Interaction between genotypes, environment and season (G x E x S) on anthocyanin corn in lowland zone of Indonesia

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Abstract. Interaction between genotypes (G), environment (E) and season (S) on anthocyanin corn has been studied under lowland zone in Indonesia. The experiment were conducted using Randomized Complete Block Design (RCBD) based on location namely Maros, Bajeng, and Polman with three replications. Ten populations of open pollinated variety (OPV) of anthocyanins purple corn included checks were used in two planting seasons (dry and rainy) in 2015/2016. The objective of the experiment is to determine which of the population is stable and has high yield to be promoted as candidate for new OPV varieties. Genotypes were planted in four rows of 5.0 m length, spacing of 75 cm x 20 cm, one plant per hill, and applied with Urea, Ponska (300 and 200 kg Ha\(^{-1}\)). Population was then selected by t test in model \( Y_{ij} = \mu + \beta I_j + \delta_{ij} \), \( \delta_{ij} \neq j \) (Y: yield, \( \mu \): mean, \( \beta \): regression coefficient, I: environmental index, \( \delta \): deviation from regression), and stability parameter \( b_i = \Sigma Y_i I_i/\Sigma I_i^2 \). The results show that there was a significant interaction of GxExS on PMU(S1).Synth.F.C1 and PPU(S1).F.C1 with yield ranging between 6.70-8.48 t ha\(^{-1}\) and grain potential found in the locations of Maros and Bajeng in the rainy season. Content of anthocyanins were between 37.15 and 51.92 µg per 100 g sample.

1. Introduction
Corn with purple coloured grain can be defined as a specialty corn which contains high content of nutritious anthocyanins. Originated from Mesoamerica, the corn with a pigment that gives colour of red, purple, and blue in the grain have health beneficial to prevent viral, cholesterol, heart disease, obesity, and cancer [1, 2]. Yasin et al. [3] reported that the corn has many excellent characters and higher economic value, that makes it as an importance raw material for food industries, textiles, paper making and feed. Germplasms had been selected to increase cycle of selection by self (S1, S2 to S4/S5). Anthocyanins corn is a recessive characteristic, production must be isolated from normal/dent to prevent losing the periculur starch and colour properties. The variety evaluation trial under some environments have been conducted and continued to provide guidance for selecting the best genotype and estimated yield. Several studies to test the yield stability of Quality Protein Maize (QPM) crops were conducted [3, 4] in seven environments under lowland tropical zone which resulted in 11 entries that found to be stable and had highly significant effect of GxExS.

The objective of the experiment is to evaluate the yield stability of ten anthocyanins corn under three environments during dry and rainy planting seasons in 2015/2016. The best population will be determined as the potential genotype for new open pollinated variety (OPV) of anthocyanins corn.
2. Methodology

The experiment was conducted in Maros (e1), Bajeng (e2) and Polman (e3) regencies in South Sulawesi Province of Indonesia. The locations are under lowland tropical zone <50 m above the sea level (asl). The genotypes (g) were 10 populations of anthocyanins corn with seed colours purple to black (table 1) based on Pixley et al.[5] that genetic population of specialty corn could be increase by initial selection of colour seeds. The experiment was set in Randomized Complete Block Design (RCBD) with three replications in dry and rainy season in 2015-2016. The genotypes were planted in four rows per se, spacing 75 x 20 cm, fertilized with Urea and Ponska with dosage of 300 and 200 kg Ha⁻¹, respectively.

Table 1. Seed texture, colour, origin and cycles population of anthocyanins corn planted in the two planting seasons in 2015/2016 in three locations under lowland of tropical zone condition.

| Genotype (g)      | Dry season, 2015 | Rainy season, 2016 |
|-------------------|------------------|-------------------|
|                   | Seed texture     | Seed colour       | Seed origin | Cycles population |
| g1. PTU(S1).F.C0  | flint            | purple to black   | China       | C0               |
| g2. PTU(S1).D.C0  | flint            | purple to black   | China       | C0               |
| g3. PMU(S1).SYN.D.C1 | semi dent       | purple           | Manado      | Syntetik C1      |
| g4. PMU(S1).SYN.F.C1 | flint           | purple           | Manado      | Syntetik C1      |
| g5. PPH.FS.C1     | flint            | purple to black   | Lembah Palu | C1               |
| g6. PPH.(S1).C1   | flint            | purple to black   | Lembah Palu | C1               |
| g7. PVU.FS.C0     | flint            | purple           | Vietnam     | C0               |
| g8. PLP.C0 (check 1) | semi flint     | purple           | Lembah Palu | C0               |
| g9. PLM.C0 (check 2) | flint          | White            | Pulut Maros | C0               |
| g10. URI1 (check 3)  | flint           | White            | UPBS        | C0               |

The variable observed were yield, plant and ear height, period of tasseling and silking, weight of 1000 seeds, water content and shelling percentage. The data were analyzed by single effect of genotype (G), two factors interaction between genotype and environment (E) (GxE) by polled analysis ei, i=1,2,3 on dry and rainy [3, 6], three factors interaction between genotype, environment and season (S) (GxES) by simple regression analysis with xi as environmental index.

Coefficient of simple regression of Gi was calculated using: 

\[ Y = \beta_0 + \beta_1 \cdot x_i \text{ for } i = 1, 2, \ldots, 10, \]

\[ \beta_i = \frac{\sum x_i y_i}{\sum x_i^2}, \text{ where } \bar{y} \text{: mean of all population and } x_i \text{: mean of each population gi [10].} \]

The hypothesis of stability was H0:β1=1 vs H1:β1≠1 by d.f : n-1. t.cal.:(β-1)/sd ; sd : VS² ; S²: 

\[ \text{Varians : } 1/n[\sum x_i^2-(\sum x_i)^2]/n. \]

Different of population in each location was compared to control with three checks by Least Significance Difference (LSD) test in level of significant of 95%. LSD: t(18)\(\sqrt{2s^2/3}\), s² : error mean square. Yield of grain in 15% water content was predicted by: 

\[ Y: ((10000/7.5)*(100-wa)/85)*\text{(shelling)}*\text{ear weight on harvested}. \]

3. Results and Discussion

3.1. GxE Interaction Analysis

The result analysis of variance and the mean square of observed variable and coefficient of variance value under dry and rainy season is shown in table 2. The analysis shows that under dry season there were significant interaction of GxE on variable of yield, tasseling, and weight of 1000 seeds. The single
effect of genotype (G) was significant on yield, plant height, silking period, and shelling percentage. In the rainy season, GxE was significant on plant height and water content of seeds only, and single effect of G shows that all variable were significant except of plant and ear height.

Table 2. The mean square and coefficient of variance (CV) of GxE interaction on anthocyanins corn in dry and rainy season in 2015/2016

| Variable                      | Dry season 2015 | Rainy season 2016 |
|-------------------------------|-----------------|-------------------|
|                               | E       | U/E    | G       | GxE     | Error | CV(%) |
| Yield                         | 10,544* | 0,275  | 4,997*  | 1,530*  | 0,660  | 12,4  |
| Plant height                  | 4699,547* | 135,890 | 156,044* | 75,877  | 85,908 | 4,9   |
| Ear height                    | 2196,466* | 1,805  | 28,173  | 42,730  | 54,802 | 8,1   |
| Tasseling                     | 12,878* | 1,056  | 1,328   | 2,051*  | 1,228  | 2,3   |
| Silking period                | 13,478* | 0,256  | 1,888*  | 0,910   | 1,095  | 2,0   |
| Water moisture                | 151,800* | 26,455 | 3,084   | 5,290   | 5,026  | 8,3   |
| Shelling percentage           | 14,042* | 1,778  | 5,404*  | 3,845   | 4,418  | 2,7   |
| Weight 1000 seeds             | 112,678 | 488,778 | 621,685 | 1330,715* | 983,049 | 10,42 |

| Variable                      | E       | R/E    | G       | ExG     | S       | ExS     | GxS     | GxE     | Error | CV(%) |
|-------------------------------|---------|--------|---------|---------|---------|---------|---------|---------|-------|-------|
| Yield                         | 12,542  | 1,901  | 12,040  | 0,967   | 0,591   | 1,990   | 2,890   | 1,147*  | 0,742 | 13,0  |
| Plant height                  | 1658,860 | 69,704 | 165,426 | 121,716 | 789,188 | 3588,287 | 79,340  | 111,366* | 90,173 | 4,9   |
| Ear height                    | 983,382 | 27,138 | 27,277  | 54,614  | 268,156 | 1231,761 | 43,222  | 50,127  | 65,871 | 8,7   |
| Tasseling                     | 3,489   | 0,567  | 2,393   | 1,859   | 2,689   | 10,289  | 7,035   | 1,338   | 1,245 | 2,3   |
| Silking period                | 2,617   | 0,428  | 4,388   | 0,907   | 1,660   | 13,039  | 7,223   | 1,045   | 1,206 | 2,1   |
| Water moisture                | 222,913 | 9,937  | 10,468  | 7,739   | 460,160 | 186,275 | 17,409  | 10,450* | 9,911 | 10,2  |
| Shelling, %                   | 8,434   | 1,757  | 4,046   | 3,058   | 11,300  | 5,879   | 6,119   | 2,316   | 5,178 | 2,8   |
| 1000 seeds                    | 232,267 | 206,961 | 1578,154 | 731,730 | 107,339 | 458,422 | 1348,722 | 970,823 | 949,441 | 10,2  |

* Significant different on 95% level, n : 180

3.2. GxE*S Interaction Analysis
Table 3 shows analysis of three factors, GxE*S, with significant variable were yield, plant height and water content of seeds. The results indicate that yield of all genotypes were different under three locations in Maros, Bajeng and Polman. The CV found for all variable was <20.0%, and be assume that there could be selected of Gi, i =1, 2, 3, …10 for new corn varieties [3]. The experiment of QPM of yellow grain from CIMMYT showed that population could be adapted in Maros after selected by S1 families and genetically improved by one cycles of selection, yield was 8.0-10.0 t Ha⁻¹ [11]. The significant interaction of GxE*S on three variables could indicate that genotypes yield, plant height and water content of seeds at harvest were significantly different under three locations.

Table 3. The mean square, coefficient of variance (CV) and interaction GxE*S on anthocyanins corn in 2015/2016

| Variable                      | E       | R/E    | G       | ExG     | S       | ExS     | GxS     | GxE     | Error | CV(%) |
|-------------------------------|---------|--------|---------|---------|---------|---------|---------|---------|-------|-------|
| Yield                         | 12,542  | 1,901  | 12,040  | 0,967   | 0,591   | 1,990   | 2,890   | 1,147*  | 0,742 | 13,0  |
| Plant height                  | 1658,860 | 69,704 | 165,426 | 121,716 | 789,188 | 3588,287 | 79,340  | 111,366* | 90,173 | 4,9   |
| Ear height                    | 983,382 | 27,138 | 27,277  | 54,614  | 268,156 | 1231,761 | 43,222  | 50,127  | 65,871 | 8,7   |
| Tasseling                     | 3,489   | 0,567  | 2,393   | 1,859   | 2,689   | 10,289  | 7,035   | 1,338   | 1,245 | 2,3   |
| Silking period                | 2,617   | 0,428  | 4,388   | 0,907   | 1,660   | 13,039  | 7,223   | 1,045   | 1,206 | 2,1   |
| Water moisture                | 222,913 | 9,937  | 10,468  | 7,739   | 460,160 | 186,275 | 17,409  | 10,450* | 9,911 | 10,2  |
| Shelling, %                   | 8,434   | 1,757  | 4,046   | 3,058   | 11,300  | 5,879   | 6,119   | 2,316   | 5,178 | 2,8   |
| 1000 seeds                    | 232,267 | 206,961 | 1578,154 | 731,730 | 107,339 | 458,422 | 1348,722 | 970,823 | 949,441 | 10,2  |
3.3. Yield Stability
The analysis of yield stability shown in table 4 shows significant differences were found in dry season and there are four candidate of genotypes (G1, G3, G4, and G7) that were significant by statistical t test, while three checks were not significant. However, in the rainy season, G1 and G3 were significant. The result indicates that population G1 (PTU(S1)F.C0) could become as a candidate for promising new OPV. The significant result indicates that the genotype yield was stable in the three locations under two seasons in rainy and dry seasons and assuming that the condition in the locations were favorable for plant growth, like well fertilized, no weeds and pest-disease resulted in maximum grain yield obtained. The statistical analysis of G1 shows that b=1, H1:b≠1.0 was rejected and R²:99.2%.

Table 4. Parameters of yield stability of anthocyanins corn. 2015-2016

|                  | R   | R²  | B    | Se.β | MSe | t.cal. |
|------------------|-----|-----|------|------|-----|-------|
| **Dry 2015**     |     |     |      |      |     |       |
| G1. PTU(S1)F.C0  | 0.996 | 0.992 | 3.050 | 0.340 | 0.001 | 6.029* |
| G2. PTU(S1)D.C0  | -0.462 | 0.213 | -1.397 | 2.677 | 5.037 | 0.894 |
| G3. PMU(S1)SYN.D.C1 | 0.906 | 0.820 | 2.469 | 1.156 | 0.939 | 1.271* |
| G4. PMU(S1)SYN.F.C1 | 0.966 | 0.934 | 1.374 | 0.366 | 0.094 | 1.023* |
| G5. PPH.FS.C1    | -0.559 | 0.313 | -0.426 | 0.631 | 0.280 | 2.260 |
| G6. PPH.(S1).C1  | -0.474 | 0.225 | -0.774 | 1.437 | 1.451 | 1.235 |
| G7. PVU.FS.C0    | 0.973 | 0.946 | 1.915 | 0.457 | 0.147 | 2.003* |
| **Check**        |     |     |      |      |     |       |
| G8. PLP.C0 (check 1) | 0.980 | 0.950 | 2.600 | 0.024 | 0.001 | 66.040 |
| G9. PLM.C0 (check 2) | -0.961 | 0.923 | -0.575 | 0.167 | 0.020 | 9.456 |
| G10. URI1 (check 3) | 0.813 | 0.661 | 0.828 | 0.597 | 0.243 | 0.300 |
| **Rainy 2016**   |     |     |      |      |     |       |
| G1. PTU(S1)F.C0  | 0.961 | 0.924 | 2.549 | 0.731 | 0.142 | 2.119* |
| G2. PTU(S1)D.C0  | 0.281 | 0.079 | 0.815 | 2.780 | 2.054 | 0.066 |
| G3. PMU(S1)SYN.D.C1 | 0.876 | 0.768 | 2.330 | 1.285 | 0.439 | 1.035* |
| G4. PMU(S1)SYN.F.C1 | 0.414 | 0.171 | 0.069 | 1.153 | 0.006 | 0.807 |
| G5. PPH.FS.C1    | -0.991 | 0.982 | -1.347 | 0.101 | 0.009 | 23.237 |
| G6. PPH.(S1).C1  | 0.546 | 0.290 | 0.772 | 1.184 | 0.373 | 0.193 |
| G7. PVU.FS.C0    | 0.669 | 0.447 | 1.834 | 2.040 | 1.106 | 0.409 |
| **Check**        |     |     |      |      |     |       |
| G8. PLP.C0 (check 1) | 0.997 | 0.995 | 1.678 | 0.122 | 0.004 | 5.568 |
| G9. PLM.C0 (check 2) | 0.979 | 0.994 | 3.747 | 0.302 | 0.024 | 9.083 |
| G10. URI1 (check 3) | 0.848 | 0.719 | 1.273 | 0.796 | 0.160 | 1.275 |

*: significant different on 95% level

3.4. Agronomic and Yield Variables
Grain yield of the genotypes including checks and statistical test by LSD of all variable in the three environments are shown in table 5. Population of G1.PTU(S1)F.C0 and G2.PTU(S1)D.C0 found to be significant in E1, E2 and E3 in rainy season, the highest yield in Maros experimental farm was 8.48 t ha⁻¹ shown by G1.PTU(S1)F.C0 which differed by 50.3% than the best yield obtained by the check genotypes in rainy season and 21.3% in dry season. This indicates that G1 was the candidate for new OPV variety of anthocyanin corn. In the dry season the G1 candidate was significant in Maros experimental farm but not significant in Bajeng and Polman.

Observation on agronomic characters of the plant ie. plant and ear height, tasseling and flowering (table 6) shows that plant height was not significant but ear height was significant between genotypes. The position of ear height was found around middle from plant height. This position of ear plant was suitable for selection of corn. Shelling percentage of G1. PTU(S1)F.C0 was higher 8.4% than check. Variable of anthesis silking interval (ASI) shows that the range of flowering and tasseling periods was
2.0 to 4.0 days. The different value of flowering and tasseling defines ASI value. ASI value < 3.0 days was very suitable for maximal grain formation while ASI value > 6.0 days showed no grain formed. Experiment results of Djamaluddin and Yasin [11] found that the ASI values of population MS-2 that ranged 5-6 days were associated with yield 2-3 t ha⁻¹. Gambin et al. [12] found that drought tolerant inbred line with ASI of 6.0 days experiencing decline in yield by 58-69%. Water content of seeds ranged between 27-34.0% and G1.PTU(S1).F.C0 found to be less than check 36.0%. The result is similar with shelling percentage of 79.3%.

| Table 5. The yield potential and statistical test of LSD on anthocyanins corn |
|-----------------|-----------------|-----------------|
| Genotype (G)    | Dry 2015         | Rainy 2016       |
|                 | E1 | E2 | E3 | E1 | E2 | E3 |
| G1. PTU(S1).F.C0| 7.95abc | 7.15 | 6.94 | 8.48abc | 8.17abc | 7.24bc |
| G2. PTU(S1).D.C0| 7.69abc | 7.21 | 6.76 | 7.65abc | 7.86abc | 6.35bc |
| G3. PMU(S1).SYN.D.C1 | 6.69 | 7.37 | 7.84abc | 7.06abc | 7.91abc | 5.75bc |
| G4. PMU(S1).SYN.F.C1 | 7.96abc | 6.28 | 7.80bc | 7.46abc | 7.76abc | 7.91abc |
| G5. PPH.FS.C1    | 6.97   | 7.70b | 6.17 | 6.95abc | 7.57abc | 5.60   |
| G6. PPH.(S1).C1  | 6.86   | 5.77  | 6.67 | 7.40abc | 7.92abc | 6.66bc |
| G7. PVU.FS.C0    | 6.75   | 7.78b | 6.71 | 6.64bc | 6.62ac  | 6.94bc |
| Check            |       |      |     |      |        |     |
| G8. PLP.C0 (check 1) | 6.56 | 6.76 | 7.13 | 5.64 | 5.60 | 6.57 |
| G9. PLM.C0 (check 2) | 5.80 | 6.17 | 5.93 | 4.86 | 5.97 | 4.99 |
| G10. URI1 (check 3) | 5.88 | 6.86 | 6.16 | 4.26 | 5.25 | 4.76 |
| CV (%)           | 12.62 | 15.25 | 17.18 | 13.08 | 11.81 | 12.08 |
| LSD 5%           | 1.06 | 1.28 | 1.42 | 1.05 | 1.01 | 0.92 |
| LSD 1%           | 1.45 | 1.75 | 1.95 | 1.44 | 1.39 | 1.26 |

a,b,c : significant vs check 1, 2 and 3
E1 : Expm Farm of Maros,
E2 : Expm Farm of Bajeng,
E3 : Farmer’s field of Polman-Sulbar

| Table 6. The average of variable of anthocyanins corns planted in Maros (E1), Bajeng (E2) and Polman (E3) under two seasons in 2015/2016 |
|-----------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| E/G             | Plant height (cm) | Ear height (cm) | Tasselling (days) | Flowering (days) | Water content (%) | Shelling (%) |
| G1. PTU(S1).F.C0 | 191.9 | 99.2 | 48.8 | 51.3 | 30.6 | 79.3 |
| G2. PTU(S1).D.C0 | 194.3 | 94.4 | 48.5 | 51.3 | 29.9 | 73.6 |
| G3. PMU(S1).SYN.D.C1 | 201.7 | 98.5 | 49.0 | 51.5 | 29.7 | 74.0 |
| G4. PMU(S1).SYN.F.C1 | 196.4 | 91.9 | 48.2 | 51.0 | 31.0 | 76.7 |
| G5. PPH.FS.C1    | 198.5   | 96.0  | 48.3  | 52.0  | 30.1  | 77.7  |
| G6. PPH.(S1).C1  | 193.3   | 95.5  | 48.5  | 51.8  | 31.0  | 77.3  |
| G7. PVU.FS.C0    | 195.3   | 95.4  | 48.5  | 51.5  | 31.1  | 75.7  |
| G8. PLP.C0 (check 1) | 193.5 | 92.9 | 48.8 | 52.2 | 31.1 | 77.3 |
| G9. PLM.C0 (check 2) | 197.6 | 96.8 | 48.3 | 51.5 | 30.6 | 75.7 |
| G10. URI1 (check 3) | 195.2 | 96.2 | 49.7 | 52.7 | 31.1 | 73.1 |
| CV (%)           | 3.0     | 3.9   | 2.0   | 2.0   | 7.8   | 4.8   |
| LSD (5%)         | 10.1    | 6.4   | 1.7   | 1.6   | 4.1   | 6.3   |
### E2. Bajeng

|       | G1. PTU(S1)F.C0 | G2. PTU(S1)D.C0 | G3. PMU(S1)SYN.D.C1 | G4. PMU(S1)SYN.F.C1 | G5. PPH.FS.C1 | G6. PPH.(S1).C1 | G7. PVU.FS.C0 | G8. PLP.C0 (check 1) | G9. PLM.C0 (check 2) | G10. URI1 (check 3) |
|-------|----------------|----------------|--------------------|--------------------|---------------|----------------|--------------|---------------------|---------------------|---------------------|
| CV (%)| 3.3            | 6.3            | 1.7                | 1.4                | 7.8           | 1.8           | 11.0         | 10.3                | 1.4                 | 3.9                 |
| LSD (5%)| 11.0          | 10.3           | 1.4                | 1.2                | 3.9           | 2.4           | 15.9         | 10.2                | 1.8                 | 2.6                 |

### E3. Polman

|       | G1. PTU(S1)F.C0 | G2. PTU(S1)D.C0 | G3. PMU(S1)SYN.D.C1 | G4. PMU(S1)SYN.F.C1 | G5. PPH.FS.C1 | G6. PPH.(S1).C1 | G7. PVU.FS.C0 | G8. PLP.C0 (check 1) | G9. PLM.C0 (check 2) | G10. URI1 (check 3) |
|-------|----------------|----------------|--------------------|--------------------|---------------|----------------|--------------|---------------------|---------------------|---------------------|
| CV (%)| 5.1            | 6.9            | 2.1                | 1.4                | 4.6           | 2.3           | 15.9         | 10.2                | 1.8                 | 2.6                 |
| LSD (5%)| 15.9          | 10.2           | 1.8                | 1.3                | 2.6           | 3.0           | 3.5         | 2.5                 | 1.4                 | 2.5                 |

3.5. Nutrition

The result of ICERI Laboratorium shows that the three genotypes that contain anthocyanins 51.92 μg g\(^{-1}\) were PMU(S1).Synth.F.C1, PMU(S1).Synth.D.C1, and PPH.(S1).C1. Compared to content of this pigment in the check genotype (12.10 μg g\(^{-1}\)), there are difference of 329.1%. According to Widowati et al. [13] and Wong [14], the amylase content are <10.0% as long as that the anthocyanins population is assumed also as waxy corn.

### 4. Conclusion

Three factors significant interaction of genetic, environment and season (GxExS) was found on grain yield parameter with highest yield of 8.17 t ha\(^{-1}\) obtained by genotype G1. PMU(S1)SYN.D.C1 in Maros which was 21.3-50.3% higher than the three check genotypes. Two genotypes, G1.PTU(S1)F.C0 and G2.PMU (S1)SYN.D.C1, were found to be stable (β=1) and showed mean yield of Gij:i:1,2 higher than mean total Gij and can be concluded as potential candidate for new superior variety of open pollinated variety of anthocyanins corn with anthocyanin content of 51.92 μg g\(^{-1}\) which is 329.1% higher than the check genotype.

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