

Multivariate analysis to determine secondary characters in selecting adaptive hybrid corn lines under drought stress

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Abstract. *Fadhli N, Farid M, Rafiuddin, Efendi R, Azrai M, Anshori MF. 2020. Multivariate analysis to determine secondary characters in selecting adaptive hybrid corn lines under drought stress. Biodiversitas 21: 3617-3624.* The development of adaptive hybrid corn varieties under drought stress needs an effective selection. Multivariate analysis has been reported can increase the effectiveness of selection in plants by attaching the secondary characters in the selection. Therefore, this concept also can be applied to develop adaptive corn varieties under drought stress. The objectives of this study are to determine the main secondary characters and select the best hybrid lines adaptive to drought stress. The experiment was arranged by a nested design, where replications nested under two environmental conditions, namely normal and drought stress. The main factor was genotypes consisted of 30 genotypes and was repeated three times. Moreover, the observations of this research consisted of 20 variables. The result of this research showed that the weight of harvested cob was an effective secondary character as a selection criterion along with productivity in selecting adaptive maize genotypes under drought stress. The number of green leaves was the character outside of the yield component could be as an alternative secondary character besides the weight of harvested cob. The selection results based on the weight of the harvested cob and productivity resulted in 12 hybrid corn lines considered adaptive to drought stress.

Keywords: Drought stress, hybrid corn, Index Tolerance, multivariate analysis, *Zea mays*

Abbreviations: AOH: age of harvesting, ASI: anthesis silking interval, DC: diameter of cob, FFD: female flowering days, HC= height of cob, LC: length of cob, LL: leaf length, Max temp: maximum temperature, MFD: male flowering days, Min temp: minimum temperature, NGL: number of green leaves, NGR: Number of grains per cob row, NRC: number of rows per cob, P: productivity, PH: plant height, SD: stem diameter, WHC: weight of harvested cob, W1000: weight of 1000 seeds, Y: yield

INTRODUCTION

Corn (*Zea mays* L.) is one of the essential cereals in the world, which is widely used as food, feed, and bioethanol production for human and animal needs. The increase in population and the industry development will have a direct impact on increasing the corn demand (Sah et al. 2020; Badr et al. 2020). Efforts to increase corn production are the primary solution in reducing import quotas in Indonesia. However, there are several obstacles to increase production. One of them is water limitations under dry season, so that corn cultivation undergo drought stress (Fahad et al. 2017).

Drought stress is the primary abiotic stress in the world, which has an impact on crop productivity, including corn. This stress causes the availability of water for plants to be reduced (Farid et al. 2019a). The drought stress will harm plant growth and metabolic processes that are highly dependent on water (Hammad et al. 2014; Sah et al. 2020). In addition, this stress also has a secondary impact on nutrient absorption, which also involves water as an intermediary for nutrients entering the plants (Waraich et

al. 2011). According to Adewale et al. (2018), drought stress can reduce the yield by 15% of average global yield. Therefore, the problem of drought stress in corn cultivation needs to be solved. One of them is by developing adaptive corn varieties under drought stress.

The adaptive variety is a variety which has stable and or increased yield productivity in different places and conditions (Lin et al. 1986). To generate the adaptive corn variety, many corn genotypes must be selected and grown under normal and stress condition in order to evaluate their responses to those conditions properly and effectively. This concept is widely applied by Anshori et al. (2019) and Anshori et al. (2020) on rice under salinity stress, Akbar et al. (2019) and Farid and Ridwan (2018) on rice under drought stress, Adhikari et al. (2019); Sah et al. (2020) and Badr et al. (2020) on corn under drought.

The selection criteria also determine the effectiveness of the selection method on adaptive maize screening under drought stress. In general, the line selection can be done with two approaches: direct selection and indirect selection (Costa et al. 2008; Fellahi et al. 2018). Direct selection is the most common selection focused on the relative

decrease of yield between normal and drought stress conditions. However, this selection method is considered ineffective because productivity has low heritability under stress condition (Fritsche-Neto and DoVale 2012; Kassahun et al. 2013). Therefore, the direct method approach needs to be integrated with the determination of secondary characters in supporting the yield stability of plants grown in a varied environment (Fellahi et al. 2018; Anshori et al. 2019).

Secondary character determination requires a precise statistical approach, one of which is the concept of multivariate analysis. Multivariate analysis can simplify, reduce, and predict the relationship between many variables and objects (Mattjik and Sumertajaya 2011). This approach has been widely used in determining character selection (Hasan et al. 2016; Kose et al. 2018; Anshori et al. 2019; Akbar et al. 2019). Multivariate analyses that can be used in identifying the best secondary characters are the biplot analysis based on principal components analysis and the path analysis. Several researchers have widely reported both analyzes in determining secondary characters, reported by Anshori et al. (2018) and Anshori et al. (2019) in determining the secondary character of rice under salinity stress, Akbar et al. (2019) in adaptive rice under drought stress, and Mollasadeghi et al. (2011), Seyedzavar et al. (2015) and Ali et al. (2017) in maize against drought stress. Based on this, The use of multivariate analysis in this study is expected to determine the characters which effective in the selection of adaptive hybrid corn lines to drought stress. Therefore, this study aims to determine the main secondary characters and select the best of adaptive hybrid corn lines under drought stress.

MATERIALS AND METHODS

The research was conducted at the Bajeng Experimental Field in the Indonesian Cereals Research Institute, Gowa Regency, from July to November 2019. The experiment design was arranged by a nested design with randomized complete block design as environmental design, where replications nested under two environmental conditions, namely normal and drought stress. The main factor was genotypes consisted of 30 genotypes (28 hybrid corn lines and two commercial hybrid corn varieties (Bisi 18 and Pioner 36)) and was repeated three times, so that there were 180 experimental units.

Procedure

Corn seeds were planted in plots measuring 5 m x 3 m with a 75 cm x 20 cm spacing. Each planting hole consisted of one seed, which was adjusted to the genotype label. Maintenance in this study included watering, fertilizing, and weeding. Irrigation systems were carried out differently between normal conditions and drought stress. Under normal conditions, watering was done at intervals of 10 days. Conversely, in plots with drought stress conditions, the watering interval is stopped at 40 days after planting (DAP) and resumed when the plants were 81 DAP. Fertilization used urea at a dose of 150 kg

ha⁻¹ and NPK 15:15:15 at a dose of 300 kg ha⁻¹ at 10 DAP and urea at a dose of 200 kg ha⁻¹ at 30 DAP.

Observation variables included plant height, height of cob, number of green leaves, stem diameter, SPAD, maximum and minimum temperature, leaf length, male flowering days, female flowering days, anthesis silking interval (ASI), harvesting days, length of cob, diameter of cob, number of rows per cob, number of grains per cob row, weight of harvested cob, prolific, yield, productivity (ton per hectare), and weight of 1000 seeds

Data analysis

The observation data were analyzed through several stages of analysis. The data were first analyzed variance through analysis of variance for parametric data with the standard error of 5%. The significant parametric characters on analysis of variance (ANOVA) interactions were converted to STI characters as an index of tolerance to drought stress (Anshori et al. 2019). The index formula follows the formula of Fernandez (1992):

$$\text{Stress tolerance index (STI)} = Y_p \times Y_s / \bar{Y}_p^2$$

Where: Y_p refers to the results of each genotype under normal conditions. Y_s refers to the results of each genotype under drought stress conditions. Furthermore, \bar{Y}_p refers to the average yield of all genotypes under normal conditions.

All STI characters were analyzed by biplot of principal component analysis and path analysis. Characters that are in the same group or as opposed to productivity were continued for path analysis. The character with the highest direct effect on productivity was chosen as the best secondary character. The secondary character was correlated with Spearman's correlation to the drought score data. If the secondary characters were significantly correlated, the analysis continued with the best genotype selection. This selection was made by slicing the best genotype based on secondary character and productivity. In this slicing, the variance of secondary character and productivity previously were normalized, and the results were mapped in two dimensions. The genotype in the positive quadrant of these characters was selected as the adaptive genotype under drought stress. Moreover, the software used in this study STAR-R 2.0.1 software for ANOVA, biplot of principal component analysis, and Spearman's correlation and R-Studio package Agricolae for path analysis (Mendiburu 2014).

RESULTS AND DISCUSSION

ANOVA results showed that almost all characters were influenced by genotypes, except the minimum temperature of leaves, ASI, and length of the cob. In addition, differences in environmental effect also almost affected to the entire growth character, except plant height, stem diameter, female flowering days, and the number of rows. Characters which did not affect by environmental variability indicated that these characters are relatively stable to environmental conditions, especially to stem

diameter and the number of rows per cob which did not show significant variance in interaction (Table 1). Meanwhile, the characters influenced by genotype and environmental interactions were plant height, the height of ear cobs, number of green leaves, male flowering days, female flowering days, anthesis silking interval, diameter of cob, number of grains per cob row, weight of harvested cob, yield, productivity, and weight of 1000 seed. The apparent interaction variances showed that the differences in responses between tolerant and sensitive genotypes to environmental differences (Ali et al. 2015; Safitri et al. 2016), which becomes the fundamental basis in the selection of secondary characters (Al-Naggar et al. 2015; Anshori et al. 2019; Akbar et al. 2019). Therefore, significant characters to interactions variance were continued for the next analysis in determining secondary characters that are adaptive to drought stress.

Stress tolerance index (STI) is one of the tolerance indexes widely used to detect the tolerant level of genotype under stress condition. This index could be a meeting point for character responses between two different environmental conditions: normal and stress (Hosseini et al. 2012; Anshori et al. 2019). The use of this index has been proven by researchers on several stresses and plants, such as Anshori et al. (2018) reported in rice under salinity stress, Farid et al. (2019b) in rice under drought stress, Anwar et al. (2020) in wheat under drought stress, and Kumar et al. (2015) on maize under drought stress.

Therefore, the formation of STIs on the characters with significant interactions can be the basis for more analysis in selecting the corn genotypes adaptive under drought stress. The results of the formation of STIs characters in this study were shown in Table 2. The overall STI characters were analyzed by multivariate analysis in determining the best secondary characters.

Based on the results of principal component biplot analysis, the stem diameter, number of grains per cob row, number of green leaves, the weight of harvested cob, and the yield had the same grouping with productivity as the main character. (Figure 1). In general, biplot analysis based on the principal component is a multivariate analysis that can combine characters and objects in two dimensions together (Mattjik and Sumertajaya 2011). The use of principal component analysis in biplots will minimize overlapping of variations so that the group determination can be more objective (Mattjik and Sumertajaya 2011; Leite and Oliveira 2015). Therefore, this analysis can facilitate the determination of characters with the same direction variance to the main characters (Kose et al. 2018), especially when using the orthogonal polygonal grouping concept of the outlier object (Leite and Oliveira 2015; Neisse et al. 2018). Based on this analysis, these five characters which have the same direction with the productivity can be as the best secondary character candidates in the selection

Table 1. ANOVA means square of hybrid corn characters in a normal and drought stress environments

Characters	Genotype (G)	Environment (E)	Interaction (GxE)	CV
PH	805.99**	1.18	345.47**	6.50
HC	452.08**	1270.42	208.63**	8.71
NGL	4.32**	897.80**	3.06**	10.00
SD	0.12**	0.02	0.05	9.17
SPAD	47.80**	14484.76**	39.07	10.56
Max Temp	13.28*	2829.82**	13.59*	8.66
Min Temp	8.57	1708.1681*	6.27	8.33
LL	143.66*	983.27*	118.64	11.20
MFD	15.00**	190.14*	8.59**	3.53
FFD	13.68**	0.67	6.03*	3.10
ASI	1.45	213.42**	3.02**	129.92
AH	3.53*	3873**	0.26	1.42
LC	1.81	738.52**	1.79	8.68
DC	20.71*	5024.45**	28.17**	7.97
NRC	2.08**	3.86	1.08	6.35
NGR	17.09*	2582.20**	20.78**	9.35
WHC	3.20**	2115.75**	2.49*	22.89
Prolific	0.06**	7.28**	0.03	18.63
Y	0.01**	0.05**	0.01**	6.14
P	2.80**	2568.89**	2.62*	6.07
W1000	21.54**	10102.66**	19.14*	11.13

Note: ** significant effect on 1% level, * significant effect on 5% level, CV: Coefficient of variance, PH: plant height, HC= height of cob, NGL: number of green leaves, SD: stem diameter, Max temp: maximum temperature, Min temp: minimum temperature, LL: leaf length, MFD: male flowering days, FFD: female flowering days, ASI: anthesis silking interval, AH: age of harvesting, LC: length of cob, DC: diameter of cob, NRC: number of rows per cob, NGR: Number of grains per cob row, WHC: weight of harvested cob, Y: yield, P: productivity, W1000: weight of 1000 seeds

Table 2. STI characters of corn that have significant interactions on ANOVA

G	PH	HC	NGL	MAX TEMP	MFD	FFD	ASI	DC	NGR	WHC	Y	P	W1000
(CIL1283)/MAL03	0.89	0.96	0.72	0.74	0.97	0.99	-15.63	0.83	0.83	0.24	1.02	0.26	0.60
CML161/MAL03	1.03	0.99	0.72	0.73	0.99	1.04	-25.00	0.80	0.97	0.12	1.01	0.26	0.69
(VL1016492)/MAL03	1.04	1.12	0.64	0.78	0.99	1.02	-9.38	0.76	0.69	0.26	0.95	0.24	0.67
(VL1016518)/MAL03	0.98	1.00	0.76	0.73	0.95	1.00	-10.94	0.82	0.81	0.25	0.97	0.27	0.55
(VL1016556)/MAL03	0.90	0.95	0.54	0.81	1.01	1.06	-10.94	0.86	0.77	0.13	0.99	0.14	0.42
(VL1016910)/MAL03	1.00	1.17	0.63	0.87	0.97	0.99	-12.50	0.73	0.76	0.17	0.97	0.15	0.55
(CAL1412)/MAL03	0.97	1.05	0.64	0.76	0.97	1.01	0.00	0.75	0.76	0.16	0.81	0.15	0.64
CML465/MAL03	0.71	0.82	0.55	0.71	0.85	0.89	12.50	0.76	0.68	0.17	1.03	0.21	0.73
CAL1427/MAL03	1.06	1.24	0.50	0.81	1.04	1.06	-62.50	0.78	0.73	0.14	0.92	0.12	0.63
VL144077/MAL03	0.96	0.91	0.62	0.84	1.00	1.04	-21.88	0.66	0.58	0.07	0.63	0.05	0.71
CAL1471/MAL03	1.14	1.32	0.78	0.71	0.92	0.97	0.00	0.81	0.82	0.25	1.01	0.24	0.61
CAL1473/MAL03	0.97	1.06	0.84	0.73	1.01	1.04	0.00	0.73	0.88	0.34	1.04	0.39	0.53
ZL132133/MAL03	0.83	0.67	0.56	0.78	0.89	0.92	-7.81	0.59	0.77	0.22	1.04	0.23	0.54
VL145755/MAL03	0.79	0.80	0.47	0.97	0.95	1.01	31.25	0.72	0.80	0.13	1.09	0.15	0.52
(CIL1283)/B112009	1.05	1.12	0.69	0.84	0.94	0.97	0.00	0.85	0.76	0.29	0.93	0.29	0.42
(CIL12148)/B112009	1.03	0.96	0.37	0.88	0.89	0.95	18.75	0.77	0.87	0.18	0.99	0.19	0.45
CML161/B112009	1.13	1.20	0.88	0.68	0.99	1.02	0.00	0.84	0.78	0.41	0.99	0.42	0.62
(VL1016518)/B112009	0.99	0.93	0.62	0.71	1.06	1.06	-31.25	0.82	0.84	0.21	0.94	0.24	0.59
(VL109288)/B112009	0.90	1.04	0.71	0.76	1.02	1.05	-6.25	0.79	0.73	0.34	1.08	0.42	0.66
(CAL1412)/B112009	1.17	1.37	0.63	0.68	1.00	1.10	-89.06	0.84	0.78	0.19	0.94	0.19	0.46
CML465/B112009	0.92	1.06	0.53	0.80	0.92	0.97	0.00	0.87	0.68	0.10	0.90	0.11	0.52
CAL1427/B112009	1.06	1.19	0.67	0.88	1.03	1.06	-7.81	0.73	1.00	0.21	0.93	0.21	0.54
CAL1473/B112009	1.20	1.38	0.69	0.75	1.00	1.02	-28.13	0.86	0.77	0.25	1.00	0.25	0.48
CAL1427/PAC	0.99	1.03	0.66	0.73	0.94	0.98	0.00	0.82	0.86	0.38	0.96	0.36	0.58
CML465/PAC	1.06	0.92	0.64	0.76	0.98	1.01	0.00	0.70	0.64	0.11	0.74	0.15	0.82
VL13687/PAC	1.04	1.21	0.71	0.83	0.94	0.96	-23.44	0.85	0.88	0.30	0.96	0.35	0.61
VL145600/PAC	0.90	0.83	0.73	0.78	0.86	0.89	14.06	0.78	0.73	0.21	0.96	0.26	0.66
MAL/PAC	1.10	1.21	0.64	0.83	0.94	0.97	9.38	0.73	0.76	0.18	0.92	0.16	0.58
BISI18	1.04	1.04	0.91	0.74	0.93	0.96	-7.81	0.79	0.94	0.25	0.95	0.31	0.65
P36	1.24	1.06	0.67	0.78	1.03	1.05	-37.50	0.75	0.83	0.24	1.03	0.23	0.62

Note: PH: plant height, HC= height of cob, NGL: number of green leaves, Max temp: maximum temperature, MFD: male flowering days, FFD: female flowering days, ASI: anthesis silking interval, DC: diameter of cob, NGR: Number of grains per cob row, WHC: weight of harvested cob, Y: yield, P: productivity, W1000: weight of 1000 seeds.

The result of biplot analysis also showed that the maximum temperature of the leaves was the only character with a variance direction in contrast to the productivity group (Figure 1). The opposite group to the main group can indicate that this group having a significant negative correlation to the main group (Mattjik and Sumertajaya 2011). In general, the low maximum leaf temperature indicates that plants could adapt to the drought stress (Effendi et al. 2019; Kögler and Söffker 2019). This response is contrary to the plant productivity in the drought condition. Stress application, especially drought stress, will increase changes in the energy conversion pattern and plant metabolism, so that the leaf temperature will increase and cause senescent in the leaves (Kögler and Söffker 2019). It indicates that the adaptive genotype to the drought stress relative has a low maximum leaf temperature, so the character is negatively correlated with productivity. Therefore, the leaves' maximum temperature character could also be a secondary candidate in the selection of

adaptive maize genotypes under drought stress. Based on the interpretation of the biplot analysis, the characters of stem diameter, number of grains per cob row, number of green leaves, the weight of harvested cob, yield, and maximum temperature of leaves could be used for path analysis.

Based on the path analysis results, the weight of harvested cob was the character with the most significant direct effect on the productivity (Table 3). The direct effect is an indicator showing the direct variance magnitude of a character influencing the main character variance (Singh and Chaudhary 2007; Manjunatha et al. 2017). It means that the the weight of harvested cob is suitable as the best secondary characters; although, the number of green leaves as the character outside of the yield component also showed a good direct effect. However, this effect value was too lower than the weight of harvested cob. Therefore, the number of green leaves was not included as the best secondary character category.

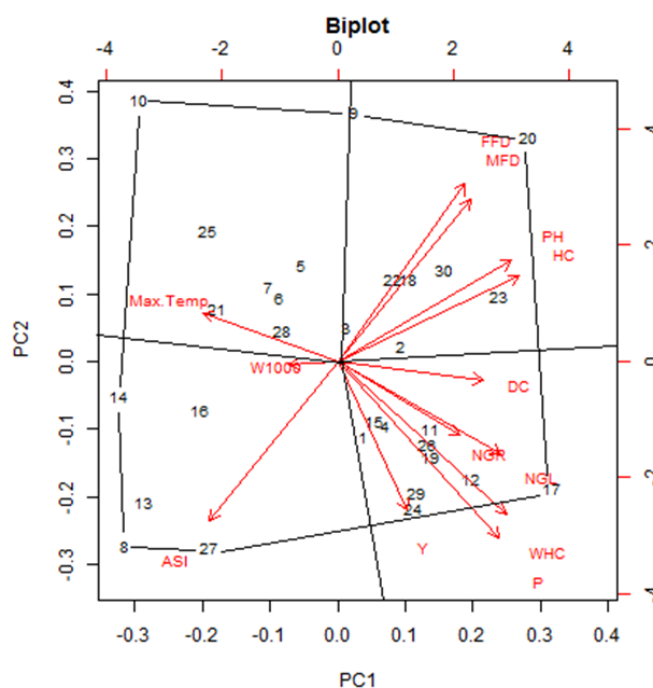


Figure 1. Biplot analysis based on the principal components of some maize characters under drought stress conditions. PH: plant height, HC= height of cob, NGL: number of green leaves, Max temp: maximum temperature, Min temp: minimum temperature, MFD: male flowering days, FFD: female flowering days, ASI: anthesis silking interval, DC: diameter of cob, NGR: Number of grains per cob row, WHC: weight of harvested cob, Y: yield, P: productivity, W1000: weight of 1000 seeds.

Table 3. Path analysis of STI productivity in determining the best secondary character under drought stress

Characters	DE	NGL	DC	NGR	WHC	Y	MAX TEMP	Residual
NGL	0.17		0.00	0.03	0.41	0.01	0.05	0.097
DC	-0.01	0.04		0.02	0.21	0.03	0.03	0.097
NGR	0.09	0.05	0.00		0.23	0.07	0.00	0.097
WHC	0.69	0.10	0.00	0.03		0.07	0.03	0.097
Y	0.15	0.02	0.00	0.04	0.32		0.01	0.097
Max temp.	-0.09	-0.09	0.00	0.00	-0.27	-0.01		0.097

Note: R²: 68.84, DE: direct effect, GL: number of green leaves, DC: diameter of cob, NGR: Number of grains per cob row, WHC: weight of harvested cob, Y: yield, MAX TEMP: maximum temperature, Residual: residual

In summary, multivariate analysis is a useful analysis for the determination of the best secondary characters in selecting the adaptive hybrid corn genotypes under drought stress. The weight of harvested cob is an effective secondary character as a selection criterion along with productivity in selecting adaptive maize genotypes under drought stress. The number of green leaves was the character outside of the yield component could be as an alternative secondary character besides the weight of harvested cob. The selection results based on the weight of the harvested cob and productivity resulted in 12 hybrid corn lines (ZL132133/MAL03, CIL1283/MAL03, CAL1471/MAL03, CAL1473/B112009, VL1016518/MAL03, VL1016493/MAL, CIL1283/B112009, VL13687/PAC, CAL1473/MAL03, VL109288/B112009, CAL1427/PAC, and CML161/B112009 as lines and Bisi) and 1 commercial varieties, namely Bisi 18, considered adaptive to drought stress.

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