Genetic Variability and Cluster Analysis for Phenological Traits of Thai Indigenous Upland Rice (Oryza sativa L.)

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ABSTRACT
Estimating genetic variability and cluster analysis of grain yield and yield contributing traits need to require for rice breeders to choose the best breeding programs. Ten upland rice genotypes were conducted from farmers’ fields during the years of 2017 at three provinces of southern Thailand. Extreme broad sense heritability and genetic gain values for flag leaf length, leaf area index, harvest index, total dry weight and filled grains showed that assortment of these yield contributing traits would be effective. Cluster analysis categorized genotypes into three groups. In each group some genotypes such as Dawk Pa-yawm or Dawk Kha 50 (group I), Nahng Kian (group II) and Khao Trai (group III) showed that genotypes had different better traits. These studies revealed that high broad sense heritability traits and the best genotypes Nahng Kian and Khao Trai would be useful for improving new upland rice varieties in southern Thailand.

Key words: Cluster analysis, Genetic advance, Genotypic coefficient, Heritability, Upland rice.

INTRODUCTION
Rice (Oryza sativa L.) is an important cereal crop and is feeding more than half of the world population. Based on planting area upland rice is one method of cultivation by which it grown in rainfed, flat or slanting, certainly well drained soils devoid of downward water accumulation and phreatic water supply also not bunded (Saito et al., 2014). According to Sohrabi et al. (2012) and Tuhina-Khatun et al. (2015) crop producers and geneticists classified upland rice genotypes based on phenological traits and concerned for improving innovative high yield genotypes with other necessary agronomic traits since this is low cost and fast, having plentiful phenological variations.

Atta et al. (2008) suggested the assessment of genetic variability traits for example phenotypic and genotypic coefficient of variation, heritability and genetic advance remains precondition for arrangement and implementation of a propagation package on behalf of the improvement of diverse qualitative as well as quantitative traits for any crop which can be used for selection of superior genotypes. According to Sneath and Sokal (1973) cluster analysis stands for a group of multivariate procedures which key determination is assembly groups based on the appearances or distances. Jain et al. (1999) defined the cluster analysis theory very well and interruption the methodologies primarily into partitional and hierarchical clustering methods. This type of study might assist the investigator for their selection strategies to improve grain yield for breeding program.

Thus, the objectives of this study were to (i) estimate the genetic variability and broad sense heritability percentages among the popular 10 Thai upland rice genotypes; and (ii) select genotypes by cluster analysis for breeding program from the three different locations of southern Thailand.

MATERIALS AND METHODS
Plant materials and conduction of experiment
The experimental material comprising ten upland rice genotypes (Table 1) were selected from the findings of Chuchert et al. (2018). Experiments were carried out at the farmers’ fields of Songkhla, Satun and Phatthalung Provinces under the rainfed upland conditions. A limited number of genotypes were used which had the characteristics of survival under upland conditions. These genotypes were already tested under rainfed upland conditions without surface water accumulation using different experiments. Another important point was that, there are limited genotypes in Thailand which are tolerant to rainfed condition. Three locations differing in latitude, longitude and elevation, from the sea level are in Satun (6° 39’ 13" N, 100° 4’ 59"E and 6 meters), in Songkhla (7.13° N, 100.26° E and 63 meters) and Phatthalung (7°37’ 04"N, 100°04’ 40”E, 14 meters). Experiment was laid down using Randomized
Complete Block Design with three replications in each environment. Each replication consisted of four rows (5 meters per row). Each genotype was planted 30 cm apart between rows and 25 cm within the rows. 15:15:15 N-P-K fertilizers were applied at the rate of 15 kg ha\(^{-1}\) as urea, super phosphate and muriate of potash before planting. Agronomic actions were done manually for example weed and insect control. Insect pests were controlled by the application of 10 ml per 1 L Cypermethrin 10% w/v EC and 2.5 ml per 1 L Benturacarb 20% w/v EC with water. At 30 days after planting, urea fertilizer 46 kg ha\(^{-1}\) was applied.

Data collection

Data were collected randomly from individual row and sixteen plants were selected from each genotype. The measured parameters were plant height (cm), tiller number (no.), panicle number (no.), panicle length (cm), flag leaf length (cm), flag leaf width (cm), leaf area index, harvest index (%), total dry weight (g), total grain weight (g), filled grains (no.), unfilled grains (no.) and grain yield (kg ha\(^{-1}\)).

Statistical analysis

Combined analysis of variance

An analysis of variance in each experiment was performed using R program with ‘agricolae’ package (Mendiburu and Simon, 2007). Homogeneity variances were analyzed with \(F_{\text{max}}\) and proved homogeneous if it was less than 5 (Tabachnick and Fidell, 2001). If they were homogeneous, the quantitative trait values in three locations were used for combined analysis of variance.

Estimation of the genotypic and phenotypic variance

From combined analysis, the variance components were substituted from the Table 2 (Bernado, 2002). For the estimation of the genetic parameters the following equations were used.

\[
\text{Genotypic variance (} \sigma^2_g \text{)} = \frac{M^2 \times M}{rl}
\]

\(M_1\) and \(M_2\) = Mean squares

\(r\) = Number of replications

\(l\) = Number of locations

\[
\text{Phenotypic variance (} \sigma^2_p \text{)} = \frac{M^2}{rl}
\]

Estimation of the coefficient of variations for genotypic and phenotypic:

\[
\text{Co-efficient of variance for genotypic (GCV)} = \frac{\sigma^2_g}{\bar{X}} \times 100
\]

\[
\text{Co-efficient of variance for phenotypic (PCV)} = \frac{\sigma^2_p}{\bar{X}} \times 100
\]

where, \(\bar{X}\) = Mean of the trait

Estimation of Broad-sense heritability base on family means (\(H^2\)):

\[
H^2 = \frac{\sigma^2_g}{\sigma^2_p} \times 100
\]

Estimation of Genetic Advance (GA)

Probable GA was assessed with the formulae given by Johnson et al. (1955) and Allard (1960).

\[
\text{Expected GA} = k \times \frac{\sigma^2_g}{\bar{X}} \times \text{Heritability}
\]

Here, \(k\) is the differential selection, for which the value is 2.06 at 5% selection intensity.

Cluster analysis

Cluster analysis was done using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Combined analysis of variance

The result of combine analysis of variance (Tables 3 and 4) revealed that highly significant differences were observed among location to location for plant height, tiller number, panicle number, panicle length, flag leaf length, flag leaf width, leaf area index, harvest index, total dry weight, total grain weight, 1000 seed weight, filled grains, unfilled grains and grain yield. The significant difference among the phenological traits of the three environments helped to find out the best genotype for the locations. This result is the same as finding of Vange (2009). While few of the phenotypic traits like flag leaf length, harvest index, total dry weight, total grain weight and filled grains had a significant difference among genotypes to genotypes. There were significant differences among harvest index, total dry weight and total grain weight of the genotype to location sites. Various environmental

Table 1: Details of 10 Thai upland rice genotypes of different provinces of Thailand.

| Genotypes                | Collection site (Province) |
|--------------------------|----------------------------|
| Dawk Pa-yawm (white rice)| Phatthalung                 |
| Mai Tahk (white rice)    | Songkhla                    |
| Bow Leb Nahng (white rice)| Satun                      |
| Dawk Kha 50 (red rice)   | Krabi                       |
| Dawk Kahm (red rice)     | Chumphon                    |
| Khaor Trai (white rice)  | Krabi                       |
| Nual Hawm (white rice)   | Songkhla                    |
| Nahng Kian (white rice)  | Chumphon                    |
| Nahng Mum (white rice)   | Chumphon                    |
| Hawm Jet Ban (red rice)  | Krabi                       |

Table 2: ANOVA when genotypes were raised at several locations for one year.

| Source               | df | Mean squares | Expectation MS |
|----------------------|----|--------------|----------------|
| Location (L)         | (l-1) | -           | -              |
| Replication/L        | (r-1) | -           | -              |
| Genotype (G)         | (g-1) | M_1          | \(\sigma^2_g + \sigma^2_p + r \sigma^2_{gl}\) |
| G × L                | (g-1) X (l-1) | M_2          | \(\sigma^2_g + \sigma^2_p + r \sigma^2_{gl}\) |
| Pooled Error         | (r-1) X (g-1) | M_3          | \(\sigma^2_p\) |

Source: Bernado (2002).
phenomenon such as, rainfall, temperature, humidity and soil types might have a great influence on the genotypes.

**Mean comparison for phenotypic parameters**

Tables 5 and 6 showed the mean comparison for the phenotypic traits of the genotypes that Nahng Kian had highest value for grain yield (6234.11 kg ha⁻¹). Khao Trai genotype for 1000 seed weight (23 g), total dry weight (44.60 g) and flag leaf width (1.99 cm). Dawk Kahm showed a highest value for plant height (131.46 cm) and filled grain (689.67 no.). Dawk Pa-yawm showed a highest value for total grain weight (19.22 g).

**Genetic Variability**

In Table 7, the maximum genotypic variabilities were found in grain yield (7735.3333), followed by filled grain (6854.0000), unfilled grains (67.3333), total dry weight (24.4444) and plant height (13.6811). Similarly, the highest phenotypic variabilities were found in grain yield (112167.4444), followed by filled grain (8764.8889), unfilled grains (424.5556), total dry weight (68.778) and plant height (21.4556). High variability in grains yield and plant height was also reported by Sumanth et al. (2017) and Girma et al. (2018).

Values more than 15% of genotypic and phenotypic coefficients of variation were considered as high, 9% to 14% as moderate and less than 9% as low. Moderate to low genotypic and phenotypic coefficients of variation were obtained for tiller number, panicle number, leaf area index and unfilled grain. The small differences observed for plant height, flag leaf length, harvest index, 1000 seed weight and filled grains indicated the presence of enough genetic variability for these traits which might facilitated selection (Yadav, 2000). The high to low differences observed only for total grain weight indicated the high influence of the environments on this trait.

**Broad sense heritability**

Johnson et al. (1955) classified broad sense heritability as low (<10%), medium (10 to 30%) and high (>30 %) showed most of the traits easily modified over selection. High heritability was observed (Table 7) for plant height (63.76), flag leaf length (55.95), leaf area index (44.04), harvest index (60.40), total dry weight (35.59) and filled grains (78.20) indicating the possibility of genetic improvement of these traits through selection. This outcome showed the similarities with the findings of Sarawgi et al. (2000) and Sao (2002). The medium heritability in panicle number (28.71) and unfilled grains (15.86) showed the more influence of environment on these traits. The low broad sense heritability observed for the tiller number (1.68), total grain weight (8.17), 1000 seed weight (8.55) and grain yield (8.90). The low heritability recorded for these traits showed direct selection for these traits was not effective.

**Genetic advance**

Johnson et al. (1955) also stated that measuring only...
Table 5: Mean comparison for plant height (PH), tiller number (TN), panicle number (PN), panicle length (PL), flag leaf length (FLL), flag leaf width (FLW) and leaf area index (LAI) of 10 Thai upland rice genotypes.

| Genotypes     | PH  | TN   | PN   | PL  | FLL | FLW | LAI |
|---------------|-----|------|------|-----|-----|-----|-----|
| Dawk Pa-yawm  | 124.23 | 6.89 | 5.51 | 25.63 | 28.84 | 1.84 | 1.66 |
| Mai Tahk      | 121.61 | 6.56 | 5.11 | 24.82 | 29.41 | 1.87 | **2.66** |
| Bow Leb Nahng | 126.46 | 7.22 | **6.81** | 24.90 | 29.13 | 1.56 | 2.07 |
| Dawk Kha 50   | 125.98 | 7.06 | 6.00 | 24.76 | **38.18** | 1.76 | 1.87 |
| Dawk Kahm     | **131.46** | 7.00 | 6.26 | 25.92 | 33.31 | 1.81 | 1.85 |
| Khao Trai     | 123.64 | 5.44 | 4.53 | 24.11 | 30.67 | **1.99** | 1.70 |
| Nual Hawm     | 126.57 | 6.67 | 5.78 | 23.84 | 28.64 | 1.68 | 2.23 |
| Nahng Khan    | 126.47 | 6.67 | 5.23 | 25.13 | 29.92 | 1.78 | 1.97 |
| Nahng Dum     | 122.52 | **7.89** | 4.56 | 24.34 | 30.64 | 1.82 | 2.07 |
| Hawm Jet Ban  | 123.84 | 7.56 | 5.39 | **26.19** | 28.79 | 1.79 | 1.79 |
| Means         | 125.28 | 6.87 | 5.72 | 24.97 | 30.75 | 1.79 | 1.99 |

LSD<sub>.05</sub> - - - - 8.39 - -

Table 6: Mean comparison for harvest index (HI), total dry weight (TDW), total grain weight (TGW), 1000 seed weight (1000 SW), filled grain (FG), unfilled grain (UFG) and grain yield (GY) of 10 Thai upland rice genotypes.

| Genotypes     | HI  | TDW | TGW | 1000 SW | FG  | UFG | GY  |
|---------------|-----|-----|-----|---------|-----|-----|-----|
| Dawk Pa-yawm  | 0.42 | 37.86 | **19.22** | 18.56 | 504.44 | 173.56 | 5546.56 |
| Mai Tahk      | 0.32 | 30.34 | 14.92 | 18.33 | 592.56 | 170.44 | 5342.89 |
| Bow Leb Nahng | 0.37 | 30.08 | 15.62 | 18.33 | 683.11 | 169.56 | 5893.44 |
| Dawk Kha 50   | 0.31 | 28.26 | 14.40 | 22.00 | 497.00 | 166.67 | 5268.22 |
| Dawk Kahm     | 0.42 | 41.74 | 17.81 | 20.44 | 689.67 | 117.44 | 6115.56 |
| Khao Trai     | 0.36 | **44.60** | 15.66 | **23.00** | 617.44 | 154.89 | 5831.22 |
| Nual Hawm     | 0.52 | 28.49 | 15.82 | 17.89 | 502.44 | 143.22 | 5854.78 |
| Nahng Khan    | 0.57 | 32.63 | 12.37 | 19.56 | **764.33** | 188.67 | **6234.11** |
| Nahng Dum     | 0.43 | 32.26 | 13.27 | 19.56 | 596.33 | 171.67 | 6115.56 |
| Hawm Jet Ban  | 0.38 | 38.62 | 14.65 | 21.44 | 513.67 | 142.22 | 6043.44 |
| Means         | 0.41 | 36.27 | 14.24 | 20.44 | 596.10 | 159.83 | 5824.58 |

LSD<sub>.05</sub> - 23.50 10.56 - 265.45 - -

LSD<sub>.01</sub> - 31.29 14.07 - 353.51 - -

Table 7: Broad sense heritability estimates from Phenotypic of 14 agronomic traits of 10 Thai upland rice genotypes.

| Trait         | Mean | σ²g | σ²p | GVC (%) | PCV (%) | H² (%) | GA (%) |
|---------------|------|-----|-----|---------|---------|--------|--------|
| Plant height  | 125.28 | 13.6811 | 21.4556 | 2.95 | 3.70 | 63.76 | 4.86 |
| Tiller number | 6.87 | 0.0077 | 0.4564 | 1.27 | 9.83 | 1.68 | 0.34 |
| Panicle number | 5.72 | 0.1399 | 0.4872 | 6.54 | 12.20 | 9.62 | 7.22 |
| Panicle length | 24.97 | 0.5920 | 0.5202 | 8.80 | 6.60 | 19.76 | 2.00 |
| Flag leaf length | 30.75 | 4.8967 | 8.7511 | 7.20 | 9.62 | 55.95 | 11.09 |
| Flag leaf width | 1.79 | 0.01149 | 0.01149 | 9.85 | 14.85 | 44.05 | 13.47 |
| Leaf area index | 1.99 | 0.0384 | 0.0873 | 9.85 | 14.85 | 44.05 | 13.47 |
| Harvest index | 0.41 | 0.0042 | 0.0069 | 15.79 | 20.31 | 60.40 | 25.27 |
| Total dry weight | 36.27 | 24.4444 | 68.6778 | 13.63 | 22.85 | 35.59 | 16.75 |
| Total grain weight | 14.24 | 1.1344 | 13.8844 | 7.48 | 26.17 | 8.17 | 4.40 |
| 1000 seed weight | 20.44 | 0.1166 | 1.3636 | 1.67 | 5.71 | 8.55 | 1.01 |
| Filled grains | 596.10 | 6854.0000 | 8764.8889 | 13.89 | 15.71 | 78.20 | 25.30 |
| Unfilled grains | 159.83 | 67.3333 | 424.5556 | 5.13 | 12.89 | 15.86 | 4.21 |
| Grain yield    | 5824.58 | 7735.3333 | 112167.4444 | 1.15 | 5.75 | 6.90 | 0.82 |

Note σ²g = variance for genotype, σ²p = variance for phenotype, GCV = variation for genotypic coefficient, PCV = variation for phenotypic coefficient, H² = broad sense heritability, GA = genetic advance and N/A = not available because of genetic variance = 0.
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Cluster analysis

Fig 1: Hierarchically cluster analysis of 10 upland rice genotypes constructed for fourteen yield contributing traits.

G1 = Dawk Pa-yawm, G2 = Mai Tahk, G3 = Bow Leb Nahng, G4 = Dawk Kha 50, G5 = Dawk Kahm, G6 = Khao Trai, G7 = Nual Hawm, G8 = Nahng Kian, G9 = Nahng Dum and G10 = Hawm Jet Ban

heritability does not indicate genetic improvement. They also classified genetic advance (GA) as low (<10%), medium (10% to 20%) and high (>20%). In this study the range of GA varied from 0.34% to 25.30%. High percentage of GA was accounted for harvest index (25.27%) and filled grains (25.30%). Flag leaf length (11.09%), leaf area index (13.47%) and total dry weight (16.75%) achieved the medium percentages of GA. On the other hand, plant height (4.86%), tiller number (0.34%), panicle number (7.22%), total grain weight (4.40%), 1000 seed weight (1.01%) and unfilled grains (4.21%) had low percentages of GA. High heritability and genetic advance were seen in harvest index and filled grains in Table 7 which indicated these traits were less influenced by the environment (Sumanth *et al.*, 2017). For better result heritability in addition with genetic advance will be more useful for the selection (Ali *et al.*, 2002).

Cluster analysis

Based on the agronomic traits, the average, maximum and minimum distances between clusters were 417.13, 978.70 and 73.74 respectively (Fig 1). Group I comprised with three genotypes (Dawk Pa-yawm, Mai Tahk and Dawk Kha 50), group II four genotypes (Nahng Kian, Hawm Jet Ban, Dawk Kahm and Nahng Dum) and Group III three genotypes (Nual Hawm, Bow Leb Nahng and Khao Trai). Each group consisted genotypes were collected from several provinces, represented the usefulness of the cluster analysis. Dendogram showed that average, maximum and minimum distances between clusters had diverged genetic materials. Group I showed the maximum value of yield contributing traits such as flag leaf length (38.18 cm), leaf area index (2.66) and total grain weight (19.22 g). Group II achieved plant height (131.46 cm), tiller number (7.89 no.), panicle length (26.19 cm), harvest index (0.57), filled grains (764.33 no.), unfilled grain (188.76 no.) and grain yield (6234.11 kg ha⁻¹). Group III showed the maximum value of panicle number (6.81), total dry weight (44.60 g) and 1000 seed weight (23.00 g). Similar results also reported by Khare *et al.* (2014), Girma *et al.* (2018) and Iqbal *et al.* (2018). These results might assist the investigator for their selection strategies to improve grain yield for breeding program. Thus, present results concluded that traits such as flag leaf length, harvest index, total dry weight, total grain weight and fills grains were useful for higher grain yield. Some genotypes such as Dawk Pa-yawm, Dawk Kha 50, Nahng Kian and Khao Trai should be used for crossing to develop new upland rice varieties of southern Thailand.

CONCLUSION

In conclusion, the present study identified the presence of adequate genetic variability in the tested genotypes. The analysis of variance revealed there were highly significant differences among location to location for all the traits. In comparison means Nahng Kian had highest value for filled grains, unfilled grains and grain yield. Khao/ Trai genotype had the maximum value for 1000 seed weight and total dry weight. The broad sense heritability and genetic advance revealed flag leaf length, leaf area index, harvest index, total dry weight and filled grains were the most important yield traits which showed moderate to high in percentage of mean, offering most effective for selection process. According to the cluster analysis results maximum genetic diversity was presented in group II with genotypes (Nahng Kian, Hawm Jet Ban, Dawk Kahm and Nahng Dum). Results obtained from the study, it can be concluded that traits like flag leaf width, total dry weight, total grain weight, 1000 seed weight, filled grains and grain yield were useful traits for higher production. Nahng Kian and Khao Trai genotypes might be used for developing new upland rice genotype in southern Thailand.
ACKNOWLEDGEMENT

Research was supported by the government budget of Prince of Songkla University (NAT610046S) and the Higher Education Research Promotion and Thailand’s Education Hub for ASEAN Countries (TEH-AC) Project Office. The authors acknowledge, Graduate School and Faculty of Natural Resources, Prince of Songkla University, Hat Yai campus, Thailand for financial support. The authors would like to thank Dr. Seppo Karrila for performing the copy-editing service of the Research and Development Office of Prince of Songkla University, Thailand.

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