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Research Article

Genetic variability and stability analysis of chili in three environments

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ABSTRACT

Chili is an important vegetable crop consumed by most Indonesian people. Chili production is affected by the limited varieties and low adaptability to growing in different environments. This study aimed to identify the variability of 22 chili pepper on morphology, the genotype x environment interaction, and the stability of 10 chili pepper genotypes in 3 different environments. The study was conducted from July 2020 to February 2022 in Sleman DIY, Bogor, and Blitar. The experimental design used a single-factor randomized complete block design, each consisting of three replicates. The genotypes evaluated were HCR 17-003, HCR 17-004, HCR 17-007, HCR 17-008, HCR17-012, HCR 17-013, HCR 17-014, HCR 17-017, F7-1, F7-2, F7-3, Ca011, Ca013, Ca020, Ca021, Cf002, Cf005, Cf007, Cf010, Cf015, Bonita, and Loblita. Ten genotypes were evaluated in stability analysis, i.e., PKHT A, PKHT B, Bara, Genie, Centil, PKHT C, PKHT D, Bonita, Sona, and Tunduk. The biplot analysis for genetic diversity study showed a total diversity was 40.5% for the two main components. The genotype x environment interaction had a significant effect on productivity. PKHT C and Sona were identified as stable based on the Francis-Kannenberg method. PKHT C, Sona, PKHT B, and Bonita were stable based on the Wricke method. PKHT A and Bonita were stable based on Finlay-Wilkilson methods. PKHT B, PKHT C, Sona, and Bonita were identified as stable based on the AMMI method. The genotype which is stable based on all stability methods was PKHT C.

Keywords: AMMI; GxE interaction; parametric stability; variety.

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INTRODUCTION

Chili pepper is a horticultural crop which is widely consumed by the community. This vegetable has an important economic significance in Indonesia that need to be increased. Chili pepper is a common element in cooking, it is used as the primary ingredient in sauces, chili powder, instant noodles, and pharmaceutical products (Saraswati et al., 2012). Prices have risen due to the increasing demand for chili peppers, which needs to be fulfilled by adequate supply. On the other hand, after a certain period, when abundant production surpasses market demand, the price of chili peppers at the farmer level falls significantly. According to the BPS (2022), Indonesia's production of chili peppers decreased by 8.09% from 2020's 1.5 million tons to 1.39 million tons in 2021. Efforts to improve the productivity of chili peppers can be made by obtaining a superior, high-yielding variety.

High-yielding varieties can be obtained from genotypes that have good performance, high yield, and are classified as stable.

Plant breeding programs, including genetic engineering through the combination of high-yielding varieties that are adaptable to various environments, can be used to control low productivity. A high-yielding variety can be identified by its appearance, agronomic characteristics, and component units. A consistent and widely adapted genotype has the best stable yield ability and relative performance at each location (Trustinah and Iswanto, 2013). A genotype's stability is its ability to survive and maintain its potential outcomes through several environmental conditions. Stability analysis can characterize variability and identify the most suitable genotypes for various environments.

Parametric analysis is one of the methods in stability analysis. The data should be spread based on the normal distribution for the tests performed (Alberts MJA, 2004). Based on the observable characteristics, stability is divided into two categories: static and dynamic. Static stability is performed when a genotype's productivity is constant in all settings. In contrast, a genotype is generally stable in dynamic statistics if it does not deviate significantly from the average response to the test environment.

The stability can be tested by interacting with the genotype and its environment (Katsenios et al., 2021). GxE interactions can be used to measure the stability of a genotype (Lin et al., 1986), because the appearance of the genotype in an environmental range depends on the magnitude of the GxE interaction. When a GxE interaction occurs, the genotype being tested has a different yield ability at each selected location. This shows that a genotype's highest yield in one environment does not necessarily equate to the best outcome in another.

The parametric approach uses a variety of methods, including Wricke ecovalence (1962), Francis-Kannenberg (1978), and Finlay-Wilkinson (1963). AMMI (Additive Main Effect Multiplicative Interaction) is a common environmental x genotype analysis method in stability analysis (Ahmed et al., 2020). Through principal component analysis, AMMI is used to clarify how genotypes and environments interact. By providing a broad overview of the genotype's pattern of environmental reaction, AMMI can show that the genotype under test is stable. To provide the best possible representation of production, it can be used to choose genotypes suited for the environment (Kusumah, 2010). This study aimed to identify the variability of 22 chili pepper on morphology, the genotype x environment interaction, and the stability of 10 chili pepper genotypes in 3 different environments.

MATERIALS AND METHODS

This study consisted of two sets of experiments. The first experiment was conducted at the field station of the Center of Tropical Horticulture Studies Tajur, Bogor, from July to December 2020. The experimental design used a single-factor randomized complete block design. The genetic material used a collection of chili peppers from The Center of Tropical Horticulture Studies, IPB University. There were 22 genotypes evaluated (HCR 17-003, HCR 17-004, HCR 17-007, HCR 17-008, HCR17-012, HCR 17-013, HCR 17-014, HCR 17-017, F7-1, F7-2, F7-3, Ca011, Ca013, Ca020, Ca021, Cf002, Cf005, Cf007, Cf010, Cf015, Bonita, and Loblita), each consisting of three replicates.

The second experiment was evaluated in 3 environments: Sleman District (Yogyakarta) 358 m above sea level (m asl), Bogor District (West Java) 250 m asl, and Blitar District (East Java) 346 m asl, from August 2021 to February 2022. The genetic material used 10 genotypes: 5 genotypes of green chili pepper (*Capsicum annuum*), i.e., PKHT A, PKHT B, Bara, Genie, Centil, and 5 genotypes of white chili (*Capsicum frutescens*), i.e., PKHT C, PKHT D, Bonita, Sona, and Tunduk. The experimental design used a single-factor randomized complete block design, each consisting of three replicates.

The cultivation followed a common cultivation method for chili plants. Chili seeds were sowed in trays for 7 weeks. The area of the beds for one experimental unit was 1 x 10 m^2 with a row spacing of 50 x 50 cm. A dose of limestone was applied (1.5 tons ha^{-1}) and base fertilizers (Urea 150 kg ha^{-1}, SP-18 300 kg $ha^{-1,}$ and KCl 200 kg ha^{-1}) before covering the planting bed with silver-black plastic mulch. Regular fertilizer application was done starting from 2 weeks after planting (WAP) on a weekly basis using NPK 16-16-16 with a 10 g L^{-1} of 250 mL for one plant. Watering, weed control, replanting, and pest diseases were applied according to field conditions.

The first experiment data analysis consisted of principal component analysis and characterization of qualitative characters. PCA was performed with R 4.0.5. Characterization was performed on qualitative characters based on the guidelines chili descriptors by International Plant Genetic Resources Institute (IPGRI, 1995). Quantitative variables included growth habit, nodule color, stem shape, stem color, leaf shape, leaf color, flower stalk attachment, flower color, general shape of fruit, young fruit skin color, harvest maturity fruit skin color, fruit apex, and fruit skin texture.

Data analysis for the second experiment consisted of analysis of variance, followed by parametric stability analysis. The parametric approach was carried out using the methods of Wricke's ecovalence (1962), Finlay-Wilkinson (1963), and Francis-Kannenberg (1978). Pearson correlation tests were performed on each stability parameter to see the relationship's closeness. Meanwhile, the multivariate analysis used is the additive main effect and multiplicative interaction (AMMI) method. ANOVA and parametric stability analysis was carried out using PKBT-STAT and PBSTAT-GE (www.pbstat.com).

RESULTS AND DISCUSSION

Qualitative characterization of 22 chili pepper genotypes

The results from observations of qualitative morphology of chili pepper are shown in Table 1. There were 2 types of growth habits, namely erect and compact. HCR 17-004 and HCR 17-008 had a compact growth type, while the other genotypes had an erect growth type. A cylindrical stem shape was prevalent among genotypes. Most leaves had ovate-shaped, HCR 17-013 and Bonita had the shape of deltoid leaves, F7-2 and F7-3 had the shape of lanceolate leaves.

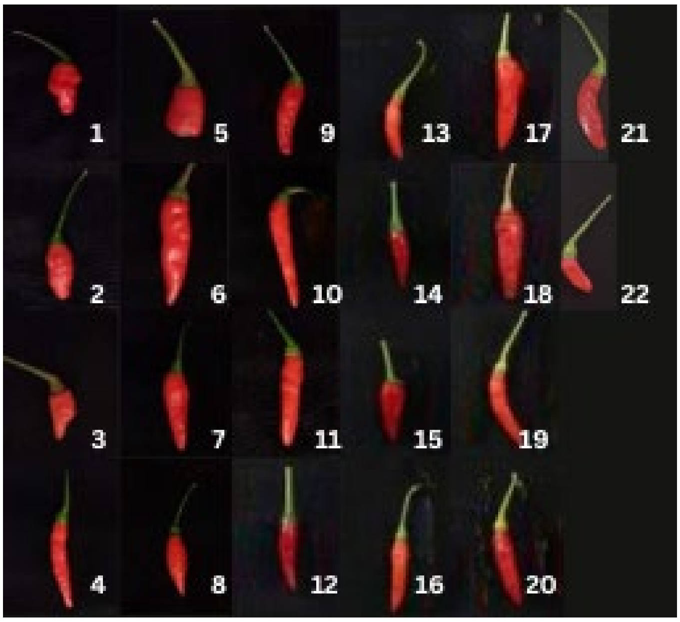

Figure 1. General shape of 22 genotypes' fruit (1= HCR 17-003; 2= HCR 17-004; 3= HCR 17-007; 4= HCR 17-008; 5= HCR17-012; 6= HCR 17-013; 7= HCR 17-014; 8= HCR 17-017; 9= F7-1; 10= F7-2; 11= F7-3; 12= Ca011; 13= Ca013; 14= Ca020; 15= Ca021; 16= Cf002; 17= Cf005; 18= Cf007; 19= Cf010; 20= Cf015; 21= Bonita; 22= Loblita).

General shape of fruit's character mostly belongs to the triangular shape (Fig 1). HCR 17-003, HCR 17-004, and HCR 17-012 had the shape of campanulate. HCR 17-008, HCR 17-013, HCR 17-014, HCR 17-017, F7-1, and F7-2 had the shape of elongate. Four types represent the characteristics of fruit apex. Most genotypes were pointy-shaped. HCR 17-003 and HCR 17-004 were sunken-shaped. HCR17-012, HCR 17-013, and F7-1 were blunt-shaped. HCR 17-014, HCR 17-017, F7-2, F7-3, Bonita, and Loblita had pointed shapes. Most fruit skin texture had a smooth texture, except HCR 17-003, HCR 17-004, Cf002, Cf010, and Loblita had wrinkled textures HCR17-012, HCR 17-017, and Ca021 had semi-wrinkled textures.

Table 1. Qualitative characters on 22 chili pepper genotypes.

Character	Type	Genotype number *)
Growth habit	Erect	1; 3; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 6; 17; 18; 19; 20; 21; 22
	Compact	2; 4
Stem shape	Cylindrical	1; 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22
Leaf shape	Ovate	1; 2; 3; 4; 5; 7; 8; 9; 12; 13; 14; 15; 16; 17; 18; 19; 20; 22
	Deltoid	6; 21
	Lanceolate	10; 11
General shape of fruit	Campanulate	1; 2; 5
	Triangular	3; 12; 13; 14; 15; 16; 17; 18; 19; 20; 21; 22
	Elongate	4; 6; 7; 8; 9; 10; 11
Fruit apex	Sunken	1; 2
	Pointy	3; 4; 12; 13; 14; 15; 16; 17; 18; 19; 20
	Blunt	5; 6; 9
	Pointed	7; 8; 10; 11; 21; 22
Fruit skin texture	Wrinkled	1; 2; 16; 19; 22
	Semi-wrinkled	5; 8; 15
	Smooth	3; 4; 6; 7; 9; 10; 11; 12; 13; 14; 17; 18; 20; 21

Note: *) see Figure 1 for genotype name.

A double-dimensional descriptive statistical technique known as biplot analysis, which can show a group of observation objects and changes in a graph on a two-dimensional frame simultaneously, can be used to analyze a characteristic of the variable and the object of observation as well as the relative position between the object of observation and the variable (Diyarti, 2003). The biplot analysis of 22 chili genotypes showed a total diversity of 40.5% for the two main components. To classify groups of genotypes, biplot analysis can categorize them according to specific criteria.

In quadrant I of Figure 2, all 10 genotypes showed strong positive correlation characteristics in stem shape (SS), stem color (SC), and young fruit skin color (YFSC). The genotypes in quadrant II, namely HCR 17-013, HCR 17-014, HCR 17-017, and F7-3, had a positive correlation in harvest maturity skin color (HMFSC), fruit skin texture (FST), general shape of fruit (GSF), leaf shape (LS), and fruit apex (FA). HCR 17-012, F7-2, Bonita, and Loblita in quadrant III were positively correlated with flower color (FC). HCR 17-003, HCR 17-004, and HCR 17-008 also showed a positive correlation with growth habit (GH) and leaf color (LC) in quadrant IV. These results indicate that the genotypes are categorized according to specific morphological characteristics seen through the vector position of objects in a quadrant. According to Hetherie (2019), accessions in the same quadrant indicate close relation, while those in a different quadrant with a 90° degree angle show distant relation.

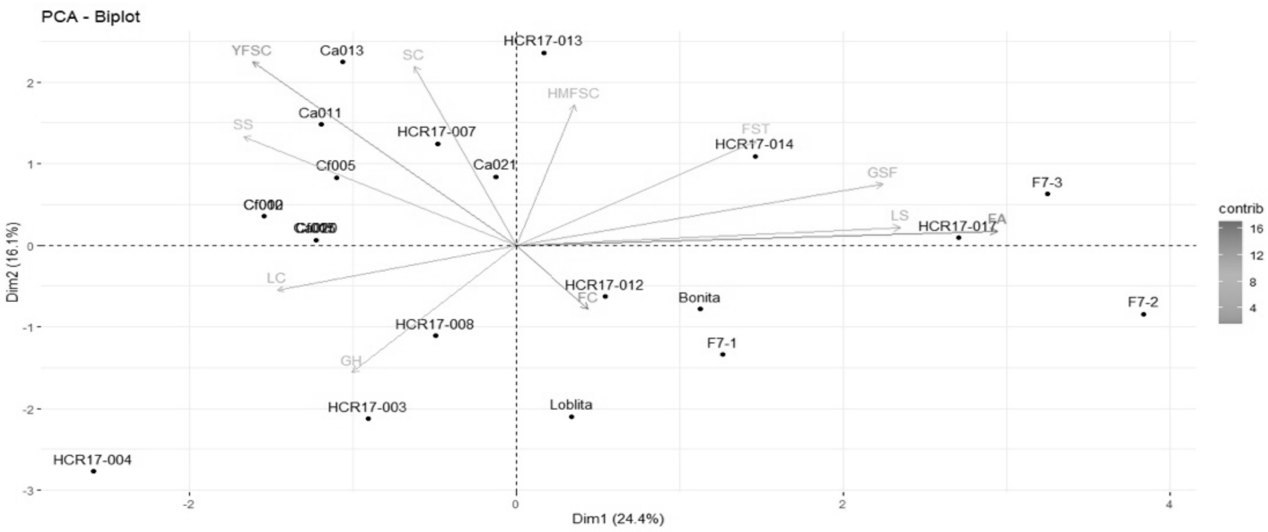

Figure 2. GT-biplot analysis on 22 chili genotypes (SS = stem shape, SC= stem color, YFSC = young fruit skin color, HMFSC = harvest maturity skin color, FST = fruit skin texture, GSF = general shape of fruit, LS = leaf shape, FA = fruit apex, FC = flower color, GH = growth habit, and LC = leaf color).

Yield performance

Three test locations were conducted in this study, each with a different climatic condition (Table 2). The climate situation was suitable for chili plants, especially in a temperature range of 24 - 30 °C (Juharni et al., 2020).

Table 2. Daily temperature, relative humidity, and monthly rainfall in three environments.

Environment	Climate		
	Temperature (°C)	Air relative humidity (%)	Monthly rainfall (mm)
Sleman	26.08 - 26.65	78.80 - 82.40	145-70 - 409.90
Bogor	25.78 - 26.40	83.70 - 85.84	150.30 - 566.50
Blitar	26.28 - 26.65	85.65 - 88.10	124.40 - 371.40

Yield performance is one of the important indicators for identifying the genotype as high-yielding. The results of green chili showed that the environment (E), genotype (G), and G x E significantly affected productivity (Table 3). The results of white chili showed that the effect of E was not significant, while the G and the E x G interaction were significant for productivity (Table 3). The significant interaction between G x E was affected by the occurrence of genotype responses to various environmental conditions at all three locations. Effect of season and geographic region on yield characteristics have been known in chili (Raghavendra et al., 2017), sweet potatoes (Andrade et al., 2016; Karuniawan and Maulana, 2020), mung bean (Anggia et al., 2020), chickpea (Erdemci, 2018), rice (Hastini et al., 2022), and corn (Syafii and Ruswandi, 2019). Sivakumar et al. (2017) stated that the significant interaction of G x E between all characters indicates that the genotypes responded differently to various environments for those characters. Several G x E interactions showed that each variety adapts differently (Thanki et al., 2010).

In chili pepper, productivity was significantly affected by G and E factors. The average productivity of chili peppers varies widely depending on the environment, from 2.61- 9.21 ton ha^{-1} (Table 4). In green chili pepper, the highest productivity was achieved by PKHT A, which was 9.21 ton ha^{-1}; PKHT B had the lowest productivity (4.36 ton ha^{-1}). Bogor reached the largest productivity of 9.59 ton ha^{-1}, followed by Sleman at 8.45 ton ha^{-1} and Blitar at 4.56 ton ha^{-1}. In white chili pepper, the highest productivity was achieved by Bonita at 7.78 ton ha^{-1} followed by PKHT C, PKHT D, Sona, and Tunduk, i.e., 7.68, 7.23, 7.04, and 2.61 ton ha^{-1}, respectively. Chili grown in Sleman District reached the largest productivity of 7 tons ha^{-1}, followed by in Bogor District 6 tons ha^{-1}. Chili grown in Blitar District exhibited low productivity for green and white chili pepper 4.56 and 6.41 tons ha^{-1}, respectively. Productivity variations can be affected by varieties, Suparwoto (2021) reported that the production of a variety is a result of its ability to adapt to environment.

Table 3. Analysis of variance for the productivity of 10 chili pepper genotypes in three environments.

Source of variation	Green chili (*Capsicum annuum*)	White chili (*Capsicum frutescens*)
Environment (E)	*	ns
Replication/E	*	ns
Genotype (G)	**	**
G x E	**	*
Coefficient of variation (%)	28.07	29.11

E = environment, G = genotype, * = significant at 5% level, ** = significant at 1% level, ns = not significant.

Table 4. Productivity of 10 chili pepper genotypes in three environments.

Genotype	Productivity (ton ha^{-1})			
	Sleman	Bogor	Blitar	Mean
Green chili (*Capsicum annuum*)				
PKHT A	7.82abc	12.56a	7.26a	9.21a
PKHT B	5.16c	4.15b	3.76a	4.36b
Bara	12.01a	9.03a	3.93a	8.32a
Genie	7.18bc	11.67a	4.20a	7.68a
Centil	10.08ab	10.52a	3.63a	8.08a
Mean	8.45A	9.59A	4.56B	
White chili (*Capsicum frutescens*)				
PKHT C	8.24a	7.39a	7.41a	7.68a
PKHT D	9.07a	4.45ab	8.17a	7.23a
Bonita	9.98a	7.67a	5.70a	7.78a
Sona	6.38a	7.97a	6.77a	7.04a
Tunduk	1.35b	2.51b	3.98a	2.61b
Mean	7.00	6.00	6.41	

Note: Numbers followed by the same lowercase letter in the same column and numbers followed by the same capital letter in the same rows are not significantly different based on Tukey test 5%.

Stability analysis of 10 chili pepper genotypes in three environments

Genetic stability in breeding programs is important. Genotype stability analysis is conducted when there is G x E interaction (Sitaresmi et al., 2016; Gupta et al., 2022) to assess whether the genotype tested is stable across all locations or specifically adapted to a certain location (Yuliasti, 2016). Parametric analysis is one of the methods for analyzing genetic stability after the statistical assumptions are fulfilled (Syukur et al., 2015).

Wricke's method (1962) used the ecovalance value (W^2i) as the stability parameter. The contribution of each genotype to the total interaction of all genotypes with the environment can be measured by ecovalence. Because their production changes less in all environments, genotypes with small ecovalence are considered stable genotypes (Fikere et al., 2014). Based on stability analysis PKHT C, Sona, PKHT B, and Bonita were the most stable genotypes (Table 5).

Table 5. Stability analysis of 10 chili pepper genotypes in three environments.

Genotype	*Yi*	*CVi*	*bi*	*P_bi*	W^2i	σ^2i
PKHT A	9.21	31.63	1.33	0.66	11.21	18.90
PKHT B	4.36	16.55	0.38	0.41	1.86	1.37
Bara	8.32	49.10	2.86	0.02	17.03	29.82
Genie	7.68	48.96	2.34	0.09	15.59	27.11
Centil	8.08	47.75	2.93	0.02	12.94	22.14
PKHT C	7.68	6.27	0.17	0.27	2.76	3.07
PKHT D	7.23	33.85	-0.66	0.04	20.05	35.48
Bonita	7.78	27.55	1.35	0.64	3.30	4.08
Sona	7.04	11.80	0.19	0.29	3.51	4.47
Tunduk	2.61	50.48	-0.89	0.02	13.11	22.46

Note: Yi= mean response, *CVi*= Francis and Kannenberg's coefficient of variation, W^2i= Wricke's ecovalence, *bi* = regression coefficient of response vs environment index, σ^2i = Shukla's stability variance.

According to the Francis-Kannenberg method (1978), a stable genotype is a genotype that has a low coefficient of variation. Genotypes of PKHT C and Sona were relatively stable because they had the lowest *CVi* compared to other genotypes (Table 5). According to Solieman et al. (2012), stability test is a static method and only evaluates each individual's response to their environment without directly comparing it to the genotype.

Finlay-Wilkinson's method (1963) measures stability parameters based on the regression coefficient (bi) with (bi) value = 1 representing the average stability, bi>1.0 stability below average, and bi<1.0 stability above average or adaptive. PKHT A and Bonita were the most stable genotypes (Table 5). When the environmental index test follows a genotype's average performance, the genotype is categorized as having dynamic stability. Genotypes that were below-average stability (bi>1.0) were Bara and Centil, while genotypes that were above-average stability (bi<1.0) were PKHT D and Tunduk. This average stability might only be applicable to the specific genotypes evaluated. Such limitation is due to the environmental index in the present study was calculated using the average of all tested genotypes. According to Juharni et al. (2020), stability characteristics may change by changing the composition of evaluated genotypes.

The three stability analyses using Wricke's method (1962), Francis-Kannenberg's method (1978), and Finlay-Wilkinson's method (1963) resulted in different categories. Nevertheless, due to the research only using one measurement of stability on the tested genotypes, further evaluation using multiple statistical models in combination might become less informative (Khalili & Pour-Aboughadareh, 2016). Therefore, combining multiple stability measurements to select stable and high-yield genotypes for testing multiple locations is recommended (Karuniawan & Maulana, 2020). Furthermore, it offers breeders more flexibility in selecting their genotypes and determining which ones have the most significant ability. The identification of stable and high-yielding genotypes has been successfully applied to sweet corn (Ruswandi et al., 2020), peanut (Ajay et al., 2020), durum wheat (Sabaghnia et al., 2013; Abate et al., 2015; Ruswandi et al., 2020), and grass pea (Ahmadi et al., 2015).

Based on the results of Spearman's correlation analysis, it can be seen that the CVi parameter was significantly correlated with bi, W^2i, and σ^2 (Table 6). The parameter s^2di significantly correlated to Di, while W^2i correlated significantly to σ^2. This indicates that the calculations for W^2i in the Wricke method and σ^2 in the Shukla method were equivalent. A significant correlation between stability parameters indicates that each parameter measures the same aspect of stability and enables using one of those parameters (Kusumah, 2010).

Table 6. Spearman correlations among parametric stability and productivity of 10 chili pepper genotypes in three environments.

	Yi	*CVi*	*bi*	s^2di	W^2i	*Di*
CVi	-0.14					
bi	0.13	0.66*				
s^2di	-0.36	0.25	-0.35			
W^2i	-0.20	0.78**	0.61	0.53		
Di	-0.36	0.25	-0.35	1.00**	0.53	
σ^2	-0.20	0.78**	0.61	0.53	1.00**	0.53

Yi= mean response, *CVi*= Francis and Kannenberg's coefficient of variation, *bi* = regression coefficient of response vs environment index, s2d = deviation from regression (Eberhart and Russel), *W2i*= Wricke's ecovalence, Di = Hanson's genotypic stability parameter, $\sigma^2 i$ = Shukla's stability variance, *= significant at 5% level, **= significant at 1% level.

Finally, stability concepts can be classified using principal component analysis (Vaezi et al., 2018). AMMI analysis can be used when there is a significant interaction between the environment and the genotype (Duma et al., 2019). AMMI biplot is a visualization of AMMI analysis used to show a stable genotype at all locations or a specific genotype at a specific location (Sadeghzadeh et al., 2018). The confidence region of the ellipse with a center point of (0.0) and the first two most significant interaction principal component (PCA) values can be utilized to interpret the AMMI2 biplot to represent the genotype's stability across all experimental locations (Suryani et al., 2022).

The contribution variability of interactions that can be explained by the main component of the interaction, which is 100% with each PC1 and PC2 were 62.3% and 37.7%. The two components dominate in explaining the diversity of interaction influences. Stable genotypes are presented by the AMMI2 biplot, which is the genotype inside the circle (ellipse). In Figure 3, the biplot shows that PKHT B, PKHT C, Sona, and Bonita were stable genotypes in all three locations and had extensive adaptations. The level of genotype stability increases with genotype proximity to the central point (Zhang et al., 2016; Mustamu et al., 2018). Therefore, it can be considered that the genotype is stable in all sampling locations. A site-specific genotype is located far from the center point yet close to the location line (Widyastuti et al., 2013). Finlay-Wilkilson, Eberhart-Russell, and AMMI methods are classified as dynamic stable or agronomic stable. Dynamic stability changes following the environmental index (Becker and Leon, 1988; Lin et al., 1986).

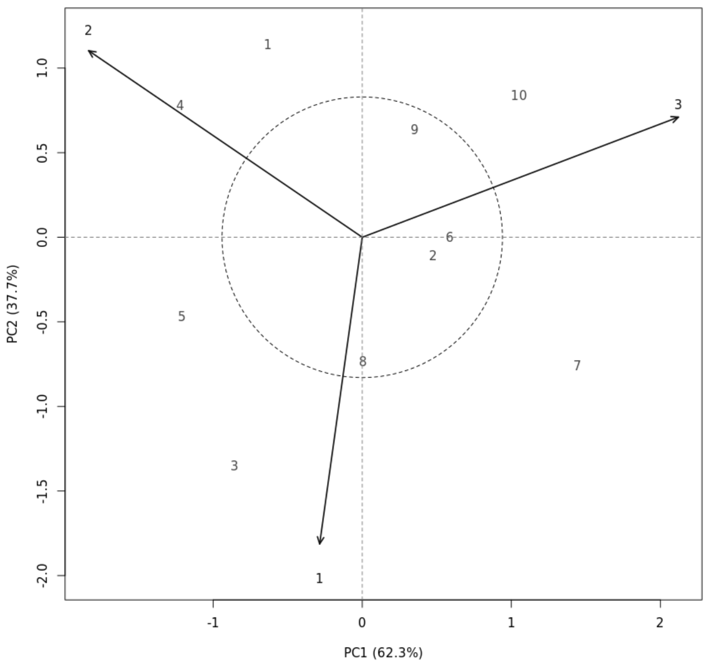

Figure 3. AMMI2 biplot interaction (PC1 and PC2) for productivity of 10 chili pepper genotypes in three environments (1 = PKHT A, 2 = PKHT B, 3 = Bara, 4 = Genie, 5 = Centil, 6 = PKHT C, 7 = PKHT D, 8 = Bonita, 9 = Sona, 10 = Tunduk).

CONCLUSIONS

The biplot analysis of 22 chili pepper genotypes showed a total diversity of 40.5% for the two main components. The genotype x environment interaction had a significant effect on productivity. PKHT C and Sona were identified as stable based on the Francis-Kannenberg method. PKHT C, Sona, PKHT B, and Bonita were stable based on the Wricke method. PKHT A and Bonita were stable based on Finlay-Wilkilson methods. PKHT B, PKHT C, Sona, and Bonita were identified as stable based on the AMMI method.

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