COVERING LETTER

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I herewith enclosed a research article,

Title:

Synthetic Corn Line Adapted Under Drought Stress Based on Morphophysiological Selection Index

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Climate change is one of the problems to increase the corn yield, especially on drought stress. Many studies have reported the negative impact of drought stress on corn yield. This problem can be solved by developing the adapted corn variety. In general, there are two concepts in developing the corn variety, namely hybrid variety and open-pollinated variety. However, the open-pollinated variety is a broader environmental adaptation than the hybrid varieties. Despite that, the combination of some characters supported the corn yield can increase the effective selection of adapted synthetic corn lines. A combination of some characters, especially from morphophysiological characters, in selection can be formulated to the selection index. However, the determination of the index weighting among traits key to increasing the effectiveness selection index. There are some reports use multivariate analysis in selecting the selected lines under stress conditions in many plants, like rice (Akbar et al. 2019; Anshori et al. 2019), hybrid corn (Fadhli et al. 2019), tropical wheat (Farid et al. 2020). Therefore, the use of multivariate in creating a selection index based on some morpho-physiology characters could increase the selection precision of synthetic corn lines under drought stress.

Based on this study, plant height, number of leaves, number of dry leaves, ear height, ear diameter, leaf rolling, chlorophyll and 1000 seed weight were characters that affected synthetic maize productivity variance under drought stress. Formed selection index based on multivariate analysis were Selection Index = 0.358 PH+ 0.362 NL - 0.315 NDL +0.352 EH + 0.263 ED -0.291 LR + 0.189 Chlorophyll + 0.293 1000SW + 0.346 productivity. It is recommended through index selection that Syn_2-2 (1.11), Syn_2-15 (1.18) dan Syn_2-16 (1.32) as drought stress adaptive lines. Therefore, the three lines can be recommended in further process in the synthetic variety release process.

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Synthetic Corn Line Adapted Under Drought Stress Based on Morphophysiological Selection Index

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Abstract. Synthetic line formation is an effort in increasing maize productivity in drought-stressed areas. The process requires systematic selection in determining adaptability levels of synthetic lines in drought stress. The selection process can involve important secondary characters formulated in the selection index. The principal component in the selection index had been widely reported, both in normal and stressed condition. Therefore, selection index development towards morpho-physiological characters based on multivariate analysis was expected to increase drought stress tolerance maize lines selection precision. The purpose of this research was to form a selection index based on morpho-physiological characters and selecting chosen synthetic lines adaptive to drought stress. This research is designed using a split-plot design with three replications. The main plot was irrigation consisted of normal and stressed irrigation. The subplot was genotype consisted of six lines and three comparing varieties: Bisma, Lamuru, and Sukamarga. Observation carried out including 18 morphological characters and four physiological characters. The result of the research was formed selection index based on multivariate analysis derived 9 characters. It is recommended through index selection that Syn_2-2 (0.79), Syn_2-15 (0.85) and Syn_2-16 (0.97) as drought stress adaptive lines. Therefore, the three lines can be recommended in further process in the synthetic variety release process.

Key words: Drought, Index selection, Morphophysiological characters, Principal component, Synthetic corn,

Commented [ASM1]: Keywords should not repeat the words in the title

Abbreviations (if any): 1000SW = 1000 seed weight, ASI = Anthesis silking interval, DAP = day after planting, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height, Pr = Productivity.

Running title: Adapted Synthetic Corn Lines under Drought Stress

INTRODUCTION

Maize or corn (*Zea mays*) is one of the important staple and feed in Indonesia. This product is one of the main ingredients in a number of industrial companies, making the crop production in Indonesia reached 30 milion ton in 2018 as a result (Agriculture Ministry 2018). However, the population growth which continually rises annually can increases the need of maize in Indonesia, so that the maize production has to be increased in order to fulfill the demand (Sah et al. 2020; Badr et al. 2020). Occurring climate change that is not possible to be controlled causes a negative impact on the plant growth environment, such as drought, salinity, submergence, etc. (Raza et al. 2018). This is a climate threat for corn production stability in Indonesia, especially drought stress (Fahad et al. 2017). Therefore, the domestic production failed in fulfilling these demands.

In general, drought stress is caused by limited water availability on agricultural land or plant inability to absorb water. However, water scarcity is a common reason for drought stress factors (Farid et al. 2019). The drought stress can inhibit some plant morphology and physiological processes, such as cell division, cell development, nutrient transfer, plant enzimatic process, plant metabolism, pollen sterility and grain development. It will impact on the plant growth and yield, including the maize (Silva et al. 2013; Fahad et al. 2017). Although the maize is C4 plant which efficient on water use (Ghannoum 2009), the drought stress in a long period, especially when it occurs in anthesis, will decrease its production (Witt et al. 2012; Souza et al. 2013; Sah et al. 2020). According to Monneveux et al. (2005) and Sah et al. (2020), the drought stress can decrease maize yield around 17–60% in tropical areas. Therefore, this problem needs to be solved, one of them is through developing corn variety adaptable in drought stress.

The maize variety can be developed through hybrid and open-pollinated concepts. The hybrid maize is a popular variety in maize (Fromme et al. 2019; Kandel 2020). However, this variety needs more input (and relative sensitive under stress condition (Kutka 2011; Sharma et al. 2019). So, it less suitable with drought area. The synthetic variety is known to be more adaptive than hybrid ones under stress conditions, making this variety suitable in developing the adaptive corn variety under drought stress (Kutka 2011; Freshley and Delgado-Serrano 2020). The adaptive variety is considered to produce good yield and growth characters in any environment, including the stress condition (Lin et al. 1986; Fadhli et al. 2020). To get this trait, combination of the yield supporting characters, known as secondary character, with the yield must be conducted. The yield is mostly polygenic traits so that the use of secondary characters that supporting the yield can keep the potential lines in any environment (Kassahun et al. 2013; Fellahi et al. 2018), especially under drought stress. The assessment of tolerance lines involved the secondary character has reported by Sabouri et al. (2008), Saad et al. (2014) Fellahi et al. (2018), Anshori et al. (2019), and Fadhli et al. (2020). Therefore, the secondary characters is an important to select the adaptive synthetic corn under drought stress.

Secondary characters can be obtained by morphology and physiology characters. These combinations presents strength that can be considered in a selection for adapted lines under stress. This concept had been previously reported by Barik et al. (2019) on rice under drought stress, Souza et al. (2013) and Sabagh et al. (2017) on maize under drought. However, to combine all secondary characters and the yield need a selection formula known as selection index. The selection index is the linear multivariate regression consisting of criteria selection with the spesific weighted in each criteria (Rajamani et al., 2016; Islam et al., 2017). The index needs sistematic analyses to determine the fit of secondary characters and selection index. One of them through the multivariate analysis. The success of this approach has reported by Sabouri et al (2008), Peternelli et al. (2017), Kose et al. (2018), Branković et al. (2018), Akbar et al. (2019), and Anshori et al. (2019). Therefore, the application of multivariate analysis to develop selection index based on morpho-physiological characters is usefull to do in selecting the adaptive synthetic corn under drought stress. The objective of this study are to develop selection index based on morpho-physiological characters and to select the adapted synthetic corn lines under drought stress.

MATERIALS AND METHODS

The study was conducted in Experimental Farm, Faculty of Agriculture, Hasanuddin University, Makassar from July – November 2017. This research is designed using split plot design with three replications. Main plot is watering (p) consisted of normal irrigation (p0) and stress irrigation (p1). Sub plots are genotype (G) consisted of six genotypes: Syn 2-1 (G1), Syn 2-2 (G2), Syn 2-4 (G3), Syn 2-8 (G4), Syn 2-15 (G5), Syn 2-16 (G6) and three comparison varieties which were bisma (G7), lamuru (G8) and sukmaraga (G9). According to the number of treatments, 18 treatment combination was present and replicated three times, resulting in 54 experimental units with ploting area for experimnetal unit as 3m x 3.5m.

Experimental Procedure

Maize Maize seeds used were given metalaxyl to prevent mildew disease. Two seeds were planted each planting hole and Carbofuran 30% was added with 15 kg ha^{-1} dosage to avoid pest attack. Each genotype treatment was planted with 80 cm x 20 cm spacing. Thinning was done 14 days after planting (DAP). Fertilizer was applied three times. First fertilizer application (basic application) done seven days after planting with dosage as follows: SP36 150 kg ha^{-1}, KCl 100 kg ha^{-1} and Urea 70 kg ha^{-1}. Second fertilizer application was done on 28 DAP with NPK 100 kg ha^{-1} and Urea 65 kg ha^{-1}. Third application was done on 40 DAP with KCl 100 kg ha^{-1} and Urea 65 kg ha^{-1}. Irrigation was done with water pump and hose by flooding the plots until reaching the height of the beds. Drought stress method was done according to CIMMYT (Bänziger et al. 2000), where irrigation was to be stopped after the plants reached 40 DAP. There was no irrigation for the next 30 days and was given on the 70th day until their physiological maturity. Plant maintenance included thinning, heaping, spraying and weeding. Weeding was done on 14 and 28 DAP. Insecticide application was adjusted according to pests present in the crop.

Commented [ASM2]: It is recommended to also explain the normal irrigation procedure

Observation

Morphology observation in this research consisted of soil water analysis, plant height, number of leaves, number of dry leaves, days of female flowering, days of male flowering, days of male flowering, Anthesis Silking Interval (ASI), days of harvesting, stem diameter, ear length, ear diameter (mm), length of seeded ear, seed rendement, 1000 seed weight, and productivity. Meanwhile, the physiology observations were leaf angle, leaf width, leaf age scoring, absorption level, reflection, stomata density, leaf chlorophyll index, and leaf roll scoring. The tools conducted in physiology characters used lab miniature leaf streptik CI 7010 and chlorophyll meter SPAD 502.

Data analysis

Recapitulated data was initially analyzed with analysis of variance at 5% error level. Characters found to be significant towards interaction effects were preceded into further analysis. Variance identification was also done under cluster analysis under normal and stressed conditions. Cluster analysis was done in Rstudio 3.6.3 with factoextra (Kassambra and Mundt 2020) and dendextend (Galili 2015). All of the characters showing significance interaction were analyzed to be stress tolerance index (STI) (Fernandez 1992). Then, the STI characters were analyzed by Pearson correlation. This analyse used Rstudio software with agricolae package (Mendiburu 2020) and corrplot (Wei and Simko 2017). Selection index was formed through principal component analysis using STAR IRRI 2.0.1 (Anshori et al. 2019). The selection index was applied to all genotypes and was evaluated by comparing synthetic maize lines index with best comparing index.

RESULTS AND DISCUSSION

ANOVA indicated that morphological and physiological characters were significantly effected by genotype variance and water condition (Table 1). However, not all characters were significantly effected by interaction variance. Morphological characters were significantly effected by the interaction variance were plant height, number of leaves, number of dried leaves, days of female flowering, days of male flowering, Anthesis Silking Interval (ASI), ear height, ear diameter, ear length, leaf rolling, 1000 seed weight and productivity. Physiological characters significantly effected by inteaction were reflection and chlorophyll. According to Al-Naggar et al. (2015), Mohamadi et al. (2017) and Anshori et al. (2019), characters significant towards genotype-environment interaction are able to exhibit response variance between genotype and difference of growing environment. This can be considered as a base in distinguishing tolerant and sensitive maize genotypes under drought stress. The same concept application was also reported by Fadhli et al. (2020) on maize under drought stress, Anshori et al. (2021) on the rice under salinity stress, Akbar et al. (2019) on the rice under drought stress.

Commented [ASM3]: Leaf rolling is also a significant physiological parameter

Table 1. Analysis of variance of morphological and physiological characters towards a number of synthetic maize genotypes in varied environments.

Characters	Irrigation (E)	Error a	Genotipe (G)	G x E	Error b	CVa	CVb
Plant Height	5998.52**	44.34	698.23**	193.06*	79.04	4%	5%
Number of Leaves	14.00**	0.02	1.45**	0.58**	0.15	1%	4%
Number of Dry Leaves	27.45**	0.07	0.49**	0.20**	0.06	13%	12%
Stem Diameter	41.93*	1.59	3.01*	0.52ns	1.11	6%	5%
Days of Female Flowering	80.67*	0.89	4.00**	3.37*	0.59	2%	1%
Days of Male Flowering	20.17**	1.06	4.21*	2.08**	0.77	2%	2%
Anthesis Silking Interval (ASI)	20.17**	0.17	1.27*	1.83**	0.51	15%	27%
Days of Harvest	136.96*	5.57	15.88*	6.59ns	4.44	2%	2%
Ear Height	1717.84*	60.87	216.49**	149.31*	59.55	8%	8%
Ear Diameter	80.87*	1.17	5.88**	5.11**	1.13	5%	5%
Leaf Angle	553.30**	5.42	22.40*	7.84ns	7.54	8%	10%
Leaf Width	90852.41*	2895.94	9762.18*	3877.43ns	4268.73	12%	14%
Leaf Aging	1.97**	0.02	0.06**	0.04ns	0.02	3%	5%
Absorbtion	1.97*	0.02	0.06**	0.04ns	0.02	18%	16%
Reflection	0.06**	0.00	0.00**	0.00*	0.00	11%	8%
Stomata Density	4129.96**	36.92	1346.99*	633.53ns	461.46	2%	8%
Leaf Rolling	22.56**	0.00	0.05**	0.02*	0.01	2%	3%
Leaf Chlorophyll Index	1244.23*	56.25	297.95**	166.79*	70.89	1%	1%
Ear Length	71.67*	0.94	4.45**	2.02**	0.35	6%	4%
Seed Rendement	365.50**	1.52	6.70*	5.01ns	2.96	2%	2%
1000 Sed weight	8151.69*	210.70	3634.07**	403.89*	143.89	4%	4%
Productivity	170.40**	0.18	3.73**	0.40**	0.08	6%	4%

Note: CV = coefficient of variance

Commented [ASM4]: Including significant physiological parameters

The second approach to detecting interaction variance of lines under normal and stress conditions can use cluster analysis. The result of cluster analysis showed that the synthetic maize genotype grouping experienced dynamic changes in both environments (Figure 1). It was shown by the connecting line between two dendrograms that showed a dynamic line without a parallel line connecting both dendrograms. Although, on the 60% dissimilarity degree, both dendrograms had the same cluster unit which was three. However, the number of group units in each dendrogram cluster had different amounts. It shows that each genotype had a different response in every environment. Based on the result, cluster analysis was considered effective in depicting response variance among genotypes in different growing environments. This was also reported by some researchers in identifying relations among objects towards vast variables in several environments or models (Silva et al. 2013; Saad et al. 2014; Anshori et al. 2020). However, the cluster analysis not yet detail explanation the specific tolerant trait under stress conditions. Thus, further analysis of the characters had to be done in evaluating the adaptability of synthetic maize under drought stress.

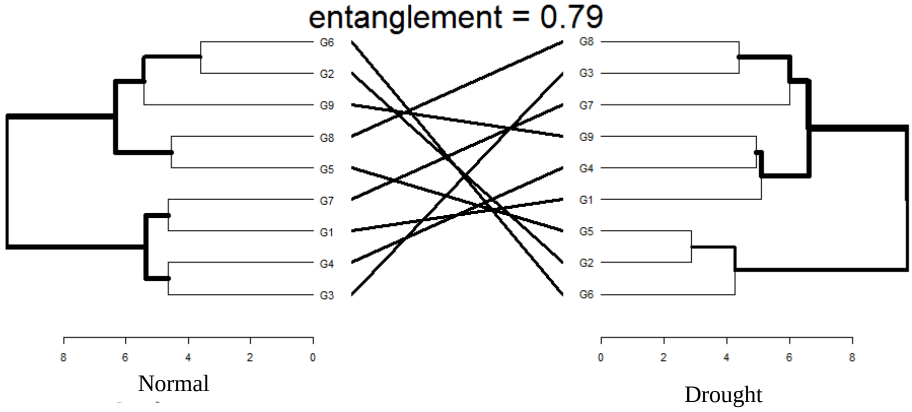

Figure 1. Cluster analysis of synthetic corn lines based on significant characters toward the interaction effect under normal and drought condition

Evaluating effectivity of tolerance and adaptability can be done with stress tolerance index (STI). The STI is one of tolerance index to detecting the tolerant lines under stress. This index has the benefit to be a midpoint in considering the line's potential under normal and stress condition (Anshori et al. 2019). Besides that, STI considers the average of all genotype responses in normal condition. It is like a dynamic concept on the stability analysis used in assessing the stability line (Hidayatullah et al. 2019; Sitaresmi et al. 2019; Kartina et al. 2019; Amzeri et al. 2020). The dynamic concept can assess the line by proportional depending on all population response included (Lin et al. 1986). So, the STI concept could be used in detecting the adapted or tolerant lines. The application of the index had been widely reported in considering genotype tolerance and adaptability under environmental stress. Anshori et al. (2018) and Anshori et al. (2019) had previously applied the method in salinity stressed rice. Kumar et al. (2015) and Fadhli et al. (2020) had used the similar on drought stressed maize and Farid et al. (2019) on drought stressed wheat. In addition, STI application on a number of characters to be further analyzed had been reported by Anshori et al. (2019) and Fadhli et al. (2020). Thus, STI application on characters with significance in this experiment was also used.

Correlation analysis STI correlation result shows that prodctivity has positive significant correlation towards plant height (0.77), number of leaves (0.76), ear height position (0.78), ear diameter (0.72) and 1000 seed weight (0.72). On the contrary, it presents negative significance only towards number of dried leaves (-0.84) (Figure 2). These correlations have also reported by some research. The correlation of ear diameter and number of leaves to productivity has been reported by Fadhli et al. (2020). Ali et al. (2017) reporteed the significant positive correlation between productivity and ear diameter. Yue et al. (2018) also reported the significant correlation between yield and plant heigh. Meanwhile, leaf rolling has negative significant correlation towards number of leaves (-0.89), plant height (-0.68), ear height (-0.68), and chlorophyll (-0.67) (Figure 2). Negative correlation exhibited in leaf rolling was due to its negative interpretation, where the more tolerant the variety under drought, the less curling (Efendi et al. 2019; Fadhli et al. 2020). Commonly, productivity is a main selection character. Yet, on a number of researchs, drought stress tolerance determination was based on leaf rolling (Obeng-Bio et al. 2011; Baret et al. 2018; Efendi et al. 2019). According to correlation analysis, indirect correlation was found between leaf rolling and productivity in drought stress. This was proven in plant height, number of leaves, and ear height which was significantly correlated with both characters. This finding indicated that drought selection accuracy can be increased with the combination of these characters. Therefore, the characters need to be combined in a selection index. This can be analyzed through principal component analysis. Anshori et al. (2019), Akbar et al. (2019), Alsabah et al (2019), and Anshori et al. (2021) had reported the index formation in this analysis.

Principal component analysis (PCA) result shows that three PC was present that were considered depicting STI characters (Table 2). This determination was based on PC reached 0.8 first time (Jolliffe 2002). Besides on eigenvalue, the PC has eigenvalue more than 1 were PC1 until PC3. So, these PCs could be as PC candidates on determining the weighting index. From 3 PC1, the PC1 was PC with most productivity eigenvector compared to PC 2 and PC 3, making this PC a base of selection index weighing (Table 2). The similar was previously reported by Anshori et al. (2019) and Akbar et al. (2019), where the formation of selection index weighing value can be based on the largest eigenvector from main characters. Despite that PC1 has a negative value, its eigenvector was still used as a base of weighing index. It also was applied by Anshori et al. (2021). According to Jolliffe (2002) that positive and negative sign is limited to variance direction of characters, making the eigenvector can be used in positive condition. However, number of leaves and leaf rolling were changed to negative due to their interpretation towards tolerance. Thus, selection index formed has a formula as follows:

Selection index = 0.358 PH+ 0.362 NL - 0.315 NDL +0.352 EH + 0.263 ED - 0.291 LR + 0.189 LCI + 0.293 1000SW + 0.346 productivity.

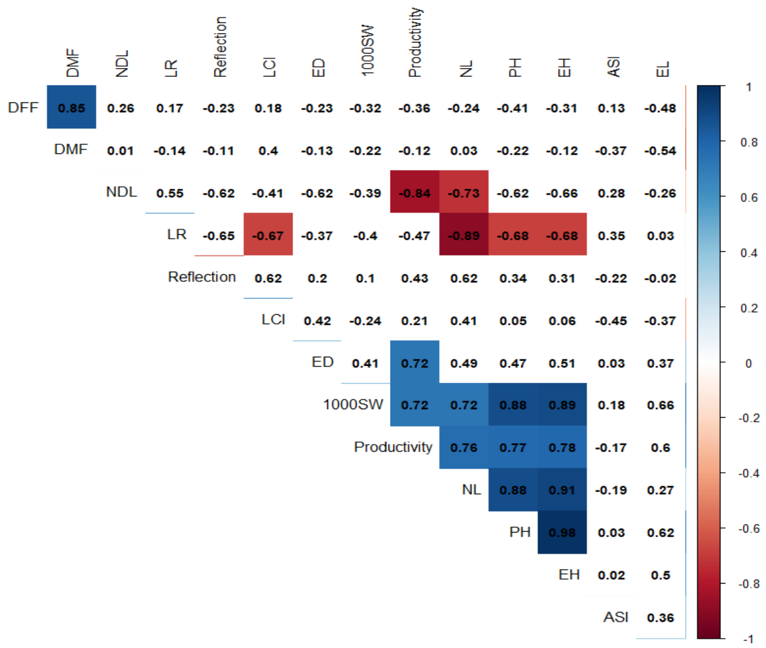

Figure 2. Heatmap based Pearson Correlation Analysis towards all STI Characters significant towards interaction (1000SW = 1000 seed weight, ASI = Anthesis silking interval, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height)

PCA application in finding a variation of an object and other variables had been widely reported in a number of researchers (Jolliffe 2002; Mattjik and Sumertajaya 2011; Singh et al. 2015; Anshori et al. 2018; Fadhli et al. 2020). This analysis was considered to be effective in preventing multicollinearity or overlapped variance (Jolliffe 2002; Mattjik and Sumertajaya, 2011). This can increase selection index objectivity in genotype selection and having relations with selection index from Smith Hazel (Godshalk and Timothy 1988). Based on the research, determination of weighing value from PC eigenvector can determine priority characters towards drought stress. Productivity relatively has low heritability value under abiotic stress (Kassahun et al. 2013; Fellahi et al. 2018), making the characters with linear variance and larger compared to productivity can increase selection effectivity in drought stress. This was reported by Alsabah et al. (2019) showing that productive tillers variety was bigger than the productivity. This was also fit with path analysis from the research. Despite that, Akbar et al. (2019) and Anshori et al. (2019) concluded that eigenvector application was combined with path analysis. However, seeing the number and status of the genotypes in the research, the analysis was considered unnecessary, due to a small amount of genotypes. Path analysis with fewer samples can cause multicollinearity (Olivoto et al. 2017; Sari et al. 2018). Therefore, this index application is considered to be suitable in increasing drought stress adaptive synthetic maize selection effectivity. Nevertheless, based on the selection index, productivity does not dominate index variance. On other hand, productivity is the main character, so, the index should be adjusted. The adjusted selection index formation has been reported by Anshori et al. (2019) on rice under salinity stress and Farid et al. (2021) on wheat under drought stress. Therefore, the adjusted selection index must be formulated in this study.

The adjusted selection index can be conducted with some analysis, one of these is correlation analysis. The use of correlation as a basis index has reported by Sabouri et al (2008) and Chaudhary et al. (2017) on rice. Based on that, the combination of PCA weighting and correlation could be done in creating the selection index. The adjusted selection index follows:

Adjusted selection index = 0.276 PH+ 0.275 NL - 0.265 NDL +0.275 EH + 0.189 ED - 0.291 LR + 0.189 LCI + 0.211 1000SW+ 0.346 productivity.

Table 2. Principal component analysis based on STI Characters significant towards interaction.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
PH	-0.358	0.125	-0.134	0.219	-0.008	0.075	0.004	0.342	-0.191
NL	-0.362	-0.130	-0.099	0.218	-0.097	-0.029	-0.024	-0.132	-0.173
NDL	0.315	0.126	-0.103	0.277	-0.135	0.427	0.436	-0.409	-0.123
DFF	0.176	-0.302	-0.543	-0.154	-0.162	-0.256	0.089	-0.100	-0.497
DMF	0.053	-0.429	-0.507	-0.019	0.162	-0.109	0.189	0.179	0.361
ASI	0.050	0.349	-0.254	-0.169	-0.772	-0.072	-0.118	0.130	0.237
EH	-0.352	0.087	-0.246	0.182	-0.011	0.076	-0.275	0.168	0.039
ED	-0.263	-0.016	-0.006	-0.662	-0.057	0.392	-0.130	-0.195	-0.307
Reflection	-0.219	-0.220	0.387	0.113	-0.391	-0.529	0.180	-0.263	-0.050
LR	0.291	0.281	-0.047	-0.322	0.175	-0.312	-0.123	-0.131	0.245
LCI	-0.189	-0.429	0.137	-0.222	-0.220	0.332	0.267	0.047	0.462
EL	-0.198	0.422	-0.044	-0.162	0.086	-0.114	0.725	0.288	-0.071
1000SW	-0.293	0.235	-0.331	0.188	0.075	0.031	-0.040	-0.580	0.327
Productivity	-0.346	0.045	-0.040	-0.277	0.275	-0.262	0.110	-0.257	0.046
PV	0.478	0.219	0.104	0.071	0.057	0.041	0.019	0.012	0.000
CV	0.478	0.697	0.801	0.872	0.929	0.970	0.988	1.000	1.000
EigenValues	6.690	3.072	1.449	0.994	0.802	0.571	0.258	0.165	0.000

Notes: PV = proportion of variance, CV = cumulative of variance, 1000SW = 1000 seed weight, ASI = Anthesis silking interval, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height.

Table 3. STI Selection index on 9 synthetic maize genotypes.

Genotype	PH	NL	NDL	EH	ED	LR	LCI	1000SW	Pr	Selection Index
Syn_2-1	0.70	0.78	2.09	0.69	0.89	1.73	0.99	0.69	0.55	0.23
Syn_2-2	0.99	1.00	1.42	0.97	0.85	1.45	1.00	0.93	0.67	0.79
Syn_2-4	0.80	0.87	2.86	0.82	0.78	1.64	0.98	0.95	0.50	0.16
Syn_2-8	0.82	0.91	2.03	0.86	0.88	1.52	1.00	0.82	0.48	0.43
Syn_2-15	1.00	1.02	1.63	1.00	1.03	1.45	1.01	1.06	0.75	0.85
Syn_2-16	1.01	1.01	1.28	1.01	0.99	1.48	1.00	1.09	0.86	0.97
Bisma	0.90	0.86	2.78	0.86	0.83	1.59	0.98	0.92	0.48	0.23
Lamuru	0.96	0.89	1.87	0.98	0.92	1.70	0.97	1.08	0.68	0.61
Sukmaraga	0.84	0.86	2.46	0.85	0.94	1.58	1.00	0.85	0.53	0.32

Note : 1000SW = 1000 seed weight, ED = Ear diameter, EH = Ear height, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height, Pr =Productivity.

Selection index result shows that there are three synthetic maize genotypes with better index compared with Lamuru as better comparing variety. These varieties were Syn_2-2 (0.79), Syn_2-15 (0.85) and Syn_2-16 (0.97). Lamuru is a composite variety with 7.6 ton ha^{-1} productivity tested and drought tolerant, making it often used in areas with long dry months (Mustikawati and Yulia 2011; Aqil et al. 2012; Prasetyo and Amin 2019). According to Suwarno et al. (2009), the use of control varieties is the common method to know the best-selected line. They have used it in detecting the best rice line with resistant blast disease line. Therefore, based on the research, it can be considered that the three synthetic maize lines to be recommended as advance line candidate under drought stress.

In conclusion, plant height, number of leaves, number of dry leaves, ear height, ear diameter, leaf rolling, chlorophyll and 1000 seed weight were characters that affected synthetic maize productivity variance in drought stress adaptability evaluation. Formed selection index based on principal component analysis were Adjusted Selection Index = 0.276 PH+ 0.275 NL - 0.265 NDL +0.275 EH + 0.189 ED - 0.291 LR + 0.189 LCI + 0.211 1000SW+ 0.346 productivity. It is recommended through index selection that Syn_2-2, Syn_2-15 and Syn_2-16 as drought stress adaptive lines. Therefore, the three lines can be recommended in further process as advance line candidate under drought stress.

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RESUBMISSION

COVERING LETTER

Dear **Editor-in-Chief,**

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Title:

Synthetic Corn Line Adapted Under Drought Stress Based on Morphophysiological Selection Index

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Climate change is one of the problems to increase the corn yield, especially on drought stress. Many studies have reported the negative impact of drought stress on corn yield. This problem can be solved by developing the adapted corn variety. In general, there are two concepts in developing the corn variety, namely hybrid variety and open-pollinated variety. However, the open-pollinated variety is a broader environmental adaptation than the hybrid varieties. Despite that, the combination of some characters supported the corn yield can increase the effective selection of adapted synthetic corn lines. A combination of some characters, especially from morphophysiological characters, in selection can be formulated to the selection index. However, the determination of the index weighting among traits key to increasing the effectiveness selection index. There are some reports use multivariate analysis in selecting the selected lines under stress conditions in many plants, like rice (Akbar et al. 2019; Anshori et al. 2019), hybrid corn (Fadhli et al. 2019), tropical wheat (Farid et al. 2020). Therefore, the use of multivariate in creating a selection index based on some morpho-physiology characters could increase the selection precision of synthetic corn lines under drought stress.

Based on this study, plant height, number of leaves, number of dry leaves, ear height, ear diameter, leaf rolling, chlorophyll and 1000 seed weight were characters that affected synthetic maize productivity variance under drought stress. Formed selection index based on multivariate analysis were Selection Index = 0.358 PH+ 0.362 NL - 0.315 NDL +0.352 EH + 0.263 ED -0.291 LR + 0.189 Chlorophyll + 0.293 1000SW + 0.346 productivity. It is recommended through index selection that Syn_2-2 (1.11), Syn_2-15 (1.18) dan Syn_2-16 (1.32) as drought stress adaptive lines. Therefore, the three lines can be recommended in further process in the synthetic variety release process.

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Synthetic Corn Line Adapted Under Drought Stress Based on Morphophysiological Selection Index

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Abstract. Synthetic line formation is an effort in increasing maize productivity in drought-stressed areas. The process requires systematic selection in determining adaptability levels of synthetic lines in drought stress. The selection process can involve important secondary characters formulated in the selection index. The principal component in the selection index had been widely reported, both in normal and stressed condition. Therefore, selection index development towards morpho-physiological characters based on multivariate analysis was expected to increase drought stress tolerance maize lines selection precision. The purpose of this research was to form a selection index based on morpho-physiological characters and selecting chosen synthetic lines adaptive to drought stress. This research is designed using a split-plot design with three replications. The main plot was irrigation consisted of normal and stressed irrigation. The subplot was genotype consisted of six lines and three comparing varieties: Bisma, Lamuru, and Sukamarga. Observation carried out including 18 morphological characters and four physiological characters. The result of the research was formed selection index based on multivariate analysis derived 9 characters. It is recommended through index selection that Syn_2-2 (0.79), Syn_2-15 (0.85) and Syn_2-16 (0.97) as drought stress adaptive lines. Therefore, the three lines can be recommended in further process in the synthetic variety release process.

Key words: Drought, Index selection, Morphophysiological characters, Principal component, Synthetic corn,

Abbreviations (if any): 1000SW = 1000 seed weight, ASI = Anthesis silking interval, DAP = day after planting, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height, Pr = Productivity.

Running title: Adapted Synthetic Corn Lines under Drought Stress

INTRODUCTION

Maize or corn (*Zea mays*) is one of the important staple and feed in Indonesia. This product is one of the main ingredients in a number of industrial companies, making the crop production in Indonesia reached 30 milion ton in 2018 as a result (Agriculture Ministry 2018). However, the population growth which continually rises annually can increases the need of maize in Indonesia, so that the maize production has to be increased in order to fulfill the demand (Sah et al. 2020; Badr et al. 2020). Occurring climate change that is not possible to be controlled causes a negative impact on the plant growth environment, such as drought, salinity, submergence, etc. (Raza et al. 2018). This is a climate threat for corn production stability in Indonesia, especially drought stress (Fahad et al. 2017). Therefore, the domestic production failed in fulfilling these demands.

In general, drought stress is caused by limited water availability on agricultural land or plant inability to absorb water. However, water scarcity is a common reason for drought stress factors (Farid et al. 2019). The drought stress can inhibit some plant morphology and physiological processes, such as cell division, cell development, nutrient transfer, plant enzimatic process, plant metabolism, pollen sterility and grain development. It will impact on the plant growth and yield, including the maize (Silva et al. 2013; Fahad et al. 2017). Although the maize is C4 plant which efficient on water use (Ghannoum 2009), the drought stress in a long period, especially when it occurs in anthesis, will decrease its production (Witt et al. 2012; Souza et al. 2013; Sah et al. 2020). According to Monneveux et al. (2005) and Sah et al. (2020), the drought stress can decrease maize yield around 17–60% in tropical areas. Therefore, this problem needs to be solved, one of them is through developing corn variety adaptable in drought stress.

The maize variety can be developed through hybrid and open-pollinated concepts. The hybrid maize is a popular variety in maize (Fromme et al. 2019; Kandel 2020). However, this variety needs more input (and relative sensitive under stress condition (Kutka 2011; Sharma et al. 2019). So, it less suitable with drought area. The synthetic variety is known to be more adaptive than hybrid ones under stress conditions, making this variety suitable in developing the adaptive corn variety under drought stress (Kutka 2011; Freshley and Delgado-Serrano 2020). The adaptive variety is considered to produce good yield and growth characters in any environment, including the stress condition (Lin et al. 1986; Fadhli et al. 2020). To get this trait, combination of the yield supporting characters, known as secondary character, with the yield must be conducted. The yield is mostly polygenic traits so that the use of secondary characters that supporting the yield can keep the potential lines in any environment (Kassahun et al. 2013; Fellahi et al. 2018), especially under drought stress. The assessment of tolerance lines involved the secondary character has reported by Sabouri et al. (2008), Saad et al. (2014) Fellahi et al. (2018), Anshori et al. (2019), and Fadhli et al. (2020). Therefore, the secondary characters is an important to select the adaptive synthetic corn under drought stress.

Secondary characters can be obtained by morphology and physiology characters. These combinations presents strength that can be considered in a selection for adapted lines under stress. This concept had been previously reported by Barik et al. (2019) on rice under drought stress, Souza et al. (2013) and Sabagh et al. (2017) on maize under drought. However, to combine all secondary characters and the yield need a selection formula known as selection index. The selection index is the linear multivariate regression consisting of criteria selection with the spesific weighted in each criteria (Rajamani et al., 2016; Islam et al., 2017). The index needs sistematic analyses to determine the fit of secondary characters and selection index. One of them through the multivariate analysis. The success of this approach has reported by Sabouri et al (2008), Peternelli et al. (2017), Kose et al. (2018), Branković et al. (2018), Akbar et al. (2019), and Anshori et al. (2019). Therefore, the application of multivariate analysis to develop selection index based on morpho-physiological characters is usefull to do in selecting the adaptive synthetic corn under drought stress. The objective of this study are to develop selection index based on morpho-physiological characters and to select the adapted synthetic corn lines under drought stress.

MATERIALS AND METHODS

The study was conducted in Experimental Farm, Faculty of Agriculture, Hasanuddin University, Makassar from July – November 2017. This research is designed using split plot design with three replications. Main plot is watering (p) consisted of normal irrigation (p0) and stress irrigation (p1). Sub plots are genotype (G) consisted of six genotypes: Syn 2-1 (G1), Syn 2-2 (G2), Syn 2-4 (G3), Syn 2-8 (G4), Syn 2-15 (G5), Syn 2-16 (G6) and three comparison varieties which were bisma (G7), lamuru (G8) and sukmaraga (G9). According to the number of treatments, 18 treatment combination was present and replicated three times, resulting in 54 experimental units with ploting area for experimnetal unit as 3m x 3.5m.

Experimental Procedure

Maize Maize seeds used were given metalaxyl to prevent mildew disease. Two seeds were planted each planting hole and Carbofuran 30% was added with 15 kg ha^{-1} dosage to avoid pest attack. Each genotype treatment was planted with 80 cm x 20 cm spacing. Thinning was done 14 days after planting (DAP). Fertilizer was applied three times. First fertilizer application (basic application) done seven days after planting with dosage as follows: SP36 150 kg ha^{-1}, KCl 100 kg ha^{-1} and Urea 70 kg ha^{-1}. Second fertilizer application was done on 28 DAP with NPK 100 kg ha^{-1} and Urea 65 kg ha^{-1}. Third application was done on 40 DAP with KCl 100 kg ha^{-1} and Urea 65 kg ha^{-1}. Irrigation was done with water pump and hose by flooding the plots until reaching the height of the beds. Drought stress method was done according to CIMMYT (Bänziger et al. 2000), where irrigation was to be stopped after the plants reached 40 DAP. There was no irrigation for the next 30 days and was given on the 70th day until their physiological maturity. Plant maintenance included thinning, heaping, spraying and weeding. Weeding was done on 14 and 28 DAP. Insecticide application was adjusted according to pests present in the crop.

Observation

Morphology observation in this research consisted of soil water analysis, plant height, number of leaves, number of dry leaves, days of female flowering, days of male flowering, days of male flowering, Anthesis Silking Interval (ASI), days of harvesting, stem diameter, ear length, ear diameter (mm), length of seeded ear, seed rendement, 1000 seed weight, and productivity. Meanwhile, the physiology observations were leaf angle, leaf width, leaf age scoring, absorption level, reflection, stomata density, leaf chlorophyll index, and leaf roll scoring. The tools conducted in physiology characters used lab miniature leaf streptik CI 7010 and chlorophyll meter SPAD 502.

Data analysis

Recapitulated data was initially analyzed with analysis of variance at 5% error level. Characters found to be significant towards interaction effects were preceded into further analysis. Variance identification was also done under cluster analysis under normal and stressed conditions. Cluster analysis was done in Rstudio 3.6.3 with factoextra (Kassambra and Mundt 2020) and dendextend (Galili 2015). All of the characters showing significance interaction were analyzed to be stress tolerance index (STI) (Fernandez 1992). Then, the STI characters were analyzed by Pearson correlation. This analyse used Rstudio software with agricolae package (Mendiburu 2020) and corrplot (Wei and Simko 2017). Selection index was formed through principal component analysis using STAR IRRI 2.0.1 (Anshori et al. 2019). The selection index was applied to all genotypes and was evaluated by comparing synthetic maize lines index with best comparing index.

RESULTS AND DISCUSSION

ANOVA indicated that morphological and physiological characters were significantly effected by genotype variance and water condition (Table 1). However, not all characters were significantly effected by interaction variance. Morphological characters were significantly effected by the interaction variance were plant height, number of leaves, number of dried leaves, days of female flowering, days of male flowering, Anthesis Silking Interval (ASI), ear height, ear diameter, ear length, leaf rolling, 1000 seed weight and productivity. Physiological characters significantly effected by inteaction were reflection and chlorophyll. According to Al-Naggar et al. (2015), Mohamadi et al. (2017) and Anshori et al. (2019), characters significant towards genotype-environment interaction are able to exhibit response variance between genotype and difference of growing environment. This can be considered as a base in distinguishing tolerant and sensitive maize genotypes under drought stress. The same concept application was also reported by Fadhli et al. (2020) on maize under drought stress, Anshori et al. (2021) on the rice under salinity stress, Akbar et al. (2019) on the rice under drought stress.

Table 1. Analysis of variance of morphological and physiological characters towards a number of synthetic maize genotypes in varied environments.

Characters	Irrigation (E)	Error a	Genotipe (G)	G x E	Error b	CVa	CVb
Plant Height	5998.52**	44.34	698.23**	193.06*	79.04	4%	5%
Number of Leaves	14.00**	0.02	1.45**	0.58**	0.15	1%	4%
Number of Dry Leaves	27.45**	0.07	0.49**	0.20**	0.06	13%	12%
Stem Diameter	41.93*	1.59	3.01*	0.52ns	1.11	6%	5%
Days of Female Flowering	80.67*	0.89	4.00**	3.37*	0.59	2%	1%
Days of Male Flowering	20.17**	1.06	4.21*	2.08**	0.77	2%	2%
Anthesis Silking Interval (ASI)	20.17**	0.17	1.27*	1.83**	0.51	15%	27%
Days of Harvest	136.96*	5.57	15.88*	6.59ns	4.44	2%	2%
Ear Height	1717.84*	60.87	216.49**	149.31*	59.55	8%	8%
Ear Diameter	80.87*	1.17	5.88**	5.11**	1.13	5%	5%
Leaf Angle	553.30**	5.42	22.40*	7.84ns	7.54	8%	10%
Leaf Width	90852.41*	2895.94	9762.18*	3877.43ns	4268.73	12%	14%
Leaf Aging	1.97**	0.02	0.06**	0.04ns	0.02	3%	5%
Absorbtion	1.97*	0.02	0.06**	0.04ns	0.02	18%	16%
Reflection	0.06**	0.00	0.00**	0.00*	0.00	11%	8%
Stomata Density	4129.96**	36.92	1346.99*	633.53ns	461.46	2%	8%
Leaf Rolling	22.56**	0.00	0.05**	0.02*	0.01	2%	3%
Leaf Chlorophyll Index	1244.23*	56.25	297.95**	166.79*	70.89	1%	1%
Ear Length	71.67*	0.94	4.45**	2.02**	0.35	6%	4%
Seed Rendement	365.50**	1.52	6.70*	5.01ns	2.96	2%	2%
1000 Sed weight	8151.69*	210.70	3634.07**	403.89*	143.89	4%	4%
Productivity	170.40**	0.18	3.73**	0.40**	0.08	6%	4%

Note: CV = coefficient of variance

The second approach to detecting interaction variance of lines under normal and stress conditions can use cluster analysis. The result of cluster analysis showed that the synthetic maize genotype grouping experienced dynamic changes in both environments (Figure 1). It was shown by the connecting line between two dendrograms that showed a dynamic line without a parallel line connecting both dendrograms. Although, on the 60% dissimilarity degree, both dendrograms had the same cluster unit which was three. However, the number of group units in each dendrogram cluster had different amounts. It shows that each genotype had a different response in every environment. Based on the result, cluster analysis was considered effective in depicting response variance among genotypes in different growing environments. This was also reported by some researchers in identifying relations among objects towards vast variables in several environments or models (Silva et al. 2013; Saad et al. 2014; Anshori et al. 2020). However, the cluster analysis not yet detail explanation the specific tolerant trait under stress conditions. Thus, further analysis of the characters had to be done in evaluating the adaptability of synthetic maize under drought stress.

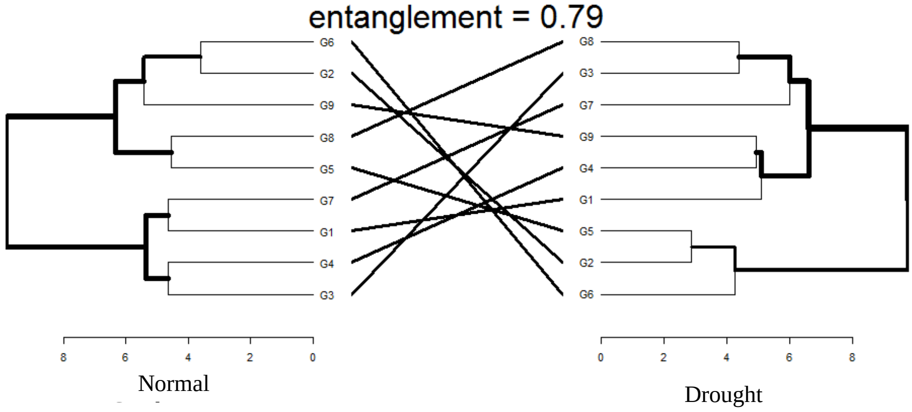

Figure 1. Cluster analysis of synthetic corn lines based on significant characters toward the interaction effect under normal and drought condition

Evaluating effectivity of tolerance and adaptability can be done with stress tolerance index (STI). The STI is one of tolerance index to detecting the tolerant lines under stress. This index has the benefit to be a midpoint in considering the line's potential under normal and stress condition (Anshori et al. 2019). Besides that, STI considers the average of all genotype responses in normal condition. It is like a dynamic concept on the stability analysis used in assessing the stability line (Hidayatullah et al. 2019; Sitaresmi et al. 2019; Kartina et al. 2019; Amzeri et al. 2020). The dynamic concept can assess the line by proportional depending on all population response included (Lin et al. 1986). So, the STI concept could be used in detecting the adapted or tolerant lines. The application of the index had been widely reported in considering genotype tolerance and adaptability under environmental stress. Anshori et al. (2018) and Anshori et al. (2019) had previously applied the method in salinity stressed rice. Kumar et al. (2015) and Fadhli et al. (2020) had used the similar on drought stressed maize and Farid et al. (2019) on drought stressed wheat. In addition, STI application on a number of characters to be further analyzed had been reported by Anshori et al. (2019) and Fadhli et al. (2020). Thus, STI application on characters with significance in this experiment was also used.

Correlation analysis STI correlation result shows that prodctivity has positive significant correlation towards plant height (0.77), number of leaves (0.76), ear height position (0.78), ear diameter (0.72) and 1000 seed weight (0.72). On the contrary, it presents negative significance only towards number of dried leaves (-0.84) (Figure 2). These correlations have also reported by some research. The correlation of ear diameter and number of leaves to productivity has been reported by Fadhli et al. (2020). Ali et al. (2017) reporteed the significant positive correlation between productivity and ear diameter. Yue et al. (2018) also reported the significant correlation between yield and plant heigh. Meanwhile, leaf rolling has negative significant correlation towards number of leaves (-0.89), plant height (-0.68), ear height (-0.68), and chlorophyll (-0.67) (Figure 2). Negative correlation exhibited in leaf rolling was due to its negative interpretation, where the more tolerant the variety under drought, the less curling (Efendi et al. 2019; Fadhli et al. 2020). Commonly, productivity is a main selection character. Yet, on a number of researchs, drought stress tolerance determination was based on leaf rolling (Obeng-Bio et al. 2011; Baret et al. 2018; Efendi et al. 2019). According to correlation analysis, indirect correlation was found between leaf rolling and productivity in drought stress. This was proven in plant height, number of leaves, and ear height which was significantly correlated with both characters. This finding indicated that drought selection accuracy can be increased with the combination of these characters. Therefore, the characters need to be combined in a selection index. This can be analyzed through principal component analysis. Anshori et al. (2019), Akbar et al. (2019), Alsabah et al (2019), and Anshori et al. (2021) had reported the index formation in this analysis.

Principal component analysis (PCA) result shows that three PC was present that were considered depicting STI characters (Table 2). This determination was based on PC reached 0.8 first time (Jolliffe 2002). Besides on eigenvalue, the PC has eigenvalue more than 1 were PC1 until PC3. So, these PCs could be as PC candidates on determining the weighting index. From 3 PC1, the PC1 was PC with most productivity eigenvector compared to PC 2 and PC 3, making this PC a base of selection index weighing (Table 2). The similar was previously reported by Anshori et al. (2019) and Akbar et al. (2019), where the formation of selection index weighing value can be based on the largest eigenvector from main characters. Despite that PC1 has a negative value, its eigenvector was still used as a base of weighing index. It also was applied by Anshori et al. (2021). According to Jolliffe (2002) that positive and negative sign is limited to variance direction of characters, making the eigenvector can be used in positive condition. However, number of leaves and leaf rolling were changed to negative due to their interpretation towards tolerance. Thus, selection index formed has a formula as follows:

Selection index = 0.358 PH+ 0.362 NL - 0.315 NDL +0.352 EH + 0.263 ED - 0.291 LR + 0.189 LCI + 0.293 1000SW + 0.346 productivity.

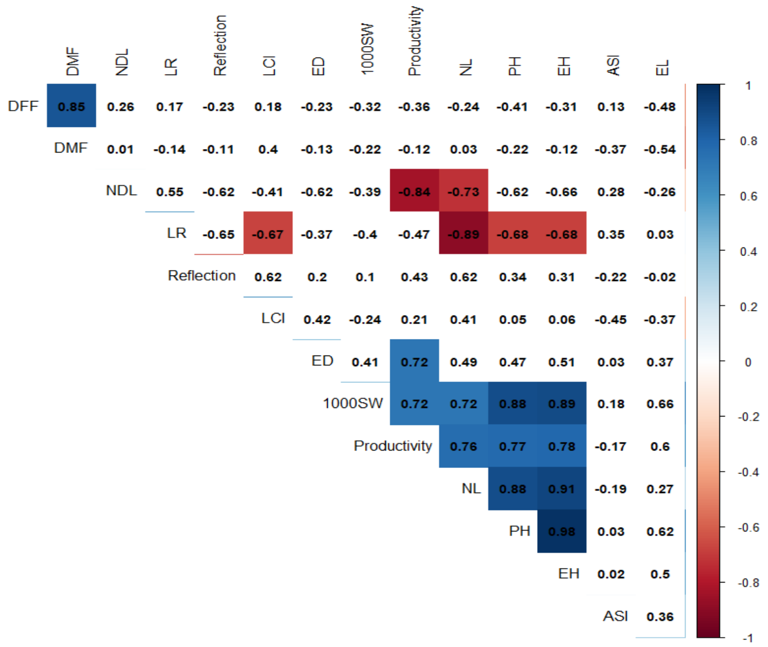

Figure 2. Heatmap based Pearson Correlation Analysis towards all STI Characters significant towards interaction (1000SW = 1000 seed weight, ASI = Anthesis silking interval, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height)

PCA application in finding a variation of an object and other variables had been widely reported in a number of researchers (Jolliffe 2002; Mattjik and Sumertajaya 2011; Singh et al. 2015; Anshori et al. 2018; Fadhli et al. 2020). This analysis was considered to be effective in preventing multicollinearity or overlapped variance (Jolliffe 2002; Mattjik and Sumertajaya, 2011). This can increase selection index objectivity in genotype selection and having relations with selection index from Smith Hazel (Godshalk and Timothy 1988). Based on the research, determination of weighing value from PC eigenvector can determine priority characters towards drought stress. Productivity relatively has low heritability value under abiotic stress (Kassahun et al. 2013; Fellahi et al. 2018), making the characters with linear variance and larger compared to productivity can increase selection effectivity in drought stress. This was reported by Alsabah et al. (2019) showing that productive tillers variety was bigger than the productivity. This was also fit with path analysis from the research. Despite that, Akbar et al. (2019) and Anshori et al. (2019) concluded that eigenvector application was combined with path analysis. However, seeing the number and status of the genotypes in the research, the analysis was considered unnecessary, due to a small amount of genotypes. Path analysis with fewer samples can cause multicollinearity (Olivoto et al. 2017; Sari et al. 2018). Therefore, this index application is considered to be suitable in increasing drought stress adaptive synthetic maize selection effectivity. Nevertheless, based on the selection index, productivity does not dominate index variance. On other hand, productivity is the main character, so, the index should be adjusted. The adjusted selection index formation has been reported by Anshori et al. (2019) on rice under salinity stress and Farid et al. (2021) on wheat under drought stress. Therefore, the adjusted selection index must be formulated in this study.

The adjusted selection index can be conducted with some analysis, one of these is correlation analysis. The use of correlation as a basis index has reported by Sabouri et al (2008) and Chaudhary et al. (2017) on rice. Based on that, the combination of PCA weighting and correlation could be done in creating the selection index. The adjusted selection index follows:

Adjusted selection index = 0.276 PH+ 0.275 NL - 0.265 NDL +0.275 EH + 0.189 ED - 0.291 LR + 0.189 LCI + 0.211 1000SW+ 0.346 productivity.

Table 2. Principal component analysis based on STI Characters significant towards interaction.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
PH	-0.358	0.125	-0.134	0.219	-0.008	0.075	0.004	0.342	-0.191
NL	-0.362	-0.130	-0.099	0.218	-0.097	-0.029	-0.024	-0.132	-0.173
NDL	0.315	0.126	-0.103	0.277	-0.135	0.427	0.436	-0.409	-0.123
DFF	0.176	-0.302	-0.543	-0.154	-0.162	-0.256	0.089	-0.100	-0.497
DMF	0.053	-0.429	-0.507	-0.019	0.162	-0.109	0.189	0.179	0.361
ASI	0.050	0.349	-0.254	-0.169	-0.772	-0.072	-0.118	0.130	0.237
EH	-0.352	0.087	-0.246	0.182	-0.011	0.076	-0.275	0.168	0.039
ED	-0.263	-0.016	-0.006	-0.662	-0.057	0.392	-0.130	-0.195	-0.307
Reflection	-0.219	-0.220	0.387	0.113	-0.391	-0.529	0.180	-0.263	-0.050
LR	0.291	0.281	-0.047	-0.322	0.175	-0.312	-0.123	-0.131	0.245
LCI	-0.189	-0.429	0.137	-0.222	-0.220	0.332	0.267	0.047	0.462
EL	-0.198	0.422	-0.044	-0.162	0.086	-0.114	0.725	0.288	-0.071
1000SW	-0.293	0.235	-0.331	0.188	0.075	0.031	-0.040	-0.580	0.327
Productivity	-0.346	0.045	-0.040	-0.277	0.275	-0.262	0.110	-0.257	0.046
PV	0.478	0.219	0.104	0.071	0.057	0.041	0.019	0.012	0.000
CV	0.478	0.697	0.801	0.872	0.929	0.970	0.988	1.000	1.000
EigenValues	6.690	3.072	1.449	0.994	0.802	0.571	0.258	0.165	0.000

Notes: PV = proportion of variance, CV = cumulative of variance, 1000SW = 1000 seed weight, ASI = Anthesis silking interval, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height.

Table 3. STI Selection index on 9 synthetic maize genotypes.

Genotype	PH	NL	NDL	EH	ED	LR	LCI	1000SW	Pr	Selection Index
Syn_2-1	0.70	0.78	2.09	0.69	0.89	1.73	0.99	0.69	0.55	0.23
Syn_2-2	0.99	1.00	1.42	0.97	0.85	1.45	1.00	0.93	0.67	0.79
Syn_2-4	0.80	0.87	2.86	0.82	0.78	1.64	0.98	0.95	0.50	0.16
Syn_2-8	0.82	0.91	2.03	0.86	0.88	1.52	1.00	0.82	0.48	0.43
Syn_2-15	1.00	1.02	1.63	1.00	1.03	1.45	1.01	1.06	0.75	0.85
Syn_2-16	1.01	1.01	1.28	1.01	0.99	1.48	1.00	1.09	0.86	0.97
Bisma	0.90	0.86	2.78	0.86	0.83	1.59	0.98	0.92	0.48	0.23
Lamuru	0.96	0.89	1.87	0.98	0.92	1.70	0.97	1.08	0.68	0.61
Sukmaraga	0.84	0.86	2.46	0.85	0.94	1.58	1.00	0.85	0.53	0.32

Note : 1000SW = 1000 seed weight, ED = Ear diameter, EH = Ear height, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height, Pr =Productivity.

Selection index result shows that there are three synthetic maize genotypes with better index compared with Lamuru as better comparing variety. These varieties were Syn_2-2 (0.79), Syn_2-15 (0.85) and Syn_2-16 (0.97). Lamuru is a composite variety with 7.6 ton ha^{-1} productivity tested and drought tolerant, making it often used in areas with long dry months (Mustikawati and Yulia 2011; Aqil et al. 2012; Prasetyo and Amin 2019). According to Suwarno et al. (2009), the use of control varieties is the common method to know the best-selected line. They have used it in detecting the best rice line with resistant blast disease line. Therefore, based on the research, it can be considered that the three synthetic maize lines to be recommended as advance line candidate under drought stress.

In conclusion, plant height, number of leaves, number of dry leaves, ear height, ear diameter, leaf rolling, chlorophyll and 1000 seed weight were characters that affected synthetic maize productivity variance in drought stress adaptability evaluation. Formed selection index based on principal component analysis were Adjusted Selection Index = 0.276 PH+ 0.275 NL - 0.265 NDL +0.275 EH + 0.189 ED - 0.291 LR + 0.189 LCI + 0.211 1000SW+ 0.346 productivity. It is recommended through index selection that Syn_2-2, Syn_2-15 and Syn_2-16 as drought stress adaptive lines. Therefore, the three lines can be recommended in further process as advance line candidate under drought stress.

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Comments and revisions of the 1st review

Drought-adapted maize line based on morphophysiological selection index

Abstract. Synthetic line formation is an effort to increase maize productivity in drought-stressed areas. This process requires systematic selection in determining adaptability levels and can involve important secondary characters formulated in the selection index. Furthermore, the principal component index had been widely reported, both in normal and stressed conditions. The selection index development to morpho-physiological characters based on multivariate analysis was expected to increase drought stress tolerance and maize lines selection precision. Therefore, the purpose of this research was to form a selection index based on morpho-physiological characters and selecting synthetic lines adaptive under drought stress. This research was designed using a split-plot with 3 replications, where the main plot consisted of normal and stressed irrigation, while the subplot was genotype consisting of 6 lines and 3 check varieties namely Bisma, Lamuru, and Sukamarga. Observations were performed on 18 morphological and 4 physiological characters, and the results showed that the selection index was formed based on multivariate analysis from 9 characters. Through index selection, Syn_2-2 (0.79), Syn_2-15 (0.85) and Syn_2-16 (0.97) were considered as drought stress adaptive lines. Therefore, the 3 lines can be recommended in the synthetic variety release process.

Keywords: Abiotic stress, Corn, Multivariate analysis, Principal component, Secondary characters

Abbreviations: 1000SW = 1000 Seed Weight, ASI = Anthesis Silking Interval, DAP = Day After Planting, DFF= Days of Female Flowering, DMF= Days of Male Flowering, ED = Ear Diameter, EH = Ear Height, EL = Ear Length, LR = Leaf Rolling, NDL= Number of Dried Leaves, NL = Number of Leaves, PH= Plant Height, Pr = Productivity

Running title: Adapted Synthetic Maize Lines under Drought Stress

INTRODUCTION

Maize (*Zea mays*) is an important feed and food in Indonesia. This commodity also is one of the main ingredients of industrial companies, so that maize makes the 3rd important cereal commodity in the world after wheat and rice (Bukhsh *et al.* 2012; Cooper *et al.* 2014). In Indonesia, maize production is considered good with a total production reach 30 million tons in 2018 (Agriculture Ministry 2018). However, as population growth annually increases, maize production also needs to further increase to meet the demand (Sah *et al*. 2020; Badr *et al*. 2020). Theclimate change negatively impacts the plant growth environment, such as drought, salinity, submergence, etc. (Raza *et al.* 2018). The temperature rise with long periods due to climate change induce drought stress. This stress can threaten maize production stability (Fahad et al. 2017) and as a result, domestic production has failed to meet the demand. Therefore, the maize production problem in drought stress condition should be solved to increase the maize yield.

Generally, water scarcity caused by limited water availability on agricultural land or plant inability to absorb water is a common drought stress factor (Farid *et al*. 2019). This stress can inhibit some plant morphology and physiological processes, such as cell division, cell development, nutrient transfer, plant enzymatic process, plant metabolism, pollen sterility, and grain development, which affects plant growth and yield, including the maize (Silva *et al*. 2013; Fahad *et al*. 2017). Although maize efficiently uses water (Ghannoum 2009), a long period of drought stress, both at vegetative stage and at the anthesis stage, will decrease production (Witt *et al.* 2012; Souza *et al*. 2013; Song et al. 2019; Sah *et al*. 2020). According to Monneveux *et al*. (2005) and Sah *et al*. (2020), drought stress can decrease maize yield by 17–60% in tropical areas. According to Song et al. (2019) also reported that the drought stress could dercrease the maize yield until

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" In Indonesia, maize production is considered good with a total production reach 30 million tons in 2018"

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"The temperature rise with long periods due to climate change induce drought stress. This stress can threaten maize production stability"

50% in 2013 to 2016. Therefore, this problem needs a solution and one way to do this is through developing adapted maize variety to drought stress.

Maize variety can be developed through hybrid and open-pollination. The hybrid is a popular variety in maize (Fromme *et al*. 2019; Kandel 2020) because it is relatively sensitive under stress conditions (Kutka 2011; Sharma *et al*. 2019). However, the synthetic variety is more adaptive under stress conditions, making it suitable for development (Kutka 2011; Freshley and Delgado-Serrano 2020). The adaptive variety produces good yield and growth characters in any environment, including stress conditions (Lin *et al*. 1986; Fadhli *et al*. 2020). To get this trait, the combination of the yield supporting characters, known as secondary characters, must be studied. The yield mostly comprised of polygenic traits, hence the use of secondary supporting characters can keep potential lines in any environment (Kassahun *et al*. 2013; Fellahi *et al*. 2018), especially under drought stress. Sabouri *et al*. (2008), Saad *et al*. (2014), Fellahi *et al*. (2018), and Anshori *et al*. (2019) have reported about the assessment of tolerance lines involved with secondary characters. Besides, according to Fadhli *et al.* (2020), the use of the secondary character in selecting adapted lines under drought stress was more effective. Therefore, these characters are important in selecting the adapted synthetic maize under drought stress.

Secondary characters can be obtained through morphology and physiology. These combinations present strength that can be considered in a selection for adapted lines under stressed conditions. Previous reports about this concept under drought stress have been made by Barik *et al*. (2019) on rice, Souza *et al*. (2013), and Sabagh *et al*. (2017) on maize. However, combining all secondary characters and yield requires a selection formula known as the selection index. This is the linear multivariate regression consisting of specific weighted criteria selection (Rajamani *et al*. 2016; Islam *et al*. 2017). Moreover, the index needs systematic analyses such as multivariate analysis to determine the fit of secondary characters and the weighting of its secondary characters. The success of this approach has been reported by Sabouri *et al* (2008), Peternelli *et al*. (2017), Kose *et al*. (2018), Branković *et al*. (2018), Akbar *et al*. (2019), and Anshori *et al*. (2021). Therefore, the application of multivariate analysis to develop a selection index based on morpho-physiological characters is useful in adaptive synthetic maize under drought stress. The objective of this study was to develop a selection index based on morpho-physiological characters and select the adapted synthetic maize lines under drought stress.

MATERIALS AND METHODS

The study was conducted in the Experimental Farm of Faculty of Agriculture, Hasanuddin University, Makassar from July to November 2017. This research was designed using a split-plot design with 3 replications where main plot watering (p) consisted of normal (p0) and stress irrigation (p1). Also, Subplots consisted of 6 genotypes (G): Syn 2-1 (G1), Syn 2-2 (G2), Syn 2-4 (G3), Syn 2-8 (G4), Syn 2-15 (G5), Syn 2-16 (G6) and 3 check varieties, i.e., bisma (G7), lamuru (G8) and sukmaraga (G9). According to the number of treatments, 18 combination was present and replicated 3 times, resulting in 54 experimental units while the plotting area for experimental unit size was 3bm x 3.5bm.

Experimental procedure

To prevent mildew disease, maize seeds used were given metalaxyl. Seeds (2) were placed in each planting hole and Carbofuran 30% was added with 15 kg ha^{-1} dosage to avoid pest attack. Furthermore, each genotype treatment was planted with 80 cm x 20 cm spacing, thinning was done 14 days after planting (DAP), and fertilizer was applied 3 times. First fertilizer application (basal application) was applied seven days after planting at dosage of SP36 150 kg ha^{-1}, KCl 100 kg ha^{-1} and Urea 70 kg ha^{-1}. The second fertilizer application was on 28 DAP with NPK 100 kg ha^{-1} and Urea 65 kg ha^{-1}, while, the third application was on 40 DAP with KCl 100 kg ha^{-1} and Urea 65 kg ha^{-1}. Also, irrigation was made with a water pump hosed by flooding the plots until it got to the height of the beds. Drought stress method was performed according to CIMMYT (Bänziger *et al*. 2000), where irrigation was stopped after the plants attained 40 DAP. After this, irrigation was avoided for the next 30 days and was given on the 70th day until physiological maturity. As for the normal condition, the irrigation used the pump for the regular watering of fields. Plant maintenance included thinning, heaping, spraying, and weeding. Weeding was performed on 14 and 28 DAP and Insecticide application was adjusted according to the crop pests.

Observation

In this research, morphology observation consisted of plant height, number of leaves, leaf angle, leaf width, number of dried leaves, days to female flowering, days to male flowering, Anthesis Silking Interval (ASI), days to harvesting, stem diameter, ear length, ear diameter, length of seeded ear, seed rendement, 1000 seed weight, and productivity. Meanwhile, the physiology observations were leaf age scoring, absorption level, reflection, stomata density, leaf chlorophyll index, and leaf roll scoring. The tools used for physiology character observations were lab miniature leaf streptik CI 7010 and chlorophyll meter SPAD 502.

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Data analysis

Recapitulated data were initially analyzed with analysis of variance at 5% error level and characters found to be significant to interaction were preceded into further analysis. Variance identification was performed using cluster analysis under normal and drought stressed conditions. This was done in Rstudio 3.6.3 with factoextra (Kassambra and Mundt 2020) and dendextend (Galili 2015). Additionally, all characters showing significant interaction effect were subjected to analysis of stress tolerance index (STI) (Fernandez 1992), as follow:

$$STI = \frac{Yp \times Ys}{\bar{Y}p^2}$$

Note: Yp = The character value of each line in normal conditions.
Ys = The character value of each line in drought stress condition.
Ȳp = Average character values of all lines in normal conditions

After which they were analyzed by Pearson correlation. This examination used Rstudio software with agricolae package (Mendiburu 2020) and corrplot (Wei and Simko 2017) while, the selection index was formed through principal component analysis using STAR IRRI 2.0.1 (Anshori *et al*. 2019). Furthermore, the selection index applied to all genotypes was evaluated by comparing the synthetic maize lines index with the best comparing index.

RESULTS AND DISCUSSION

Analysis of variance indicated that morphological and physiological characters were significantly affected by genotypic variance and water condition (Table 1). However, not all characters were affected significantly by interaction variance. Morphological characters that were affected included plant height, number of leaves, number of dried leaves, days to female flowering, days to male flowering, Anthesis Silking Interval (ASI), ear height, ear diameter, ear length, leaf rolling, 1000 seed weight and productivity. Meanwhile, the physiological characters affected were reflection and chlorophyll. According to Al-Naggar *et al*. (2015), Mohamadi *et al.* (2017), Anshori *et al.* (2019) and Anshori *et al*. (2021), characters significant towards genotype-environment interaction can exhibit response variance between genotype and difference of growing environment. This is a base in distinguishing adapted or tolerant and sensitive maize genotypes under drought stress. A similar concept application was reported by Fadhli *et al*. (2020) on maize under drought stress, Anshori *et al*. (2021) on the rice under salinity stress, and Akbar *et al.* (2019) on the rice under drought stress. Therefore, all characters significantly affected by the interaction of genotype - water condition variance could be continued in further analysis.

Table 1. Analysis of variance of morphological and physiological characters of a number of synthetic maize genotypes in varied environments.

Characters	Irrigation (E)	Error a	Genotipe (G)	G x E	Error b	CVa	CVb
Plant Height	5998.52**	44.34	698.23**	193.06*	79.04	4%	5%
Number of Leaves	14.00**	0.02	1.45**	0.58**	0.15	1%	4%
Number of Dried Leaves	27.45**	0.07	0.49**	0.20**	0.06	13%	12%
Stem Diameter	41.93*	1.59	3.01*	0.52ns	1.11	6%	5%
Days to Female Flowering	80.67*	0.89	4.00**	3.37*	0.59	2%	1%
Days to Male Flowering	20.17**	1.06	4.21*	2.08**	0.77	2%	2%
Anthesis Silking Interval (ASI)	20.17**	0.17	1.27*	1.83**	0.51	15%	27%
Days of Harvest	136.96*	5.57	15.88*	6.59ns	4.44	2%	2%
Ear Height	1717.84*	60.87	216.49**	149.31*	59.55	8%	8%
Ear Diameter	80.87*	1.17	5.88**	5.11**	1.13	5%	5%
Leaf Angle	553.30**	5.42	22.40*	7.84ns	7.54	8%	10%
Leaf Width	90852.41*	2895.94	9762.18*	3877.43ns	4268.73	12%	14%
Leaf Aging	1.97**	0.02	0.06**	0.04ns	0.02	3%	5%
Absorbtion	1.97*	0.02	0.06**	0.04ns	0.02	18%	16%
Reflection	0.06**	0.00	0.00**	0.00*	0.00	11%	8%
Stomata Density	4129.96**	36.92	1346.99*	633.53ns	461.46	2%	8%
Leaf Rolling	22.56**	0.00	0.05**	0.02*	0.01	2%	3%
Leaf Chlorophyll Index	1244.23*	56.25	297.95**	166.79*	70.89	1%	1%
Ear Length	71.67*	0.94	4.45**	2.02**	0.35	6%	4%
Seed Rendement	365.50**	1.52	6.70*	5.01ns	2.96	2%	2%
1000 Sed weight	8151.69*	210.70	3634.07**	403.89*	143.89	4%	4%
Productivity	170.40**	0.18	3.73**	0.40**	0.08	6%	4%

Note: CV = coefficient of variance; * significant effect at 5% level; ** significant effect at 1% level; ns = not significant

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Commented [MFA12R11]: Thank you for your correction. We have adding explanation according to your suggestion "* significant effect at 5% level; ** significant effect at 1% level; ns = not significant"

Cluster analysis was the second approach to detecting interaction variance lines under normal and stress conditions. The results showed that synthetic maize genotype grouping experienced dynamic changes in both environments (Figure 1), and there was no straight line connecting the two dendrograms. Although on the 60% dissimilarity degree, both dendrograms had 3 cluster units with different group units in each. This shows that each genotype had a different response in every environment. Based on the result, cluster analysis was effective in depicting response variance in different growing environments. This has been reported by some research identifying relationships among objects towards vast variables in several environments or models (Silva *et al*. 2013; Saad *et al*. 2014; Anshori *et al*. 2020). However, the simple dendrogram could not explain the specific adapted trait under stress conditions. It proves that further analysis was required to evaluate the adaptability of synthetic maize under drought stress.

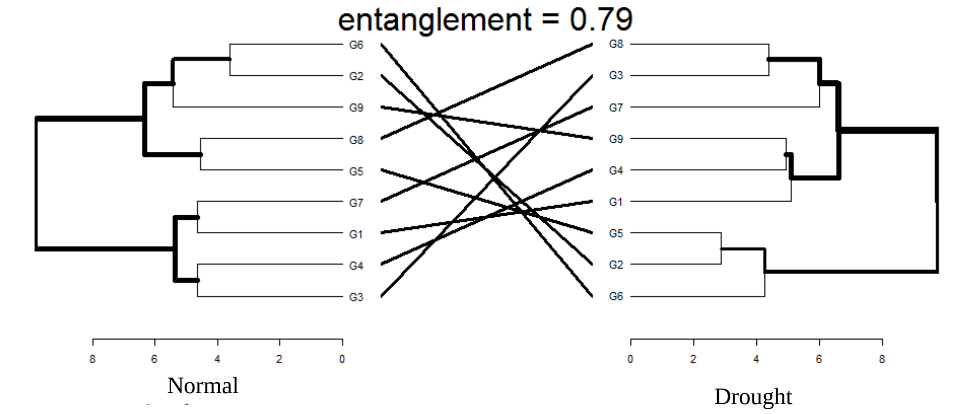

Figure 1. Cluster analysis of synthetic maize lines based on significant characters toward the interaction effect under normal and drought condition

The effectiveness of tolerance and adaptability can be evaluated with Stress Tolerance Index (STI). This detects tolerant lines under stress and has a midpoint benefit in considering the line's potential under normal and stress conditions (Anshori *et al*. 2019). Besides, STI considers the average responses of all genotypes under normal conditions. This is similar to a dynamic concept on the stability analysis used in assessing the stability lines (Hidayatullah *et al*. 2019; Sitaresmi *et al*. 2019; Kartina *et al*. 2019; Amzeri *et al*. 2020). Furthermore, the dynamic concept can assess the line's potential based on the average population responses (Lin *et al*. 1986), hence, the STI concept could be used in detecting adapted or tolerant lines. The application of the index to genotype tolerance and adaptability under environmental stress has been widely reported. Anshori *et al.* (2018) and Anshori *et al*. (2019) had previously applied this method in salinity-stressed rice, while, Kumar *et al*. (2015). A similar application has been made by Fadhli *et al*. (2020) on drought-stressed maize and Farid *et al.* (2019) on drought-stressed wheat. In addition, STI application on several characters had been reported by Anshori *et al.* (2019) and Fadhli *et al*. (2020). Therefore, STI application on characters with significantly affected by the treatments was also used in this research.

Correlation analysis of STI results shows that productivity was correlated positively to plant height (0.77), the number of leaves (0.76), ear height position (0.78), ear diameter (0.72), and 1000 seed weight (0.72). However, a negative sign was presented to the number of dried leaves (-0.84) (Figure 2). These correlations have also been reported by some research, such as the correlation of ear diameter and number of leaves to productivity reported by Fadhli *et al*. (2020). Additionally, Ali *et al.* (2017) reported a significant positive correlation between productivity and ear diameter, while Yue *et al.* (2018) reported a significant correlation between yield and plant height. Leaf rolling has a negative correlation towards the number of leaves (-0.89), plant height (-0.68), ear height (-0.68), and chlorophyll (-0.67) (Figure 2). This was due to its negative interpretation, where the more adapted the variety under drought, the less curling occurred (Efendi *et al*., 2019; Fadhli *et al*., 2020). Commonly, productivity is the main selection character, but various research was based on drought stress tolerance determination on leaf rolling (Obeng-Bio *et al*., 2011; Baret *et al*. 2018; Efendi *et al*. 2019). According to correlation analysis, an indirect correlation was discovered between leaf rolling and productivity in drought stress. This was proven in plant height, number of leaves, and ear height which was significantly correlated with both characters. Furthermore, this indicated that drought selection accuracy can be increased by the combination of these characters. Therefore, the characters need to be combined in a selection index, and this can be analyzed through principal component analysis. Anshori *et al*. (2019), Akbar *et al*. (2019), Alsabah *et al* (2019), and Anshori *et al.* (2021) had reported the index formation in this analysis.

Principal Component Analysis (PCA) result shows that 3 PC depicted STI characters (Table 2). This determination was based on the early PC that attained 0.8 cumulative variances (CV) (Jolliffe 2002). Based on CV value, PC1 to PC3 were determined as the potential candidates on the weighting index. Furthermore, the PC1 was the most productive eigenvector compared to PC 2 and PC 3, making this PC the basis for the weighing index (Table 2). A similar trend was previously reported by Anshori *et al*. (2019), Akbar *et al*. (2019), and Anshori *et al*. (2021), where the formation of selection index weighting value was based on the largest eigenvector from the main characters. Despite this, PC1 had a negative value, but its eigenvector was still used as a base of the weighing index, as was applied by Anshori *et al*. (2021). According to Jolliffe (2002), positive and negative sign was limited to variance direction of characters, making the eigenvector useful in positive condition. However, due to their interpretation of tolerance, the number of leaves and leaf rolling were changed. Therefore, the selection index formed had the following formula:

Selection index = 0.358 plant height (PH) + 0.362 number of leaves (NL) - 0.315 number of dried leaves (NDL) + 0.352 ear height (EH) + 0.263 ear diameter (ED) - 0.291 leaf rolling (LR) + 0.189 leaf chlorophyll index (LCI) + 0.293 1000 seed weight (1000SW) + 0.346 productivity.

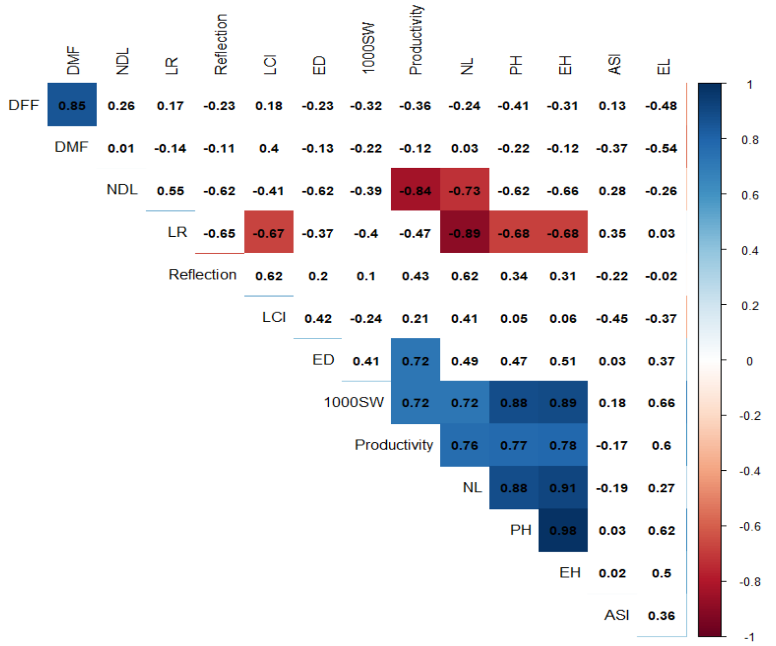

Figure 2. Heatmap based Pearson Correlation Analysis towards all STI Characters significant towards interaction (1000SW = 1000 seed weight, ASI = Anthesis silking interval, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height)

Various research has reported PCA application in finding a variation of an object and other variables (Jolliffe 2002; Mattjik and Sumertajaya 2011; Singh *et al.* 2015; Anshori *et al*. 2018; Fadhli *et al*. 2020). This analysis was effective in preventing multicollinearity or overlapped variance (Jolliffe 2002; Mattjik and Sumertajaya, 2011), can increase selection index objectivity in genotype, and is linked with the index from Smith Hazel (Godshalk and Timothy 1988). Based on the research, determination of weighing value from PC eigenvector can affect priority characters towards drought stress. Productivity relatively has a low heritability value under abiotic stress (Kassahun *et al*. 2013; Fellahi *et al*. 2018), hence, the utilization of the characters with linear and larger variance can increase selection effectiveness under drought stress. Furthermore, Alsabah *et al*. (2019) reported that the variety of productive tillers was larger than productivity, and this was in line with path analysis results. According to Akbar *et al.* (2019) and Anshori *et al*. (2019), eigenvector application can be combined with path analysis. However, due to the small number of genotypes in the research, the analysis was considered unnecessary. Path analysis with fewer samples can cause multicollinearity (Olivoto *et al*. 2017; Sari *et al.*

2018), hence, this index application could increase the selection effectiveness of synthetic maize under drought stress. Based on the selection index, productivity does not dominate index variance but is the main character, as a result, the index requires adjustment. This has been reported by Anshori *et al.* (2019) on rice under salinity stress and Farid *et al*. (2021) on wheat under drought stress. Therefore, the adjusted selection index was formulated in this study.

The adjusted concept is important because this analysis could avoid the overestimate interpretation (Anshori *et al*. 2019). The adjusted selection index can be conducted with some analysis. One of these adjusted analyses that could be conducted is correlation analysis. This analysis has been reported by Sabouri *et al.* (2008) and Chaudhary *et al.* (2017) on rice. Based on this, the combination of PCA weighting and correlation was performed in creating the selection index. The adjusted selection index was as follows:

Adjusted selection index = (0.358 x 0.77) PH + (0.362 x 0.76) NL – (0.315 x 0.85) NDL + (0.352 x 0.78) EH + (0.263 x 0.72) ED - 0.291 LR + 0.189 LCI + (0.293 x 0.72) 1000SW + 0.346 productivity.

Adjusted selection index = 0.276 PH+ 0.275 NL - 0.265 NDL +0.275 EH + 0.189 ED - 0.291 LR + 0.189 LCI + 0.211 1000SW+ 0.346 productivity.

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"Adjusted selection index = (0.358 x 0.77) PH + (0.362 x 0.76) NL – (0.315 x 0.85) NDL + (0.352 x 0.78) EH + (0.263 x 0.72) ED - 0.291 LR + 0.189 LCI + (0.293 x 0.72) 1000SW + 0.346 productivity"

Table 2. Principal component analysis based on STI Characters significant towards interaction

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
PH	-0.358	0.125	-0.134	0.219	-0.008	0.075	0.004	0.342	-0.191
NL	-0.362	-0.130	-0.099	0.218	-0.097	-0.029	-0.024	-0.132	-0.173
NDL	0.315	0.126	-0.103	0.277	-0.135	0.427	0.436	-0.409	-0.123
DFF	0.176	-0.302	-0.543	-0.154	-0.162	-0.256	0.089	-0.100	-0.497
DMF	0.053	-0.429	-0.507	-0.019	0.162	-0.109	0.189	0.179	0.361
ASI	0.050	0.349	-0.254	-0.169	-0.772	-0.072	-0.118	0.130	0.237
EH	-0.352	0.087	-0.246	0.182	-0.011	0.076	-0.275	0.168	0.039
ED	-0.263	-0.016	-0.006	-0.662	-0.057	0.392	-0.130	-0.195	-0.307
Reflection	-0.219	-0.220	0.387	0.113	-0.391	-0.529	0.180	-0.263	-0.050
LR	0.291	0.281	-0.047	-0.322	0.175	-0.312	-0.123	-0.131	0.245
LCI	-0.189	-0.429	0.137	-0.222	-0.220	0.332	0.267	0.047	0.462
EL	-0.198	0.422	-0.044	-0.162	0.086	-0.114	0.725	0.288	-0.071
1000SW	-0.293	0.235	-0.331	0.188	0.075	0.031	-0.040	-0.580	0.327
Productivity	-0.346	0.045	-0.040	-0.277	0.275	-0.262	0.110	-0.257	0.046
PV	0.478	0.219	0.104	0.071	0.057	0.041	0.019	0.012	0.000
CV	0.478	0.697	0.801	0.872	0.929	0.970	0.988	1.000	1.000
EigenValues	6.690	3.072	1.449	0.994	0.802	0.571	0.258	0.165	0.000

Notes: PV = proportion of variance, CV = cumulative of variance, 1000SW = 1000 seed weight, ASI = Anthesis silking interval, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height.

Table 3. STI Selection index on 9 synthetic maize genotypes.

Genotype	PH	NL	NDL	EH	ED	LR	LCI	1000SW	Pr	Selection Index
Syn_2-1	0.70	0.78	2.09	0.69	0.89	1.73	0.99	0.69	0.55	0.23
Syn_2-2	0.99	1.00	1.42	0.97	0.85	1.45	1.00	0.93	0.67	0.79
Syn_2-4	0.80	0.87	2.86	0.82	0.78	1.64	0.98	0.95	0.50	0.16
Syn_2-8	0.82	0.91	2.03	0.86	0.88	1.52	1.00	0.82	0.48	0.43
Syn_2-15	1.00	1.02	1.63	1.00	1.03	1.45	1.01	1.06	0.75	0.85
Syn_2-16	1.01	1.01	1.28	1.01	0.99	1.48	1.00	1.09	0.86	0.97
Bisma	0.90	0.86	2.78	0.86	0.83	1.59	0.98	0.92	0.48	0.23
Lamuru	0.96	0.89	1.87	0.98	0.92	1.70	0.97	1.08	0.68	0.61
Sukmaraga	0.84	0.86	2.46	0.85	0.94	1.58	1.00	0.85	0.53	0.32

Note : 1000SW = 1000 seed weight, ED = Ear diameter, EH = Ear height, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height, Pr =Productivity.

The selection index result revealed 3 synthetic maize genotypes with a better index compared to Lamuru. These varieties were Syn_2-2 (0.79), Syn_2-15 (0.85), and Syn_2-16 (0.97). Lamuru is a composite variety with 7.6 ton ha^{-1} productivity and is tolerant to drought, making it frequently planted in areas with long dry months (Mustikawati and Yulia 2011; Aqil *et al*. 2012; Prasetyo and Amin 2019). According to Suwarno *et al*. (2009), the use of control varieties is the common method for best-selected lines and has been used in detecting the best rice line resistant to blast disease. Therefore, based on the research, the 3 synthetic maize lines were recommended as advance line candidates under drought stress.

In conclusion, plant height, number of leaves, number of dried leaves, ear height, ear diameter, leaf rolling, chlorophyll, and 1000 seed weight were characters that affected synthetic maize productivity variance in drought stress adaptive. Meanwhile, the principal component analysis resulted in Adjusted Selection Index = 0.276 plant height (PH) + 0.275 number of leaves (NL) - 0.265 number of dried leaves (NDL) +0.275 ear height (EH) + 0.189 ear diameter (ED) - 0.291 leaf rolling (LR) + 0.189 leaf chlorophyll index (LCI) + 0.211 1000 seed weight (1000SW) + 0.346 productivity. Through index selection Syn_2-2, Syn_2-15 and Syn_2-16 were considered as drought stress adaptive lines. Therefore, these 3 lines can be recommended in further process as candidates for drought stress.

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Corrected proofreading

Drought-adapted maize line based on morphophysiological selection index

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Abstract. *Padjung R, Farid M, Musa Y, Anshori MF, Nur A, Masnenong A. 2021. Drought-adapted maize line based on morphophysiological selection index. Biodiversitas 22: xxxx*. Synthetic line formation is an effort to increase maize productivity in drought-stressed areas. This process requires systematic selection in determining adaptability levels involving important secondary characters formulated in the selection index. Furthermore, the principal component index had been widely reported, both in normal and stressed conditions. The selection index development on morpho-physiological characters based on multivariate analysis was expected to increase drought stress tolerance and maize lines selection precision. Therefore, the purpose of this research was to form a selection index based on morpho-physiological characters and selecting synthetic lines adaptive under drought stress. This research was designed using a split-plot with 3 replications, where the main plot consisted of normal and stressed irrigation, while the subplot was genotype consisting of 6 lines and 3 check varieties namely Bisma, Lamuru, and Sukamarga. Observations were performed on 18 morphological and 4 physiological characters, and the results showed that the selection index was formed based on multivariate analysis from 9 characters. Through index selection, Syn_2-2 (0.79), Syn_2-15 (0.85) and Syn_2-16 (0.97) were considered as drought stress adaptive lines. Therefore, the 3 lines can be recommended in the synthetic variety release process.

Keywords: abiotic stress, corn, multivariate analysis, principal component, secondary characters

Abbreviations: 1000SW = 1000 Seed Weight; ASI = Anthesis Silking Interval; DAP = Day After Planting; DFF= Days of Female Flowering; DMF= Days of Male Flowering; ED = Ear Diameter; EH = Ear Height; EL = Ear Length; LR = Leaf Rolling; NDL= Number of Dried Leaves; NL = Number of Leaves; PH= Plant Height; Pr = Productivity

INTRODUCTION

Maize (*Zea mays* L.) is an important feed and food in Indonesia. This commodity also is one of the main ingredients of industrial companies, which makes it the 3rd important cereal commodity in the world after wheat and rice (Bukhsh et al. 2012; Cooper et al. 2014). In Indonesia, maize production is considered good with a total production reach 30 million tons in 2018 (Agriculture Ministry 2018). However, as population growth increases, annually maize production also needs to further increase to meet the demand (Sah et al. 2020; Badr et al. 2020). The climate change negatively impacts the plant growth environment, such as drought, salinity, submergence, etc. (Raza et al. 2018). The prolonged temperature rise due to the climate change induces drought stress, which in turn threaten the maize production stability (Fahad et al. 2017), and as a result, domestic production has failed to meet the demand. Therefore, the maize production problem in drought stress condition should be solved to increase the maize yield.

Generally, water scarcity caused by limited water availability on agricultural land, or by plant inability to absorb water, is a common drought stress factor (Farid et al. 2019). This stress can inhibit some plant morphology and physiological processes, such as cell division, cell development, nutrient transport and translocation, plant enzymatic process, plant metabolism, pollen sterility, and grain development, which affect the growth and yield of plants, including the maize (Silva et al. 2013; Fahad et al. 2017). Although maize is known to use water efficiently (Ghannoum 2009), a long period of drought stress, both at vegetative stage and at the anthesis stage, will decrease production (Witt et al. 2012; Souza et al. 2013; Song et al. 2019; Sah et al. 2020). According to Monneveux et al. (2005) and Sah et al. (2020), drought stress can decrease maize yield by 17–60% in tropical areas. Song et al. (2019) also reported that the drought stress could dercrease the maize yield up to 50% during 2013 to 2016. Therefore, this problem needs to be solved through, among others, developement of drought stress adapted maize variety.

Maize variety can be developed through hybridization and open-pollination. The hybrid is a popular variety in maize (Fromme et al. 2019; Kandel 2020) because it is relatively sensitive under stress conditions (Kutka 2011; Sharma et al. 2019). However, the synthetic variety is more adaptive under stress conditions, making it suitable for development (Kutka 2011; Freshley and Delgado-Serrano 2020). The adaptive variety produces good yield and growth characters in any environment, including stress conditions (Lin et al. 1986; Fadhli et al. 2020). To produced the variety possesing this trait, the combination of the yield supporting characters, known as secondary characters, must be studied. The yield are mostly comprised of polygenic traits, hence the use of secondary supporting characters can keep potential lines in any environment (Kassahun et al. 2013; Fellahi et al. 2018), especially under drought stress condition. Sabouri *et al.* (2008), Saad et al. (2014), Fellahi et al. (2018), and Anshori et al. (2019) have reported the assessment of tolerance lines involving the secondary characters. Besides, according to Fadhli et al. (2020), the use of the secondary character in selecting adapted lines under drought stress was more effective. Therefore, these characters are important in selecting the adapted synthetic maize under drought stress.

Secondary characters can be obtained through morphology and physiology characterization. These combinations present strength that can be considered in a selection for adapted lines under stress conditions. Previous reports on this concept under drought stress have been made by Barik et al. (2019) on rice, Souza et al. (2013), and Sabagh et al. (2017) on maize. However, combining all secondary characters and yield requires a selection formula known as the selection index. This is the linear multivariate regression consisting of specific weighted criteria selection (Rajamani et al. 2016; Islam et al. 2017). Moreover, the index needs systematic analyses such as multivariate analysis to determine the fit of secondary characters and the weighting of its secondary characters. The success of this approach has been reported by Sabouri et al. (2008), Peternelli et al. (2017), Kose et al. (2018), Branković et al. (2018), Akbar et al. (2019), and Anshori et al. (2021). Therefore, the application of multivariate analysis to develop a selection index based on morpho-physiological characters is useful in adaptive synthetic maize under drought stress. The objective of this study was to develop a selection index based on morpho-physiological characters and select the adapted synthetic maize lines under drought stress.

MATERIALS AND METHODS

The study was conducted in the Experimental Farm of Faculty of Agriculture, Hasanuddin University, Makassar from July to November 2017. This research was designed using a split-plot design with 3 replications where main plot watering (p) consisted of normal (p0) and stress irrigation (p1). Also, Subplots consisted of 6 genotypes (G): Syn 2-1 (G1), Syn 2-2 (G2), Syn 2-4 (G3), Syn 2-8 (G4), Syn 2-15 (G5), Syn 2-16 (G6) and 3 check varieties, i.e., bisma (G7), lamuru (G8) and sukmaraga (G9). According to the number of treatments, 18 combination was present and replicated 3 times, resulting in 54 experimental units while the plotting area for experimental unit size was 3bm x 3.5bm.

Experimental procedure

To prevent mildew disease, maize seeds used were given metalaxyl. Seeds (2) were placed in each planting hole and Carbofuran 30% was added with 15 kg ha^{-1} dosage to prevent pest infestation. Furthermore, each genotype treatment was planted in a 80 cm x 20 cm spacing, the thinning was done 14 days after planting (DAP), and fertilizer was applied 3 times. First fertilizer application (basal application) was applied seven days after planting at dosage of SP36 150 kg ha^{-1}, KCl 100 kg ha^{-1} and Urea 70 kg ha^{-1}. The second fertilizer application was on 28 DAP with NPK 100 kg ha^{-1} and Urea 65 kg ha^{-1}, while, the third application was on 40 DAP with KCl 100 kg ha^{-1} and Urea 65 kg ha^{-1}. Also, irrigation was made with a water pump hosed by flooding the plots until it got to the height of the beds. Drought stress method was performed according to CIMMYT (Bänziger *et al.* 2000), where irrigation was stopped after the plants attained 40 DAP. Afterwards, irrigation was avoided for the next 30 days and was given on the 70th day until physiological maturity. As for the normal condition, the irrigation was done by using the pump for the regular watering of fields. Plant maintenance included thinning, heaping, spraying, and weeding. Weeding was performed on 14 and 28 DAP and Insecticide application was adjusted according to the crop pests present in the experimental field.

Observation

Observations were done morphological and agronomical characters consisted of plant height, number of leaves, leaf angle, leaf width, number of dried leaves, days to female flowering, days to male flowering, Anthesis Silking Interval (ASI), days to harvesting, stem diameter, ear length, ear diameter, length of seeded ear, seed rendement, 1000 seed weight, and productivity. Meanwhile, the physiological character's observations were done on leaf age scoring, absorption level, reflection, stomata density, leaf chlorophyll index, and leaf roll scoring. The tools used for physiology character observations were lab miniature leaf streptik CI 7010 and chlorophyll meter SPAD 502.

Data analysis

Recapitulated data were subjected to analysis of variance and characters significantly affected were preceded into further analysis. Variance identification was performed using cluster analysis under normal and drought stressed conditions. This was done in Rstudio 3.6.3 with factoextra (Kassambra and Mundt 2020) and dendextend (Galili 2015). Additionally, all characters showing significant interaction effect were subjected to analysis of stress tolerance index (STI) (Fernandez 1992), as follow:

$$STI = \frac{Yp \times Ys}{\bar{Y}p^2}$$

Note:

Yp : The character value of each line in normal conditions.

Ys : The character value of each line in drought stress condition.

Ȳp : Average character values of all lines in normal conditions

After which they were analyzed by using a Pearson correlation analysis. This analysis was perfomred using Rstudio software with agricolae package (Mendiburu 2020) and corrplot (Wei and Simko 2017) while, the selection index was formed through principal component analysis using STAR IRRI 2.0.1 (Anshori et al. 2019). Furthermore, the selection index applied to all genotypes was evaluated by comparing the synthetic maize lines index with the best comparing index.

RESULTS AND DISCUSSION

Analysis of variance indicated that morphological and physiological characters were significantly affected by genotypic variance and water condition (Table 1). However, not all characters were affected significantly by interaction variance. Morphological characters that were affected included plant height, number of leaves, number of dried leaves, days to female flowering, days to male flowering, Anthesis Silking Interval (ASI), ear height, ear diameter, ear length, leaf rolling, 1000 seed weight and productivity. Meanwhile, the physiological characters affected were reflection and chlorophyll. According to Al-Naggar et al. (2015), Mohamadi et al. (2017), Anshori et al. (2019) and Anshori et al. (2021), characters significantly affected by genotype-environment interaction can exhibit response variance between genotype and growing environment factors. This is a base in distinguishing adapted or tolerant and sensitive maize genotypes under drought stress. A similar concept application was reported by Fadhli et al. (2020) on maize under drought stress, Anshori et al. (2021) on rice under salinity stress, and Akbar et al. (2019) on rice under drought stress. Therefore, all characters significantly affected by the interaction of genotype - water condition variance could be continued in further analysis.

Cluster analysis was the second approach to detecting interaction variance lines under normal and stress conditions. The results showed that synthetic maize genotype grouping experienced dynamic changes in both environments (Figure 1), and there was no straight line connecting the two dendrograms. Although on the 60% dissimilarity degree, both dendrograms had 3 cluster units with different group units in each. This shows that each genotype had a different response in every environment. Based on the result, cluster analysis was effective in depicting response variance in different growing environments. This has been reported by some research identifying relationships among objects towards many variables in several environments or models (Silva et al. 2013; Saad et al. 2014; Anshori et al. 2020). However, the simple dendrogram could not explain the specific adapted trait under stress conditions. This proves that further analysis was required to evaluate the adaptability of synthetic maize under drought stress.

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Table 1. Analysis of variance of morphological and physiological characters of a number of synthetic maize genotypes in varied environments

Characters	Irrigation (E)	Error a	Genotipe (G)	G x E	Error b	CVa	CVb
Plant Height	5998.52**	44.34	698.23**	193.06*	79.04	4%	5%
Number of Leaves	14.00**	0.02	1.45**	0.58**	0.15	1%	4%
Number of Dried Leaves	27.45**	0.07	0.49**	0.20**	0.06	13%	12%
Stem Diameter	41.93*	1.59	3.01*	0.52ns	1.11	6%	5%
Days to Female Flowering	80.67*	0.89	4.00**	3.37*	0.59	2%	1%
Days to Male Flowering	20.17**	1.06	4.21*	2.08**	0.77	2%	2%
Anthesis Silking Interval (ASI)	20.17**	0.17	1.27*	1.83**	0.51	15%	27%
Days of Harvest	136.96*	5.57	15.88*	6.59ns	4.44	2%	2%
Ear Height	1717.84*	60.87	216.49**	149.31*	59.55	8%	8%
Ear Diameter	80.87*	1.17	5.88**	5.11**	1.13	5%	5%
Leaf Angle	553.30**	5.42	22.40*	7.84ns	7.54	8%	10%
Leaf Width	90852.41*	2895.94	9762.18*	3877.43ns	4268.73	12%	14%
Leaf Aging	1.97**	0.02	0.06**	0.04ns	0.02	3%	5%
Absorbtion	1.97*	0.02	0.06**	0.04ns	0.02	18%	16%
Reflection	0.06**	0.00	0.00**	0.00*	0.00	11%	8%
Stomata Density	4129.96**	36.92	1346.99*	633.53ns	461.46	2%	8%
Leaf Rolling	22.56**	0.00	0.05**	0.02*	0.01	2%	3%
Leaf Chlorophyll Index	1244.23*	56.25	297.95**	166.79*	70.89	1%	1%
Ear Length	71.67*	0.94	4.45**	2.02**	0.35	6%	4%

Seed Rendement	365.50**	1.52	6.70*	5.01ns	2.96	2%	2%
1000 Sed weight	8151.69*	210.70	3634.07**	403.89*	143.89	4%	4%
Productivity	170.40**	0.18	3.73**	0.40**	0.08	6%	4%

Note: CV = coefficient of variance; * significant at 5%; ** highly significant at 1% level; ns = not significant

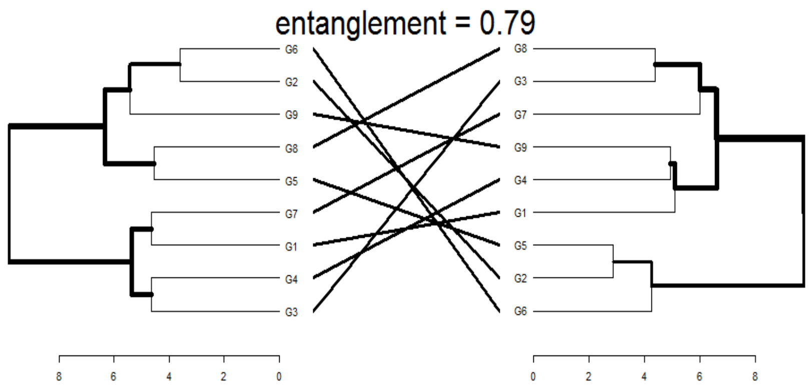

Figure 1. Cluster analysis of synthetic maize lines based on significant characters toward the interaction effect under normal and drought condition

The effectiveness of tolerance and adaptability can be evaluated with Stress Tolerance Index (STI). This detects tolerant lines under stress and has a midpoint benefit in considering the line's potential under normal and stress conditions (Anshori et al. 2019). Besides, STI considers the average responses of all genotypes under normal conditions. This is similar to a dynamic concept on the stability analysis used in assessing the stability lines (Hidayatullah et al. 2019; Sitaresmi et al. 2019; Kartina et al. 2019; Amzeri et al. 2020). Furthermore, the dynamic concept can assess the line's potential based on the average population responses (Lin et al. 1986), hence, the STI concept could be used in detecting adapted or tolerant lines. The application of the index to genotype tolerance and adaptability under environmental stress has been widely reported. Anshori et al. (2018) and Anshori et al. (2019) had previously applied this method in salinity-stressed rice, while, Kumar et al. (2015). A similar application has been made by Fadhli *et al.* (2020) on drought-stressed maize and Farid et al. (2019) on drought-stressed wheat. In addition, STI application on several characters had been reported by Anshori et al. (2019) and Fadhli et al. (2020). Therefore, STI application on characters significantly affected by the treatments were also used in this research.

Correlation analysis of STI values and the significantly affected characters showed that productivity was correlated positively to plant height (0.77), the number of leaves (0.76), ear height position (0.78), ear diameter (0.72), and 1000 seed weight (0.72). However, a negative sign was observed on the number of dried leaves (-0.84) (Figure 2). These correlations have also been reported by some research, such as the correlation of ear diameter and number of leaves to productivity reported by Fadhli *et al.* (2020). Additionally, Ali et al. (2017) reported a significant positive correlation between productivity and ear diameter, while Yue et al. (2018) reported a significant correlation between yield and plant height. Leaf rolling has a negative correlation with the number of leaves (-0.89), plant height (-0.68), ear height (-0.68), and chlorophyll (-0.67) (Figure 2). This was due to its negative interpretation, where the more adapted the variety under drought, the less the leaf curling occurred (Efendi et al. 2019; Fadhli et al. 2020). Commonly, productivity is the main selection character, but various research was based on drought stress tolerance determination on leaf rolling (Obeng-Bio et al. 2011; Baret et al. 2018; Efendi et al. 2019). According to correlation analysis, an indirect correlation was discovered between leaf rolling and productivity in drought stress. This was proven in plant height, number of leaves, and ear height which was significantly correlated with both characters. Furthermore, this indicated that drought selection accuracy can be increased by the combination of these characters. Therefore, the characters need to be combined in a selection index, and this can be analyzed through principal component analysis. Anshori et al. (2019), Akbar et al. (2019), Alsabah et al. (2019), and Anshori et al. (2021) had reported the index formation in this analysis.

Principal Component Analysis (PCA) result showed that 3 PC depicted STI characters (Table 2). This determination was based on the early PC that attained 0.8

cumulative variances (CV) (Jolliffe 2002). Based on CV value, PC1 to PC3 were determined as the potential candidates on the weighting index. Furthermore, the PC1 was the most productive eigenvector compared to PC 2 and PC 3, making this PC the basis for the weighing index (Table 2). A similar trend was previously reported by Anshori et al. (2019), Akbar et al. (2019), and Anshori et al. (2021), where the formation of selection index weighting value was based on the largest eigenvector from the main characters. Despite this, PC1 had a negative value, but its eigenvector was still used as a base of the weighing index, as was applied by Anshori et al. (2021).

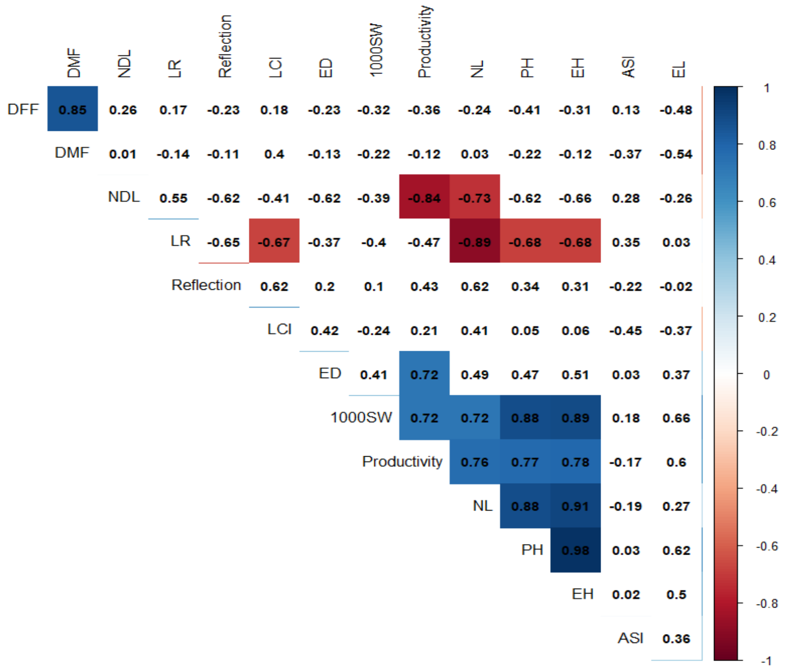

Figure 2. Heatmap based Pearson Correlation Analysis towards all STI Characters significant towards interaction (1000SW = 1000 seed weight, ASI = Anthesis silking interval, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height)

According to Jolliffe (2002), positive and negative sign was limited to variance direction of characters, making the eigenvector useful in positive condition. However, due to their interpretation of tolerance, the number of leaves and leaf rolling were changed. Therefore, the selection index formed had the following formula:

Selection index = 0.358 plant height (PH) + 0.362 number of leaves (NL) - 0.315 number of dried leaves (NDL) + 0.352 ear height (EH) + 0.263 ear diameter (ED) - 0.291 leaf rolling (LR) + 0.189 leaf chlorophyll index (LCI) + 0.293 1000 seed weight (1000SW) + 0.346 productivity.

Various research has reported PCA application in finding a variation of an object and other variables (Jolliffe 2002; Mattjik and Sumertajaya 2011; Singh et al. 2015; Anshori et al. 2018; Fadhli et al. 2020). This analysis was effective in preventing multicollinearity or overlapped variance (Jolliffe 2002; Mattjik and Sumertajaya 2011), can increase selection index objectivity in genotype, and is linked with the index from Smith Hazel (Godshalk and Timothy 1988). Based on the research, determination of weighing value from PC eigenvector can affect priority characters towards drought stress. Productivity relatively has a low heritability value under abiotic stress (Kassahun et al. 2013; Fellahi et al. 2018), hence, the utilization of the characters with linear and larger variance can increase selection effectiveness under drought stress. Furthermore, Alsabah et al. (2019) reported that the variety of productive tillers was larger than productivity, and this was in line with path analysis results. According to Akbar et al. (2019) and Anshori et al. (2019), eigenvector application can be combined with path analysis. However, due to the small number of genotypes in the research, the analysis was considered unnecessary. Path analysis with fewer samples can cause multicollinearity (Olivoto et al. 2017; Sari et al. 2018), hence, this index application could increase the selection effectiveness of synthetic maize under drought stress. Based on the selection index, productivity does not dominate index variance but is the main character, as a result, the index requires adjustment. This has been reported by Anshori et al. (2019) on rice under salinity stress and Farid et al. (2021) on wheat under drought stress. Therefore, the adjusted selection index was formulated in this study.

Table 2. Principal component analysis based on STI Characters significant towards interaction

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
PH	-0.358	0.125	-0.134	0.219	-0.008	0.075	0.004	0.342	-0.191
NL	-0.362	-0.130	-0.099	0.218	-0.097	-0.029	-0.024	-0.132	-0.173
NDL	0.315	0.126	-0.103	0.277	-0.135	0.427	0.436	-0.409	-0.123
DFF	0.176	-0.302	-0.543	-0.154	-0.162	-0.256	0.089	-0.100	-0.497
DMF	0.053	-0.429	-0.507	-0.019	0.162	-0.109	0.189	0.179	0.361
ASI	0.050	0.349	-0.254	-0.169	-0.772	-0.072	-0.118	0.130	0.237
EH	-0.352	0.087	-0.246	0.182	-0.011	0.076	-0.275	0.168	0.039
ED	-0.263	-0.016	-0.006	-0.662	-0.057	0.392	-0.130	-0.195	-0.307
Reflection	-0.219	-0.220	0.387	0.113	-0.391	-0.529	0.180	-0.263	-0.050
LR	0.291	0.281	-0.047	-0.322	0.175	-0.312	-0.123	-0.131	0.245
LCI	-0.189	-0.429	0.137	-0.222	-0.220	0.332	0.267	0.047	0.462
EL	-0.198	0.422	-0.044	-0.162	0.086	-0.114	0.725	0.288	-0.071
1000SW	-0.293	0.235	-0.331	0.188	0.075	0.031	-0.040	-0.580	0.327
Productivity	-0.346	0.045	-0.040	-0.277	0.275	-0.262	0.110	-0.257	0.046
PV	0.478	0.219	0.104	0.071	0.057	0.041	0.019	0.012	0.000
CV	0.478	0.697	0.801	0.872	0.929	0.970	0.988	1.000	1.000
EigenValues	6.690	3.072	1.449	0.994	0.802	0.571	0.258	0.165	0.000

Notes: PV = proportion of variance, CV = cumulative of variance, 1000SW = 1000 seed weight, ASI = Anthesis silking interval, DFF= Days of female flowering, DMF= Days of male flowering, ED = Ear diameter, EH = Ear height, EL = Ear length, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height.

Table 3. STI Selection index on 9 synthetic maize genotypes

Genotype	PH	NL	NDL	EH	ED	LR	LCI	1000SW	Pr	Selection index
Syn_2-1	0.70	0.78	2.09	0.69	0.89	1.73	0.99	0.69	0.55	0.23
Syn_2-2	0.99	1.00	1.42	0.97	0.85	1.45	1.00	0.93	0.67	0.79
Syn_2-4	0.80	0.87	2.86	0.82	0.78	1.64	0.98	0.95	0.50	0.16
Syn_2-8	0.82	0.91	2.03	0.86	0.88	1.52	1.00	0.82	0.48	0.43
Syn_2-15	1.00	1.02	1.63	1.00	1.03	1.45	1.01	1.06	0.75	0.85
Syn_2-16	1.01	1.01	1.28	1.01	0.99	1.48	1.00	1.09	0.86	0.97
Bisma	0.90	0.86	2.78	0.86	0.83	1.59	0.98	0.92	0.48	0.23
Lamuru	0.96	0.89	1.87	0.98	0.92	1.70	0.97	1.08	0.68	0.61
Sukmaraga	0.84	0.86	2.46	0.85	0.94	1.58	1.00	0.85	0.53	0.32

Note : 1000SW = 1000 seed weight, ED = Ear diameter, EH = Ear height, LCI= Leaf chlorophyll index, LR = Leaf rolling, NDL= Number of dried leaves, NL = Number of leaves, PH= Plant height, Pr =Productivity.

The adjusted concept is important because this analysis could avoid the overestimate interpretation (Anshori et al. 2019). The adjusted selection index can be conducted with some analysis. One of these adjusted analyses that could be conducted is correlation analysis. This analysis has been reported by Sabouri et al. (2008) and Chaudhary et al. (2017) on rice. Based on this, the combination of PCA weighting and correlation was performed in creating the selection index. The adjusted selection index was as follows:

Adjusted selection index = (0.358 x 0.77) PH + (0.362 x 0.76) NL – (0.315 x 0.85) NDL + (0.352 x 0.78) EH + (0.263 x 0.72) ED - 0.291 LR + 0.189 LCI + (0.293 x 0.72) 1000SW + 0.346 productivity.

Adjusted selection index = 0.276 PH+ 0.275 NL - 0.265 NDL +0.275 EH + 0.189 ED - 0.291 LR + 0.189 LCI + 0.211 1000SW+ 0.346 productivity.

The selection index result revealed 3 synthetic maize genotypes with a better index compared to Lamuru sa check variety. These varieties were Syn_2-2 (0.79), Syn_2-15 (0.85), and Syn_2-16 (0.97). Lamuru is a composite variety with 7.6 ton ha^{-1} productivity and is tolerant to drought, making it frequently planted in areas with long dry months (Mustikawati and Yulia 2011; Aqil *et al*. 2012; Prasetyo and Amin 2019). According to Suwarno et al. (2009), the use of control/check varieties is the common method for best-selected lines and has been used in detecting the best rice line resistant to blast disease. Therefore, based on the research, the 3 synthetic maize lines were recommended as advance line candidates under drought stress.

In conclusion, plant height, number of leaves, number of dried leaves, ear height, ear diameter, leaf rolling, chlorophyll, and 1000 seed weight were characters that affected synthetic maize productivity variance in drought stress condition. Meanwhile, the principal component analysis resulted in Adjusted Selection Index = 0.276 plant height (PH) + 0.275 number of leaves (NL) - 0.265 number of dried leaves (NDL) +0.275 ear height (EH) + 0.189 ear diameter (ED) - 0.291 leaf rolling (LR) + 0.189 leaf chlorophyll index (LCI) + 0.211 1000 seed weight (1000SW) + 0.346 productivity. Through index selection, Syn_2-2, Syn_2-15 and Syn_2-16 were considered as drought stress adaptive lines. Therefore, these 3 lines can be recommended for further process as candidates of drought tolerant varieties.

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