 ^{ns}	0.37 ^{ns}	312.32**	0.00**	162.61**
Error (Ev)	1.46	0.61	0.01	0.48	0.66	234.80	0.00	2.94
Source	NPT	FA	HA	SFT	GWP	W100	Y	
Concentration (C)	47.24**	2441.55**	3858.09**	9569.54**	4.19**	90.30**	4.61**	
Error (Ec)	0.35	6.30	52.31	59.31	0.00	0.08	0.02	
Varieties (V)	3.55**	2801.87**	31.57 ^{ns}	59.40**	2.03**	3.31**	2.26**	
c×v	0.03 ^{ns}	73.53**	4.09 ^{ns}	32.20 ^{ns}	0.94**	0.12**	0.01**	
Error (Ev)	0.12	18.81	95.84	25.28	0.18	0.02	0.00	

**Significant effect at 1%, *Significant effect at 5%, Note PH: Plant height, NSP: Number of spikelet per panicle, SD: Stomatal density, PL: Panicle length, NT: Number of tillers, NGP: Number of grains per panicle, PEF: Percentage of empty floret, NPT: Number of productive tillers, FA: Flowering age, HA: Harvest age, SFT: Seed filling time, GWP: Grain weight per panicle, W100: Weight of 100 grains, Y: Yield, ns: Not significant

Table 2: Yield and relative reduction of yield at each PEG concentration

Genotypes	Yield at each PEG concentration(g per plant)				Relative reduction (%) at PEG concentration		
	0%	5%	10%	15%	5%	10%	15%
G1	0.51	0.41	0.21	0.11	19.61	58.82	78.43
G2	0.53	0.38	0.19	0.09	28.30	64.15	83.02
G3	0.98	0.78	0.57	0.25	20.41	41.84	74.49
G4	1.53	1.38	1.19	0.93	9.80	22.22	39.22
G5	0.51	0.36	0.19	0.13	29.41	62.75	74.51
G6	0.51	0.36	0.17	0.04	29.41	66.67	92.16
G7	0.64	0.49	0.25	0.14	23.44	60.94	78.13
G8	1.43	1.24	1.05	0.79	13.29	26.57	44.76
G9	1.31	1.13	0.94	0.67	13.74	28.24	48.85
G10	1.60	1.41	1.22	0.84	11.88	23.75	47.50
G11	1.07	0.86	0.67	0.38	19.63	37.38	64.49
G12	1.27	1.08	0.89	0.65	14.96	29.92	48.82
G13	0.87	0.75	0.47	0.13	13.79	45.98	85.06
G14	0.77	0.62	0.37	0.10	19.48	51.95	87.01
G15	1.09	0.91	0.72	0.29	16.51	33.94	73.39
G16	0.51	0.39	0.20	0.03	23.53	60.78	94.12
G17	0.51	0.39	0.20	0.04	23.53	60.78	92.16
G18	1.85	1.67	1.48	1.21	9.73	20.00	34.59
G19	0.51	0.38	0.25	0.15	25.49	50.98	70.59
G20	0.51	0.38	0.28	0.14	25.49	45.10	72.55
G21	2.00	1.82	1.63	1.27	9.00	18.50	36.50
G22	0.51	0.38	0.19	0.09	25.49	62.75	82.35
G23	1.15	0.96	0.77	0.55	16.52	33.04	52.17
G24	1.23	1.05	0.76	0.41	14.63	38.21	66.67
G25	0.51	0.41	0.20	0.13	19.61	60.78	74.51
Average	0.96	0.80	0.60	0.38	19.07	44.24	67.84

decrease in the yield. The yield relative decrease of three levels of PEG concentration was 19.07% for PEG 5%, 44.24% for PEG 10% and 67.84% for 15%. Based on these, the potential concentration of PEG as a selection environment for drought stress was at a concentration of 10 and 15%. Both concentrations were analyzed further intensely to determine the best PEG concentration for a drought stress selection environment.

Analysis of correlation: Correlations were carried out independently on PEG concentrations of 10% (Fig. 1) and 15% (Fig. 2) based on the Stress Tolerance Index (STI). Based on

Fig. 1, the Stress Tolerance Index (STI) characters, at a concentration of 10%, that had a significant correlation to the yield were plant height (0.52), stomatal density (0.58), grain weight per panicle (0.9), number of grain per panicle (0.79), proline (0.82), the weight of 100 grains (0.94) and the percentage of empty floret (-0.74). As for the 15% PEG concentration, STI characters that showed significant correlation to the yield as shown in Fig. 2, were stomatal density (0.51), grain weight per panicle (0.85), number of grain per panicle (0.65), proline (0.88), weight of 100 grains (0.93) and percentage of empty floret (-0.70).

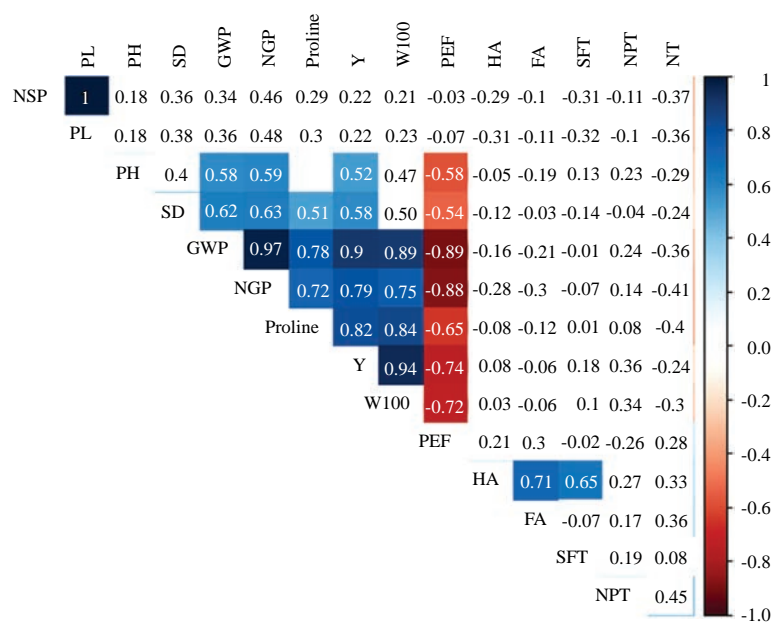


Fig. 1: Pearson correlation of all Stress Tolerance Index (STI) characters at PEG concentration of 10%

Colour in table indicated significant correlation at $\alpha = 1\%$, PH: Plant height, NSP: Number of spikelet per panicle, SD: Stomata density, PL: Panicle length, NT: Number of tillers, NGP: number of grains per panicle, PEF: Percentage of empty floret, NPT: Number of productive tillers, FA: Flowering age, HA: Harvest age, SFT: Seed filling time, GWP: Grain weight per panicle, W100: Weight of 100 grains, Y: Yield, CP: Cumulative proportion, PC: Principal component

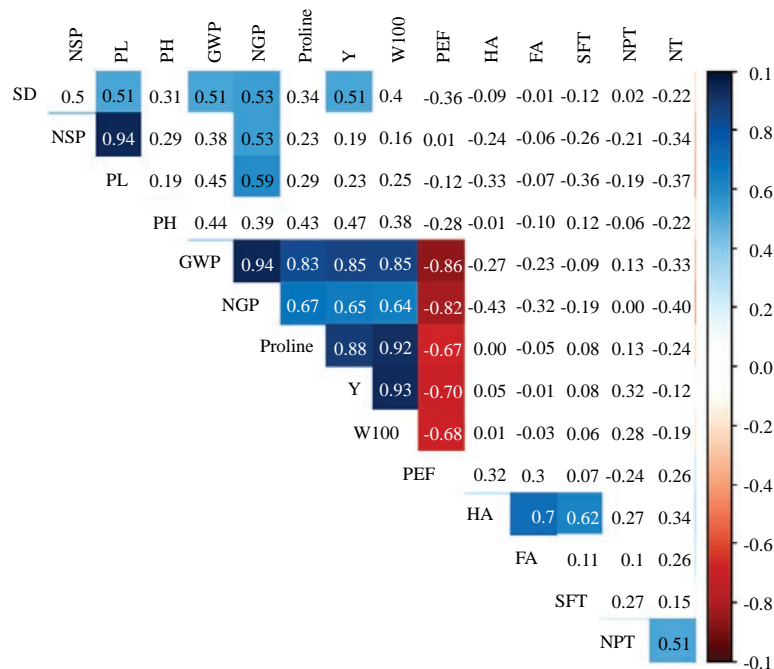


Fig. 2: Pearson correlation of all Stress Tolerance Index (STI) characters at PEG concentration of 15%

Colour in table indicated significant correlation at $\alpha = 1\%$, PH: Plant height, NSP: Number of spikelet per panicle, SD: Stomata density, PL: Panicle length, NT: Number of tillers, NGP: Number of grains per panicle, PEF: Percentage of empty floret, NPT: Number of productive tillers, FA: Flowering age, HA: Harvest age, SFT: Seed filling time, GWP: Grain weight per panicle, W100: Weight of 100 grains, Y: Yield, CP: Cumulative proportion, PC: Principal component

Table 3: Path analysis on Stress Tolerance Index (STI) of yield character at PEG concentration of 10%

Character	Direct effect	Indirect effect							
		W100	GWP	NGP	PEF	PH	SD	Proline	Residual
W100	1.28		-1.06	0.69	0.04	0.01	0.05	-0.07	0.0994
GWP	-1.19	1.14		0.89	0.05	0.02	0.06	-0.07	0.0994
NGP	0.91	0.96	-1.15		0.05	0.02	0.07	-0.06	0.0994
PEF	-0.06	-0.92	1.06	-0.80		-0.02	-0.06	0.06	0.0994
PH	0.03	0.60	-0.69	0.54	0.03		0.04	-0.03	0.0994
SD	0.10	0.64	-0.74	0.58	0.03	0.01		-0.04	0.0994
Proline	-0.09	1.07	-0.93	0.66	0.04	0.01	0.05	-0.09	0.0994
IE accumulation	-	3.49	-3.51	2.46	0.24	0.05	0.21	-	-

R²: 68.47% or 0.6847, IE: Indirect effect, W100: Weight of 100 grains, GWP: Grain weight per panicle, NGP: Number of grains per panicle, PEF: Percentage of empty floret, PH: Plant height, SD: Stomata density

Table 4: Path Analysis on Stress Tolerance Index (STI) of yield character at PEG concentration of 15%

Character	Direct effect	Indirect effect							
		W100	GWP	NGP	PEF	SD	Proline	Residual	
W100	-0.43		2.02	-1.06	0.00	0.09	0.31	0.04	
GWP	2.37	-0.36		-1.55	0.00	0.12	0.28	0.04	
NGP	-1.65	-0.27	2.23		0.00	0.12	0.22	0.04	
PEF	0.00	0.29	-2.04	1.35		-0.08	-0.22	0.04	
SD	0.23	-0.17	1.21	-0.87	0.00		0.11	0.04	
Proline	0.33	-0.39	1.97	-1.11	0.00	0.08		0.04	
IE accumulation	-	-0.90	5.39	-3.24	0.00	0.33	0.70	-	

R²: 77.59% or 0.7759, IE: Indirect effect, W100: Weight of 100 grains, GWP: Grain weight per panicle, NGP: Number of grains per panicle, PEF: Percentage of empty floret, PH: Plant height, SD: Stomata density

Path analysis: Path analysis was also carried out independently at each PEG concentration (Table 3 and 4). At the 10% PEG concentration (Table 3), weight of 100 grains (W100), Number of Grains per Panicle (NGP), Plant Height (PH) and Stomatal Density (SD) have positive direct effect with values as 1.28, 0.91, 0.03 and 0.1, respectively. In the other hand, Grain Weight per Panicle (GWP), Percentage of Empty Floret (PEF) and proline have negative direct influence with values as -1.19, -0.06 and -0.09, respectively. As for the indirect effect, the W100 and NGP have a high of positive indirect effect accumulation to the yield correlation as 3.49 and 2.46, respectively. In addition, the GWP has the highest negative indirect effect accumulation on the yield correlation of -3.51. For characters of PEF, PH and SD the characters have positive indirect effect accumulation, however, these values were lower than W100 and NFP (0.24, 0.05 and 0.21, respectively). The W100 and NGP have the highest indirect influence to GWP of 1.14 and 0.89, respectively. Based on direct and indirect effect in Table 3, the weight of 100 grains and the number of grains per panicle have well-impacted effect in supporting the yield.

The path analysis in the concentration of 15% showed that the grain weight per panicle, percentage of the empty floret, stomatal density and proline have positive direct effect with values of 2.37, 0.00, 0.23 and 0.33, respectively. In the other hand, the weight of 100 grains and number of grains per

panicle have negative direct influence with values of -0.43 and -1.65, respectively (Table 4). For the indirect effect in this concentration, the GWP and NGP dominantly have the highest positive and negative of indirect effect accumulation of 5.39 and -3.24, respectively. As for the proline, SD, PEF and W100 have indirect effect accumulation value in the $1 < X < -1$ interval, namely 0.70, 0.33, 0.00 and -0.90, respectively. The GWP has the highest indirect influence to NGP of 2.23. Based on direct effect and indirect effect in Table 4, grain weight per panicle has dominantly impact in supporting the yield (Table 4). However, based on the comparison of both concentrations, the 10% PEG concentration was considered to be more suitable to be used as a basis for character selection.

Principal component analysis: The formation of a drought adaptability selection index in hydroponic culture was carried out by principal component analysis. Based on the results of the Principal Component Analysis (PCA) in Table 5, there are five principal components that can be used as a basis for an index weighting.

The first component (PC1) was the best component for the selection index weighting base. Based on PC1, the adaptability selection index formed were $0.3474 \text{ yield} + 0.3408 \text{ weight of 100 grains (W100)} + 0.3746 \text{ Number of Grain per Panicle (NGP)}$. This index was then corrected based on the value of the direct effect of secondary characters and the

Table 5: Principal Component Analysis (PCA) on Stress Tolerance Index (STI) characters at PEG Concentration of 10%

Character	PC1	PC2	PC3	PC4	PC5
Yield (Y)	0.3474	-0.2167	0.0518	-0.0183	0.0548
W100	0.3408	-0.1881	0.0396	-0.0012	0.1231
GWP	0.3801	-0.0752	-0.0230	0.065	0.0345
NGP	0.3746	0.0294	-0.0304	0.0562	-0.0437
PEF	-0.3303	0.1286	0.2574	-0.1749	-0.0852
NSP	0.1815	0.3637	0.4406	-0.0895	-0.3071
PL	0.1892	0.3624	0.4342	-0.0602	-0.3017
SFT	-0.0172	-0.3727	-0.1375	-0.5372	-0.4307
HA	-0.0918	-0.4550	0.3292	-0.392	0.0188
FA	-0.1014	-0.2509	0.5737	0.0029	0.3964
NPT	0.0695	-0.3655	0.1562	0.4814	-0.4378
NT	-0.1842	-0.2702	0.1799	0.5101	-0.1156
PH	0.2492	-0.0944	-0.1002	-0.0503	-0.3271
SD	0.2705	0.0463	0.1483	-0.0131	0.2704
Prolin	0.3279	-0.0673	0.0146	-0.1144	0.2408
PC	0.4330	0.6186	0.7254	0.8036	0.8663
Eigenvalue	6.4955	2.7835	1.6012	1.1736	0.9411

PH: Plant height, NSP: Number of spikelet per panicle, SD: Stomata density, PL: Panicle length, NT: Number of tillers, NGP: Number of grains per panicle, PEF: Percentage of empty floret, NPT: Number of productive tillers, FA: Flowering age, HA : Harvest age, SFT: Seed filling time, GWP: Grain weight per panicle, W100: Weight of 100 grains, Y: Yield, CP: Cumulative proportion, PC: Principal Component

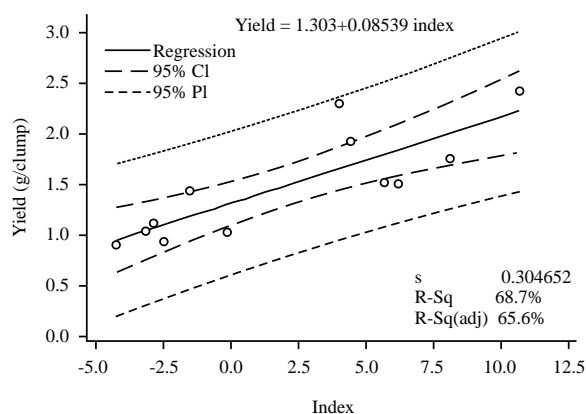


Fig. 3: Regression analysis of selection index on yield of wheat genotypes on soil media at 80% water availability status

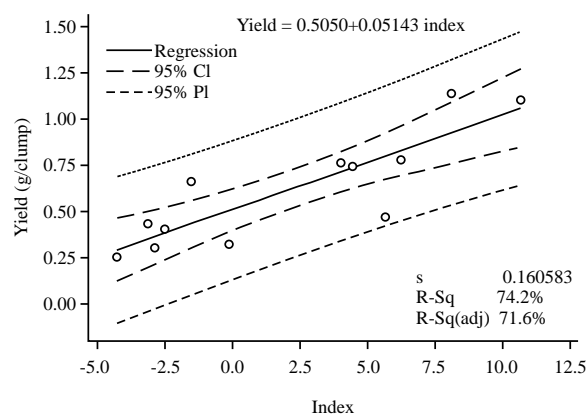


Fig. 4: Regression analysis of selection index on yield of wheat genotypes on soil media at 60% water availability status

determination of the analysis (R^2) (Table 3 and 4). Based on the results of the selection index correction, the final index formed was calculated from the selection index = $0.3474 \text{ yield} + (0.3408 \times 1.28 \times 0.6847) \text{ W100} + (0.3746 \times 0.91 \times 0.6847) \text{ NGP}$ to corrected selection index = $0.3474 \text{ yield} + 0.2987 \text{ W100} + 0.2334 \text{ GWP}$.

Based index selection (Table 6) G21, G18, G10, G4, G9 and G8 have positive index value as 10.70, 8.15, 6.25, 5.68, 4.46, 4.05 and 1.82, respectively. In this index, Nias variety was considered as the best check variety. However, this variety has negative index value. Therefore, based on the index selection, the seven wheat lines were adaptive to drought stress in hydroponic cultures.

Validity test of the hydroponic screening method: Validation was carried out on two water availability statuses in the screening of drought stress in the pot through regression analysis. Based on a regression analysis of 80% water availability, the hydroponic selection index proven can predict yield per clump significantly with a determination value of 65.6% (Fig. 3). As for the regression analysis on 60% water availability, the hydroponic selection index also showed a significant regression of yield per cluster with a determination value of 71.6% (Fig. 4). Based on the two regression analysis, the hydroponic selection index was considered quite good in screening the drought stress of wheat in hydroponic culture.

Table 6: Adaptability Selection Index on 25 wheat genotypes

Genotypes	Stress tolerance index (STI)			Standardisation of STI			
	Yield	W100	NGP	Yield	W100	NGP	Index
G21	3.56	1.32	0.98	14.07	10.76	11.12	10.70
G18	2.99	1.28	0.78	11.11	9.56	6.16	8.15
G10	2.13	1.27	0.73	6.66	9.39	4.85	6.25
G4	1.99	1.14	0.87	5.91	5.51	8.48	5.68
G9	1.35	1.10	0.92	2.57	4.32	9.74	4.46
G8	1.64	1.11	0.75	4.10	4.63	5.30	4.05
G12	1.24	1.04	0.61	2.00	2.45	1.67	1.82
G15	0.86	0.98	0.50	0.04	0.63	-1.14	-0.06
Nias	1.02	1.03	0.36	0.89	2.14	-4.59	-0.12
Selayar	0.97	1.00	0.35	0.61	1.23	-4.86	-0.55
G3	0.61	0.89	0.56	-1.24	-1.98	0.39	-0.93
G11	0.78	0.92	0.37	-0.34	-1.12	-4.40	-1.48
G13	0.45	0.86	0.56	-2.09	-2.97	0.41	-1.52
G2	0.11	0.87	0.59	-3.83	-2.69	1.36	-1.82
G19	0.14	0.91	0.48	-3.68	-1.41	-1.54	-2.06
G7	0.17	0.95	0.35	-3.50	-0.40	-4.75	-2.44
G17	0.11	0.87	0.44	-3.83	-2.75	-2.56	-2.75
G6	0.09	0.78	0.56	-3.91	-5.29	0.53	-2.81
Munal	0.11	0.91	0.36	-3.83	-1.41	-4.65	-2.84
G14	0.31	0.82	0.41	-2.79	-4.07	-3.30	-2.96
G5	0.11	0.79	0.50	-3.86	-5.15	-0.95	-3.10
G1	0.12	0.81	0.46	-3.80	-4.62	-1.96	-3.16
G16	0.11	0.82	0.36	-3.83	-4.21	-4.50	-3.64
G20	0.16	0.74	0.37	-3.60	-6.47	-4.43	-4.22
Dewata	0.11	0.76	0.29	-3.86	-6.08	-6.37	-4.64

W100: Weight of 100 grains NGP: Number of grains per panicle

DISCUSSION

Significant interaction is the initial indicator in the screening analysis of stress. This indicates that each genotype has a different response pattern to differences in the drought stress environment, especially in the character of yield (Table 1). Yield is the main component in a plant which determines its economic value, so this character is generally used as the main selection character. In addition to the selected character, the effectiveness of stress tolerance selection is also determined by the selection environment. Some researchers used a relative reduction of 50% as the best environmental determinant of selection^{11,17,13}. Therefore, the concentration of PEG which reached a relative reduction of 50% is used as the best drought selection environment for hydroponic screening. Based on the results in Table 2, a 50% relative reduction was found in PEG concentrations of 10 and 15%. Therefore, both concentrations are considered as concentrations of selection. Hence, in-depth analysis of the two concentrations was carried out before determining the best concentration.

Assessment of the genotype responses to stress should use a tolerance index. This has been done by some researchers in evaluating the response of tolerance or adaptability of a

genotype to a particular stress^{14,18,19}. The use of tolerance index is able to become a meeting point between two environments so that the selected genotype has a good response to the normal environment and the drought stress environment¹⁴. Stress Tolerance Index (STI) is one of the tolerance indexes that is widely used in evaluating a genotype response to a particular stress^{20,21,18,22}. The STI has the ability to select genotypes that have high yield under normal conditions and stress²³, so that it is consistent with the concept of adaptability under drought stress. Therefore, this STI can be a good basis for in-depth analysis of both PEG concentrations.

Correlation analysis is a general analysis used as a basis for other in-depth analyses such as path analysis and principal component analysis²⁴. Based on the results of the correlation analysis, stomata density characters, grain weight per panicle, number of grain per panicle, proline, weight of 100 grains and percentage of empty floret were characters consistent with the selection of adaptability to grain drought stress in hydroponic culture (Fig. 1 and 2). However, the identification of specific characters that influence the yield STI per cluster requires in-depth analysis such as path analysis.

In the path analysis, the direct effect becomes a specific parameter to find out the supporting characters that independently influence the variance of the main characters

(the yield)²⁵⁻²⁷. Based on the results of the path analysis, the concentrations of 10 and 15% had different results (Table 3 and 4). However, the 10% concentration was considered to have a more suitable path analysis partitioning model than the 15% concentration. This is due to a direct effect value that was too high in the 15% PEG concentration that almost reached value of 2. A very large direct effect might be due to the presence of multicollinearity in both characters^{28,29}, so that it can emerge overestimate of data interpretation. Based on a 10% PEG concentration, the appropriate character to be selected as a character was weight of 100 grains and the number of grain per panicle. This has also been reported by Nofouzi³⁰ on wheat under drought stress. Therefore, both characters are considered worthy to be used as selection characters together with yield characters in forming the selection index.

The yields are very polygenic characteristic³¹, so that the genotype selection under drought stress needs some supporting characters. The use of the yield supporting characters aims to make the stability of genotype potential yield when planted or tested in other environments¹⁴. The index selection is a selection method considered wiser in selecting genotype based on several characters^{32,33}. However, the crucial to the formation of a selection index is the determination of the index weights on each selection character. Akbar *et al.*³⁴ and Anshori *et al.*¹⁴ have developed a method of determining the weighting of indexes through an eigenvector from the principal component analysis.

The use of principal component analysis in the formation of a selection index has a good fit and correlation with the Smith and Hazel selection index³⁵. Smith and Hazel selection indexes have a concept of selection indexes based on genetic variance components³⁶. So, the use of the principal component concept can also be applied in this experiment. Determination of the weighting value in the analysis of the principal components was based on components that have an Eigenvalue above 1. The variance of the components was determined by the optimal variance of the main characters i.e., yield (Table 5). This is what underlies the selection of PC1 as the basic component of the weighting value of the selection index³⁴. However, the eigenvector cannot be used directly on the secondary characters. This needs to be corrected by the direct influence of the secondary character on the main character. This concept has been carried out by Dao *et al.*¹³ on drought tolerant hybrid corn screening and Anshori *et al.*¹⁴ in rice which is adaptive to salinity stress. Based on both studies, the use of direct influence as a selection index was considered quite well in selecting the desired rice genotype. Therefore,

the correction of the secondary characters weighting was also carried out in this study.

Based on the index selection, seven wheat genotypes were considered adaptive to drought stress (Table 6). However, the result of selection index needs to be validated to measure the effectiveness of the model formed³⁷. In this study, the validation of drought adaptability was directed at the soil screening method in pots. Based on the results of a regression analysis at two levels of water availability, this index was considered to study significantly in predicting the yield of clumps in drought screening in pots (Fig. 3 and 4). This is evidence that the use of the selection index is good for use in selecting adaptive genotypes under drought stress. In general, soil screening has a more complex environment, so stress levels in soil screening are difficult to control³⁸. Therefore, screening of hydroponic culture in drought selection is considered more effective and efficient than screening in soil.

CONCLUSION

Based on the overall results it can be concluded that the 10% PEG concentration is the best PEG concentration in selecting wheat genotypes that are adaptive to drought stress in hydroponic cultures. The character of the weight of 100 seeds and the number of grain per panicle are secondary characters that support productivity. The selection index formed in the screening of adaptive wheat under drought stress through hydroponic culture is $0.3474 \text{ yield} + 0.2987 \text{ weights } 100 \text{ grains} + 0.2334 \text{ number of grain per panicle}$. This index is able to select seven adaptive genotypes and has good validation for drought screening in pots. In addition, drought screening of wheat through hydroponic culture is considered to be able to replace the screening of wheat drought on the soil in pots.

SIGNIFICANCE STATEMENT

This article reports on the use of hydroponic methods in performing screening on wheat mutant lines adaptive to tropical Indonesian lowland under drought condition. The paper contained significant information regarding an approach that can be used in the screening process including some statistical methods to determine the genetic parameters that can be used to properly select the mutants adaptive to drought. The results reported in this article will be somehow important for the development of screening methods in the plant breeding program. Hence, this study contributes in the improvement of wheat crop production in tropical region.

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