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APPLICATION OF COMBINED ANALYSIS WITH BIPLOT METHOD FOR EXPLAINING AGRONOMIC TRAITS OF THE UPLAND RICE FROM MINORITY FARMERS IN PRACHUAP KHIRI KHAN, THAILAND

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ABSTRACT

Genetic studies in upland rice in Thailand, both inside and outside the farming area, can provide the fundamental knowledge for management of the upland rice under the ongoing changes and pressing issues, such as low yield, land use policy in upland areas, and the impact of social issues. The present study exploys the combined analysis with biplot methods (modeling of main effect of genotype and the genotype-by-environment interaction; GGE) to obtain information on the response of upland rice genetics and characteristics under changing planting environments. In this study, seven native upland rice varieties were received from hill tribe farmers in Prachuap Khiri Khan Province, Thailand. These rice varieties were subsequently replanted in plots located in three different sites, in two different provinces for three consecutive years in the rainy season. The results indicate that three traits viz, seed number panicle^{-1} (SNP), percent of seed filling (PSF), and plant height (PH) varied significantly under genetic x environment (GxE) analysis. For seed yield per hill (SYH) in polygon form, three varieties (*Aung Jerng Yai*, *Khao Niaw Pala-U* and *Beu Ge*) were higher most adaptive genotypes in each environment (Environments 1, 2, and 3, respectively). Among these three varieties, *Khao Niaw Pala-U* exhibited the best mean and stability for yielding as determined by biplot method.

Key words: *Oryza sativa* (L.), indigenous varieties, upland rice, combine analysis, GxE interaction

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INTRODUCTION

Rice (*Oryza sativa* L.) is an important staple crop; cultivated in 95 countries around the world (Gadal *et al.*, 2019; Kakar *et al.*, 2019), and is considered a reserved food for farmers' households as well. More than 100 million households across Asia, Africa, and Latin America rely on its cultivation not only for nutrition, but for employment and livelihood (Nguyen, 2002; Makino, 2011). Rice cultivation may be classified mainly into two basic types: lowland and upland rice. These types vary greatly in their genetic constitution and cultivation practices, including water supply and planting area requirements (Saito *et al.*, 2018). Upland rice has a lower yield and much smaller production area than lowland rice does. Upland rice accounts for only about eight percent of the total area for rice planting when evaluating from countries in many continents (IRRI, 1998). While, upland rice in Thailand accounts for approximately 11 percent; compared with total rice planting area (Saito *et al.*, 2018). Even so, upland rice is considered an important source of food security and poverty alleviation for farmers and communities in many countries (Saito *et al.*, 2018). In recent years, however, the planting area of upland rice in many continents has decreased (Guimarães *et al.*, 2016; Saito *et al.*, 2018). Reasons cited for the reduction include low yields (stemming from factors such as inappropriate genetic varieties and biotic or abiotic stress), increasing demand of lowland rice, and the policy to reduce of deforestation for agriculture, upon which upland rice cultivation often relies (Van Vliet *et al.*, 2012; Van Oort *et al.*, 2017).

In light of the foregoing, finding ways to improve both genetic conservation and productivity have become pressing. This includes more effective resource management and cultivation that reduces environmental impact. Genetic conservation or breeding under environments mirroring farmers' cultivation areas are likely to be the most promising approaches to such end.

It should be noted, however, that the extensive capitalist system that spans throughout Thailand nowadays has adversely impacted the lives of rural people, their agriculture, and their environment (Podhisita, 2017). Such commercialized developments have contributed to the loss of genetic variation within species, resulting in loss of indigenous rice varieties and possibility for genetic selection in the future. Urgent measures are now required to conserve the genetics along with either genetic study or genetic improvement, even though these have to be conducted outside the distribution or planting areas.

The purpose of this study is to assess the genetic (varieties) response on some agronomic characteristics more accurately–either in a wide range of environments (broad adaptation) or a single, specific environment–to improve rice productivity. Moreover, the genotype x environment interaction, the response of different phenotypes among genotypes when grown in different environments has been determined using combined analysis together with the explicit explanation by Biplot method. Combined analysis with Biplot method can efficiently help the assessment of the agronomic characteristics for planting and genetic selection.

MATERIALS AND METHODS

Seed materials, experimental area, and planting practice: Five indigenous rice varieties were selected: *Nah San, Beu Ge*, *Khao Niaw Pala-U*, *Beu Gaw Bi* and *Beu Soo Ser Lah*. Also, two non-native species were selected, which the local farmers had introduced: *Aung Jerng Yai* (from Petchaburi Province) and *Row Su Ya* (from Chiang Mai Province). These seven upland rice varieties were planted at the farmers' fields in 2014 at Pala-U village, Prachuap Khiri Khan Province, Thailand (Latitude 12° 30.642´N and Longitude 99° 29.839´E). The planting area is about 300 meters above sea level.

The said seven upland rice varieties were planted in rainy season (July to November) over three successive years, in three different planting areas: (1) Pala-U village, Prachuap Khiri Khan Province in 2015; (2) Nong Ya Plong, Phetchaburi Province in 2016 (12° 59´N and 99° 42´E and 80 meters above sea level); and (3) Cha-am, Phetchaburi Province in 2017 (12° 54´N and 99° 55´E and at 10 meters above sea level) (both provinces are located in Thailand's Southeast coast). In each case, 5-7 seeds were sown (per hill) into the soil at depths of between 2-3 cm with a spacing between row and hill of 50x30 cm. After two weeks, thinning was done to maintain two plants per hill. Weeds were controlled manually after seed emergence and once per month after that. Rainfall served as the sole source of irrigation in all fields.

Assessment characteristics: **Some agronomic traits, yield and yield components:** In all three studied years, at harvesting stage in each variety, many characteristics were collected including some agronomic traits, yield and yield components. All traits were evaluated by collecting the samples from 20 hills in the inner row of each plot: [1] seed yield hill^{-1} (SYH), [2] seed weight panicle^{-1} (SWP), [3] tiller number hill^{-1} (TNH), [4] seed number panicle^{-1} (SNP), [5] 100 seed weight (100-SW), [6] percent of seed filling (PSF), [7] panicle length-as measured from the panicle base to the end of the tip of the panicle (PL), and [8] plant height –as measured from soil base to the end of the highest leaf (PH).

Statistical analysis: A randomized complete block design (RCBD) was arranged for seven upland rice varieties with four replications for each year while. Bartlett's Chi-square test was used for homogeneity of variances of each trait across the experiment's three-year time span. After that, a combined analysis for treatments variance–varieties grown under three years (or three environments) –was performed. For each trait, a mixed model was used for combined analysis, whereby the different varieties and environments used fixed effect and random effect, respectively. This study used a biplot method to display multivariate data for GGE modeling; both the main effect of genotypes (G) and the genotype-by-environment interaction (GE). The present GGE, in two-way data were the main effect of genotype (G) and the (seven) genotypes interaction by the (three) environments (GE) in 2015-2017. In addition, a statistical software– R program version 3.5.1 (R Core Team, 2018) was employed.

RESULTS AND DISCUSSION

The variance of traits over the three years were tested for homogeneity (data not shown). After the homogeneity analysis, results showed no difference in the variances in all three years (or three environments); combined analysis was subsequently performed.

Results of the combined analysis for the eight traits are presented in Table 1. Four traits–SYH, 100-SW, PSF, and PL–exhibited statistically significant variances affected by the different planting environments. Environmental differences, in this study, refer to the differences in both the location and the planting year. As such, this makes it difficult to ascertain the specific factors responsible for the differences in yield and agronomic traits. Six traits–SWP, SNP, 100-SW, PSF, PL, and PH–exhibited significant variances affected by different varieties. Meanwhile, the interaction between genetic and environment (GxE) was significant in three traits: SNP, PSF, and PH. *Nah San*, which exhibited high values of SNP and PH when grown in 2015 and 2017, also exhibited high values of SYH in these years (data not shown). Similarly, *Beu Gaw Bi*, which exhibited high values of SNP and PSF when grown in 2016-2017, also exhibited high SYH in these years (data not shown). These observations suggest that three traits–SNP, PSF, and PH–were important and have a close effect on yield. Phenotypic variability in rice grain yield, however, is affected by many yield-contributing traits, and also by environmental conditions, referred to as 'quantitative traits' (Singh *et al.*, 2013). Using these traits as benchmarks may facilitate the selection of superior varieties for yield improvement. The GxE on these traits, however, requires the breeding process be done with caution. As such, traits may adversely affect the breeding process (Kang, 1990).

The mean of traits in different varieties and the results from the combined analysis that were evaluated on average across environments (three years) are presented in Table 2. Although there was no statistical difference among varieties on SYH, there were three varieties that tended to lead high-yield varieties, namely: *Aung Jerng Yai*, *Khao Niaw Pala-U*, and *Beu Ge*. Focusing on traits that were not significantly affected by GxE, higher yielding varieties showed superior results on traits related to panicle character including SWP, 100-SW, and PL (Tables 1-2). These results reflected relatively stable expression of these traits (SWP, 100-SW, and PL) in these varieties. It should also be noted that the SWP and PL traits were reported to have broad-sense heritability values with a wide range (low to high) in rice, (SWP; Hb ~ 0.17-0.74 and PL; Hb ~ 0.06-0.47) (Lestari *et al.*, 2015; Adhikari *et al.*, 2018), which these traits, although controlled by a relatively high genetic influence, still affected by environmental influences.

Table 1. Combined ANOVA among 7 upland rice varieties under 3 environments in Prachuap Khiri Khan and Phetchaburi provinces; The mix model were fixed effect (varieties) and random effect (environment).

Source	Df	Mixed model (F-test)	Mean squares							
			SYH (g)	SWP (g)	TNH	SNP	100-SW (g)	PSF (%)	PL (cm)	PH (cm)
Environment (E)	2	YMS/RMS	8.598**	4.03NS	40.47NS	6.01NS	0.52**	1.57**	60.94**	0.43NS
Rep w/n Envi.	9	RMS/EMS	0.820NS	1.26*	12.86NS	1.82NS	0.06NS	0.09NS	4.10NS	0.37**
Varieties (G)	6	GMS/EMS	1.392NS	1.23*	12.25NS	18.76**	1.16**	0.40**	29.60**	1.43**
GxE	12	GxE MS/ EMS	1.037NS	0.56NS	2.34NS	3.90**	0.10NS	0.25**	2.89NS	0.40**
Pool error	54	EMS	0.851	0.49	6.36	0.96	0.09	0.08	3.22	0.12

SYH = seed yield hill^{-1}, SWP = seed weight panicle^{-1}; TNH = tiller number hill^{-1}; SNP = seed number panicle^{-1}; 100-SW = 100 seed weight; PSF = percent of seed filling; PL = panicle length, PH = plant height, SYH, PH, SNP and PSF were transform by square root before analysis.
*, **, significant difference at 95 ($P \leq 0.05$) and 99 ($P \leq 0.01$) percent of confidence, respectively.
NS, non-significant difference at 95 percent of confidence ($P>0.05$).

Table 2. Means of traits from combined analysis of upland rice varieties grown in rainy seasons of 2015-2017 at Prachuap Khiri Khan and Phetchaburi provinces.

Varieties	SYH (g)	SWP (g)	TNH	SNP	100-SW (g)	PSF (%)	PL (cm)	PH (cm)
Nah San	22.65	3.78bc	8.3	221a	2.31d	77b	27.1bcd	132bc
Beu Ge	29.06	3.98abc	10.4	143c	3.32a	86a	29.0a	127c
Khao Niaw Pala-U	29.43	4.06ab	9.8	186b	2.63bcd	85a	28.5ab	135b
Row Su Ya	22.44	3.47c	8.7	140c	2.82b	82ab	24.4e	143a
Aung Jerng Yai	30.17	4.24ab	9.0	194ab	2.66bc	83a	26.6cd	119d
Beu Gaw Bi	22.22	4.44a	8.6	217a	2.50cd	79b	25.7de	139ab
Beu Soo Ser Lah	25.62	3.78bc	10.9	150c	2.67bc	85a	27.8abc	136ab
Mean	25.87	3.97	9.4	179.63	2.70	82	27.0	133
P-value	0.157	0.034	0.093	<0.001	<0.001	<0.001	<0.001	<0.001
F-test	1.635NS	2.49*	1.93NS	19.62**	12.29**	4.92**	9.20**	12.37**
CV (%)	18.54	17.72	26.95	7.36	11.35	3.14	6.63	2.95

NS, non-significant difference at 95 percent of confidence ($P>0.05$). *, **, significant difference at 95 ($P \leq 0.05$) and 99 ($P \leq 0.01$) percent of confidence, respectively. SYH = seed yield hill^{-1}, SWP = seed weight panicle^{-1}; TNH = tiller number hill^{-1}; SNP = seed number panicle^{-1}; 100-SW = 100 seed weight; PSF = percent of seed filling; PL = panicle length, PH = plant height, SYH, PH, SNP and PSF were transformed by square root before analysis.

A biplot method was used to display multivariate data for modeling GGE in the R program. The display in GGE presents, using two-way data, the main effect of genotype (G) and the (seven) genotypes interaction by (three) environments (GE) in 2015-2017. The GGE biplot is recommended for use by breeders for ranking of parameters of cultivar among environments; either by location or years (Frutos *et al*., 2013).

The polygon form of genotypes in several environments: Those traits showing a significant effect due to GxE interaction (Table 1) were plotted in a polygon form (genotypes markers connection) to determine the most adaptive genotype in each environment (Figure 1). Grain yield is a complex trait because of its quantitative nature. Although the statistical analysis showed that SYH was not significantly different, either on testing in each year of its had effected by varieties or GxE, it was also included in the polygon study. For SYH (Figure 1, upper left), six perpendicular lines divided the biplot. Three best genotypes located at the respective vertex for three sectors responded to each environment (separated by the perpendicular lines) (Yan and Tinker, 2006). The top three varieties in each of three environments were analyzed including *Aung Jerng Yai* (V5), *Khao Niaw Pala-U* (V3), and *Beu Ge* (V2) in Environments 1, 2, and 3, respectively. For SNP, *Nah San* (V1) showed itself to be the best adaptive genotype by forming the highest values in Environments 1 and 3 (Figure 1, upper right). Meanwhile, *Beu Gaw Bi* (V6) was the best adaptive genotype on SNP in Environment 2 (Figure 1, upper right). For PSF, the best adaptive performances in Environment 1 (Figure 1, lower left) was achieved by *Beu Ge* (V2). Meanwhile, *Khao Niaw Pala-U* (V3) was the best adaptive genotype showed high values on PSF in Environments 2 and 3 (Figure 1, lower left). Although plant height is not considered a yield component, it was reported as a primary trait along with the yield trait) for selection. This is because plant height affects other characteristics that directly affect the productivity, such as effect on stem lodging resistance in rice (Kashiwagi *et al*., 2008). For PH, the best adaptive genotype showing high value in Environment 1 was *Beu Gaw Bi* (V6) (Figure 1, lower right). Meanwhile, *Row Su Ya* (V4) showed as the best adaptive genotype in Environments 2 and 3 (Figure 1, lower right).

Mean and stability: The mean and stability of varieties were derived from the mean against stability [from the single-arrow line which showed the average-environment coordination (AEC) view]. The varieties with the highest mean and stability on traits: SYH, SWP, SNP, 100-SW, and PSF, across environments, were presented in Figure 2. These traits, except on SYH, had significant difference affected by varieties (Tables 1-2).

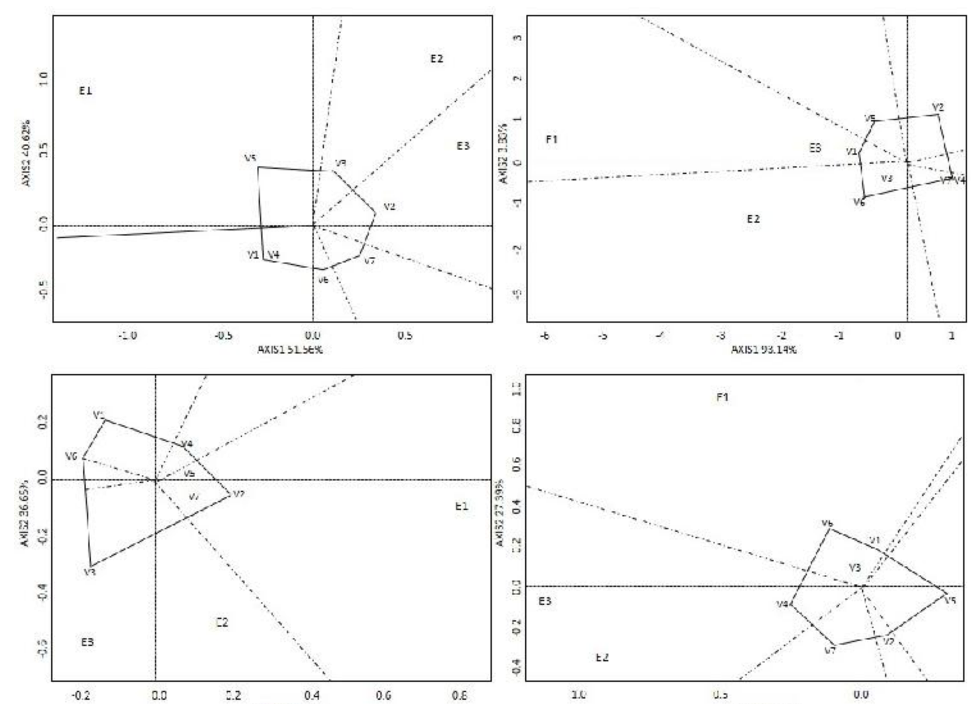

Figure 1. **The polygon form of upland rice genotypes in three environments**: **seed yield hill^{-1} (SYH) (upper left); seed number panicle^{-1} (SNP) (upper right); percentage of seed filling (PSF) (lower left); and plant height**

(PH) (lower right). SYH, SNP, PSF, and PH were transformed by square root before plotting. V1-V7: varieties; E1-E3: environments

For SYH, *Khao Niaw Pala-U* (V3) had the highest mean yield, followed by *Aung Jerng Yai* (V5) and *Beu Ge* (V2), respectively (Figure 2, upper left). Other varieties had lower means than average yield across environments and are plotted below the perpendicular line to the AEC abscissa. The order of SYH in varieties resulting from biplot is slightly different from that resulting from combined analysis, where the highest mean yields were *Aung Jerng Yai* (V5) and *Khao Niaw Pala-U* (V3), respectively. The stability of any varieties on characteristics was measured on the length of line plotted from the plot origin in either direction, which is perpendicular from AEC abscissa (Frutos *et al.*, 2013). For SYH, *Beu Gaw Bi* (V6) and *Khao Niaw Pala-U* (V3) had less variabilities (better stability), which was shown by the shorter length of the line from the plot origin than other varieties. In the opposite direction, *Aung Jerng Yai* (V5), *Beu Ge* (V2), *Row Su Ya* (V4), *Beu Soo Ser Lah* (V7), and *Nah San* (V1) had greater variability or highly unstable on SYH. High yield and high stability are desirable targets in breeding for wide adaptation, as they help achieve consistently good yields across a wide range of environments. In contrast, the interaction between the genotype x environment or the unstable genotypes generally contributes to sub-optimal results (Oladosu *et al.*, 2017). However, for yield component traits such as SWP, SNP, 100-SW and PSF, there was a sequence of varieties that were high mean or high stability varying in various traits studied (Figure 2). Of note, *Khao Niaw Pala-U* (V3) showed a higher mean on many traits, but with higher variability on these yield components (except on PL) compared with other varieties. Those traits showing the most stable results were: SNP, 100-SW, and PL. For consideration of both biplot and no significance on GxE interaction by combining analysis (Table 1), the traits performed high stability in varieties were on 100-SW and PL. Whether or not these stable traits on varieties are suitable as criteria traits for selection, it should be determined based on yield performance.

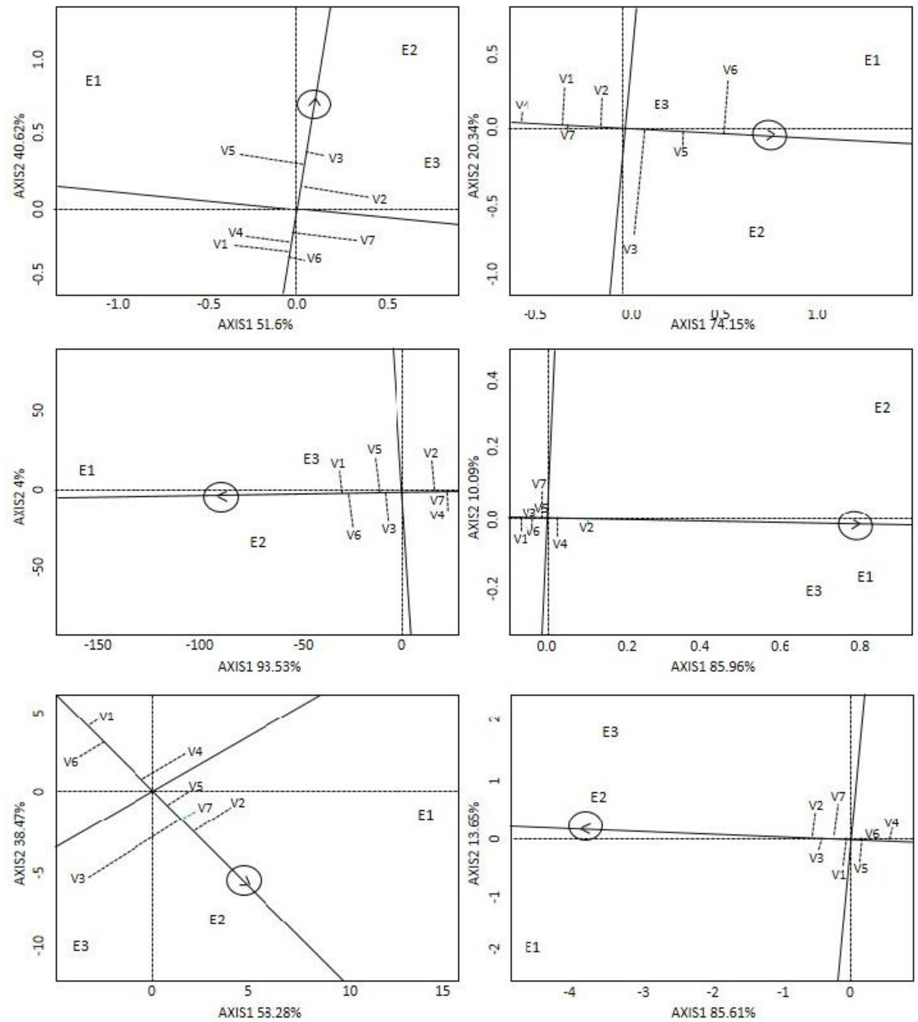

Figure 2. Mean and stability of upland rice genotypes in three environments: seed yield hill^{-1} (SYH) (upper left); seed weight panicle^{-1} (SWP) (upper right); seed number panicle^{-1} (SNP) (middle left); 100-seed weight (100-SW) (middle right); percentage of seed filling (PSF) (lower left); and plant length (PL) (lower right). SYH, SNP and PSF were transformed by square root before plot. V1-V7: varieties; E1-E3: environments.

Ranking genotypes from a wide range of environments (ideal genotype): The plotting of the various genotypes under ideal cultivation conditions was showed in Figure 3. At point zero of the average-environment coordination (AEC), the projection on the AEC was zero, which means the genotype is absolutely stable (Frutos *et al*., 2013). Thus, for SYH of different upland rice varieties, *Khao Niaw Pala-U* (V3) achieved better stability than *Aung Jerng Yai* (V5) and *Beu Ge* (V2) did. *Khao Niaw Pala-U* (V3), *Aung Jerng Yai* (V5), and *Beu Ge* (V2) achieved better stability than other varieties, while *Beu Gaw Bi* (V6), *Row Su Ya* (V4), *Beu Soo Ser Lah* (V7), and *Nah San* (V1) were categorized in the poorest genotypes based on stability performance. The distant point of Environment 1 showed that the SYH is different from that of Environments 2 and 3 (Figure 3). Even so, the result of the combined analysis showed that the SYH did not show any interaction between varieties and environments (Table 1). The GGE biplot showed different yield stability in different varieties (Figure 3), which were also indicative of GxE interaction.

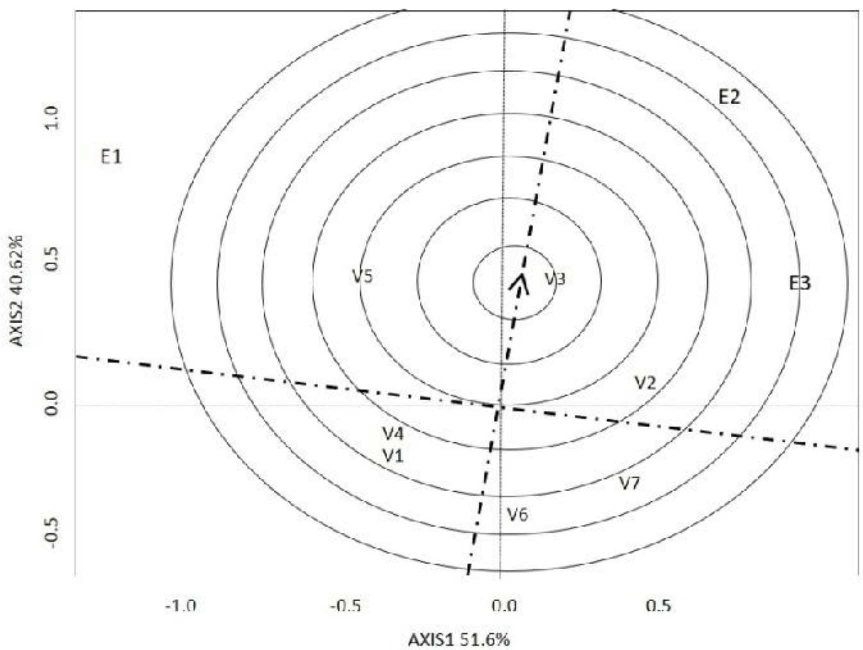

Figure 3. **Ranking genotypes relative to the ideal genotype on seed yield hill^{-1} (SYH)**. **V1-V7**: **varieties; E1-E3**: **environments.**

The results of this study should help to facilitate the selection of the optimal cultivation varieties for each of the analyzed environments. To achieve ongoing genetic improvement, the breeder should be heedful to select the most appropriate variety for yield, stability, and adaption in different environments (Temesgen *et al*., 2015). However, the GxE interaction in many traits, especially in quantitative traits, could limit genetic improvement through selecting those traits as a parameter (Kang, 1990). High-yielding and high agronomic characteristics and high adaptability and stability can be achieved by breeders by selecting genotypes as parental lines for the breeding program (Abuali *et al*., 2014).

Conclusion: The study of the ability to adapt to various environments of plant genetics has many advantages. Stability studies, for example, using both combined analysis and GGE modeling, help identify patterns of use of those genotypes. In this study, three traits such as SNP, PSF, and PH-showed an interaction between genetics and the environment (GxE) from the combined analysis. The polygon form of genotypes in three environments in GxE significant traits (SNP, PSF, and PH) and SYH showed different varieties responded differently to higher values of traits in each environment. For SYH, different varieties were more adaptive in each environment (high means and high stability) such as *Aung Jerng Yai*, *Khao Niaw Pala-U*, and *Beu Ge* for Environments 1–3, respectively. Regarding the yield component traits, *Khaw Niaw Pala-U* and *Aung Jerng Yai* exhibited a higher frequency ranking of means than the other varieties did, with *Khao Niaw Pala-U* exhibiting the best yielding stability.

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