Variability of Grain Protein Content in Improved Upland Rice Genotypes and Its Response to Locations

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Research Article

Variability of Grain Protein Content in Improved Upland Rice Genotypes and Its Response to Locations

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Abstract:
To search for genetic resources of high grain protein content in upland rice, the variability and genotype × location interaction of the protein content was studied by a field experiment. The materials used were 10 improved genotypes and two reference upland rice cultivars. The experiment was carried out at seven different locations in rainy season of 2008/2009. Genetic variability for grain protein content among the genotypes was confirmed which leads to the possibility for breeding of high grain protein content in upland rice.Genotype × location interaction influenced grain protein content of upland rice indicating that the genotypes gave different responses to the variation of locations.The genotypes namely UNRAM 4E, UNRAM 9E, UNRAM 17E were found to be the high protein content resources. These three genotypes, with stable protein content across locations, may be considered as genetic material for further development of high protein content upland rice cultivar. This finding might be very useful to improve nutritional status of poor people in the rural areas especially in east Indonesia (Lombok, Sumbawa) and semiarid areas with low rainfall.

Key word:
Upland rice, protein content, G x E interaction

Introduction

In Indonesia, rice is a staple food which serves as the most important commodity. It contributes to the total consumption of energy, protein, and iron in an amount of 63.1%, 37.7%, and 21.5%, respectively (Indrasari *et al.*, 2006). It is also the main source of income of approximately 18 million household farmers. Therefore, rice production and availability are very much connected with the issue of national food security. Moreover, rice is also very much associated with the issues of livelihood and rural development as more than 60% out of 26.6 million household farmers involve in rice production with only 0.3 ha average land-holding per farmer (Damarjati, 2006).

One of the possibilities to meet the food needs for the anticipated increase in rice deficits is development of upland rice cultivars with better phenotypic characteristics. About55.6 million ha upland in Indonesia is not utilised for cultivation. Statistical data showed that upland rice harvest area is only 1.2 million ha. With a productivity of 2.27 t/ha, it produce 2.6 million tons per year which is not more than 5 % of national rice production.

Besides low productivity, the weekness of upland rice that leads to limitation of harvest area is, cultivation of local cultivars with low yield potential and quality. Therefore, it is important, to develop high yielding upland rice cultivar with high grain quality.High yielding aromatic upland rice lines were developed by crossing Poso (high-yielding upland rice, tolerant to drought, Indica type) and Mentikwangi (low-land aromatic rice, Javanica type) (Totok and Utari, 2005; Totok et al., 2008).

One of the important characteristics of rice in relation to nutritional quailty is grain protein content. In Indonesia and several Asian countries, rice contribute less than 37.7 % of protein intake, especially in the rural area. The mean grain protein content of rice is 7 %. On recognition of world-wide protein malnutrition greateremphasis has been given for improving rice protein content,either through breeding for high-protein cultivars or through manipulation of environment. The advantage of protein from rice is high digistability, non alergic and may be the only protein source with hypoalergenic. Furthermore, protein from rice also has fiber suitable for digestion and reducing colesterol (Tobiason, 2006). The existence of varietal differences in protein content of rice was reported by De Data *et al*., 1972 and Gomez and De Data, 1975. A high-yielding improved line 'IR480-5-9'has high protein content in the grain (De Datta, 1972).

As the performance of an individual is influenced by genotype, environment and its genotype by environment interaction (Fehr, 1987; Wilkinson, 1994), it is important to study genotype × environment interaction on the characteristics of a crop. Studies on cultivar × environment interactions for yield of upland rice (Lafitte and Courtois, 2002), dry matter production of upland rice (Kato *et al*., 2006a), grain yield of upland rice (Kato et al., 2006b), and yield of chickpea (Kashiwagi et al., 2008) have been reported.

The information on genotype × environment interaction may be very useful for cultivar development program, such as adoption of appropriate selection method and identification of area where the new cultivars may be extended. Genotypes or cultivars which show few environmental interactions, and consistently rank high among all cultivars over the environments , are considered as agronomically stable (Zuders and Sherwood, 1985). Gomez and De Datta (1972) reported the grain yield and protein content of several promising rice cultivars. They found that crop season, location, N fertilizer, water management, and weed control were the major sources of variation in rice protein content.

In this study, four high-yielding improved genotypes of upland rice with wide adaptability (Totok et al., 2008) together with several improved breeding materials were evaluated for varietal difference and genotype × location interaction of the grain protein content.

Material and Methods

Material: Ten rice lines with various genetic backgrounds were used in this experiment. The lines were evolved from breeding program of different institutions. Situ Patenggang and Way Raremwere also used as reference genotypes. Both were upland rice cultivars released in 2002 and 1994, respectively by Ministry of Agriculture, Indonesia. Thus, 12 genotypes in total were used in this field experiment. The specification of material is presented in Table 1.

Methods and Design: The 12 genotypes were planted in the upland at five different locations in islands of Java, Bali, Lombok and Sumbawa, Indonesia in October 2008 and harvested in February 2009. The locations were Regency of :KulonProgo (KPr) and Cilacap (Cil) as representative of Java island, East Lombok (ELo) as representative of Lombok island, West Sumbawa (WSu) as representative of Sumbawa island, and Bangli (Bgl) as representative of Bali island, respectively. The map of locations was shown in figure 1. Average rainfall in a month was about 267.2 mm in KPr, 224.8 mm in Cil, 141.6 mm in ELo, 170.0 mm in WSu, and 122.4 mm in Bgl, respectively.

At each location, seeds of each genotype were sown directly in the rate 3 – 4 seeds per hole in October, 2008. Seedlings were then thinned to two seedlings per genotype. The plot size was 5 x 2.5 m^2 and planting distance was 25 cm between rows and between plants. Fertilizer was applied at the rate of 200 kg ha^{-1} of N, 100 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} K_2O. Half of N dosage together with P_2O_5 and K_2O was applied before sowing. Half of N dosage was applied at 2 weeks after sowing. No irrigation was given. Water supply was rainfall dependent. The experiment was a randomized complete block design with three replications. Therefore, five identical field experiments were conducted at five different locations of four islands.

Data for protein content of grain were collected from five randomly chosen plants from each plot. Protein content was calculated by multiplying total nitrogen content by 5.95, the constant to convert nitrogen content into protein content in rice, and expressed on a dry matter basis of milled rice. Total nitrogen content was estimated by Kjeldahls method. Yield (t ha^{-1}) was calculated from yield of 5 × 2.5 m plot.

The data of protein content and yield were subjected to analysis of variance. Means were separated by Duncan's Multiple Range Test (DMRT) when the variance analysis revealed significant differences (Steel and Torrie, 1980).

Results and Discussion

The analysis of variance for mean protein content data is presented in Table 2. It showed that difference among the genotypes for protein content is significant. It indicated the significance of varietal difference for protein content. It meant that the protein content of upland rice may be genotype-dependent character.

It was also found significant effect of location to the protein content, revealed the environment variability. It revealed that the protein content of upland rice may be changed due to the change in sowing location.

The results also showed that protein content of upland rice was significantly influenced by genotype × location interaction. It meant that difference of protein content among genotypes was depending on the location where each genotype was sown. Interaction may be defined as the differences in the magnitude of genotypes under different locations. The effect of genotype × location interaction for yield and yield components of aromatic upland rice has been reported by Totok et al., 2008.

Effect of genotype × location interaction on protein content may be showed in the changing of the genotype rank for protein content under different locations or changing of varied magnitude of protein content when the location was changed (Table 3).

Table 3 showed that in KPr, protein content of UNRAM 1E, UNRAM 4E, UNRAM 9E, UNRAM 17E and UNSOED G39 is higher than those of cultivar Situ Patenggang. But, only UNRAM 1E and UNRAM 9E showed as high protein content as cultivar Way Rarem. In Cil, protein content of UNRAM 1E, UNRAM 4E, UNRAM 9E, UNRAM 17E, UNSOED G10, UNSOED G136, and B124986-MR-1 is higher than those of cultivar Situ Patenggang. But, only UNRAM 1E, UNRAM 4E, UNRAM 9E, UNRAM 17E, UNSOED G136, and B124986-MR-1 showed higher protein content than cultivar Way Rarem. In ELo, all genotypes showed lower protein content compared with both cultivars Situ Patenggang and Way Rarem, except UNRAM 4E and UNRAM9E. In WSu, UNRAM 4E, UNRAM 9E, and UNRAM 17E showed higher protein content compared with cultivar Situpatenggang, but only UNRAM 4E and UNRAM 9E showed similar with cultivar Way Rarem. In Bgl, even the protein content of UNRAM 4E, UNRAM 9E, and UNRAM 17E is higher than cultivar Situ Patenggang, but no genotype showed similar or higher protein content than cultivar Way Rarem.

Mean protein content ranged from 5.35% (B12644F-MR-2 in WSu) to 9.73% (Way Rarem in ELo). For each genotype in all locations, protein content varied from 5.91% (B12644F-MR-2) to 8.93 % (UNRAM 17E), as shown in Fig. 2. The variation of rice protein content was reported by Gomez and De Datta (1975) where the protein content of IR8 ranged from 4.8 to 12.1% and of IR480-5-9 ranged from 6.4 to 17.4%.

The improved genotypes, without reference cultivars, may be separated into two groups, namely genotypes with protein content higher than 8% and genotypes with protein content lower than 8%. UNRAM 1E, UNRAM 4E, UNRAM 9E, and UNRAM 17E are genotypes belonging to the high protein content group which have similar or higher than that of reference cultivars. UNSOED G10, UNSOED G19, UNSOED G39, UNSOED G136, B12644F-MR-2 and B12498C-MR-1 belong to the low protein content which have lower than that of reference cultivars.

The presence of varietal difference for protein content in each location among genotypes indicated the possibility of breeding for high protein content of upland rice.

The protein content of genotypes in KPr varied from 6.29 % (Unsoed G10) to 7.79% (Way Rarem) with 7.00% mean value and in Cilit varied from 6.48% (B12644F-MR-2) to 9.46% (Unram 4E) with 7.90% mean value (Table 3 and Fig.3).

Mean protein content in each location for all genotypes varied from 7.00 to 8.35 (Fig. 3). Based on the effect of each location to the protein content, locations may be separated into two groups, namely locations which are suitable for producing protein content higher than 8% and locations which produce protein content lower than 8%. ELo, WSu, and Bgl are locations suitable for producing high protein content. Whereas, KPr and Cil are locations which produce lower protein content. Gomez and De Datta (1975) already mentioned that location is one of the major source of variation for rice protein content.

The three locations produced high protein content, namely ELo, WSu, and Bgl, have monthly rainfall of 141.6, 170.0, and 122.4 mm, respectively. These values were lower than that of the two locations which produced low protein content, namely KPr and Cil, which have monthly rainfall of 267.0 and 224.8 mm, respectively. It is found that in this study, the effect of location to the protein content was related to the rainfall. Location with lower monthly rainfall produced higher protein content, and *vice versa*.

Locations influence variation of grain protein content in upland rice, it is also depended on their genetic background. Watanabe et al. (2006) studied grain protein content of interspecific progenies derived from the cross of African rice and Asian rice. They concluded that protein content was considered as a genetic character of interspecific progeny though it was also affected by environment. Transgressive segregation of protein content is observed in the interspecific progenies that are attributed to a certain mechanism to concentrate protein in grains with a genetic background.

The genotypes having grain protein content similar or above that for the reference cultivars over all locations is considered to have high protein content stability. Our study showed that UNRAM 4E, UNRAM 9E, UNRAM 17 E had protein content above that of the cultivar Situ Patenggang and similar with cultivar Way Rarem over all locations. It shows that these genotypes may have high stability across locations (Fig. 4).

The performance of yield of all genotypes is shown in table 4. The yield varied from 3.23 (B12498C-MR-1) to 5.92 t ha^{-1} (Unsoed G39) in KPr, from 3.90 (B12644F-MR-2) to 6.04 t ha^{-1} (Unsoed G10) in Cil, from 3.14 (Unram 4E) to 5.40 (Stp) in ELo, from 4.08 (Wr) to 5.83 t ha^{-1} (Unsoed G39) in WSu, and from 4.34 (B12644F-MR-2) to 5.85 t ha^{-1} (Unsoed G10) in Bgl, respectively. Based on the difference in locations, the yield in average ranked as follows: Cil (4.99 t ha^{-1}), Bgl (4.95 t ha^{-1}), WSu (4.86 t ha^{-1}), KPr (4.53 t ha^{-1}) and ELo (4.20 t ha^{-1}).

Table 4 also showed that Unsoed G10 (5.11 t ha^{-1}) ,Unsoed G39 (5.14 t ha^{-1}) and B12498C-MR-1 (4.90 t ha^{-1}), Unram 1E (4.96 t ha^{-1}), and Unram 17E (4.88 t ha^{-1}) lines showed comparable yield with those of reference cultivars, Stp (5.29 t ha^{-1}) and Wr (5.05 t ha^{-1}). This result was comparable with previous study reported by Totok et al (2008).

UNRAM 4E, UNRAM 9E, UNRAM 17 E are genotypes developed through breeding program, for ecotype of East Indonesia, in Mataram University, with high protein content, high stability, and adapted to the low rainfall areas.These genotypes may be considered as candidates for new upland rice cultivars with high grain protein content character. Also, they may be considered as genetic resources for further development of high protein content upland rice cultivars.

It is concluded that genetic variability for grain protein content among genotypes was confirmed and the possibility for breeding of high grain protein content in upland rice. Genotype × location interaction influenced grain protein content of upland rice indicating that the genotypes gave different responses to the variation of locations. UNRAM 4E, UNRAM 9E, UNRAM 17E were stable genotypes as they showed consistently higher protein content across five locations. These three genotypes may be considered as genetic resources for further development of high protein content upland rice cultivars. This finding might be very useful in effort to improve nutritional quality of poor people in the rural area especially in East Indonesia (Nusa Tenggara) and semiarid area with low rainfall.

Acknowledgements

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References

Damarjati, D.S. 2006. Learning from Indonesian experiences in achieving rice self sufficiency. *In*Sumarno, Suparyono, A.M. Fagi, and M.O. Adnyana (Eds.).Rice industry, culture, and environment.Book 1.Indonesian Center for Rice Research. P. 21-32.

De Datta, SK, Obcemea, WN and Jana RK.1972. Protein content of rice grain as affected by nitrogen fertilizer and some triazines and substituted ureas. *Agron.J.*, **64**:785-788.

Gomez, K.A. and De Datta, S.K. 1975. Influence of Environment on protein content of rice. *Agron, J.*, **67**:565-568.

Fehr, W.R. 1987. Principles of cultivar development.Vol.1. Theory and technique.Collier Macmillan Publishers.London. 249-259.

Indrasari, S.D., Daradjat, A.A. and I. Hanarida. 2006. Prospect of iron biofortification on rice. In Sumarno, Suparyono, A.M. Fagi, and M.O. Adnyana (Eds.).Rice industry, culture, and environment.Book 1.Indonesian Center for Rice Research. P. 267-273.

Kashiwagi, J., Krishnamurthy, N., Gaur, P.M., Umezaki, T., Zheng, S.H., Hiraoka, Y., Terauchi, T. and Iwama, K. 2008.Genotype-environment Interaction in Chickpea (C. arietinum L.) for Adaptation to Humid Temperate and Semi-arid Tropical Environments.*Trop. Agr. Develop*., **52** (4): 89-96.

Kato, Y., Kamoshita, A., Yamagishi, J. and Abe, J. 2006a. Growth of three rice (*Oryza sativa* L) cultivars under upland condition with different levels of water supply 1.Nitrogen content and dry matter production.*Plant Proc. Sci.,* **9**: 422 – 434.

Kato, Y., Kamoshita, A., Yamagishi, J. and Abe, J. 2006b. Growth of three rice (*Oryza sativa* L) cultivars under upland condition with different levels of water supply 2.Grain yield.*Plant Proc. Sci.,* **9**: 435 – 445.

Lafitte, H.R. and Courtois, B. 2002. Interpreting cultivar × environment interactions for yield in upland rice: assigning value to drought-adaptive traits. *Crop Sci*., **42**:1409-1420.

Steel, R.G.D. and Torrie, J.H.1980.Principles and procedures of statistic.A biometrical approach.2nd.ed. McGrow-Hill, New York.401-437.

Tobiason, L. 2006. Rice Protein Guide – Rice Protein Enters the Winner's Circle. N101, Inc.

Totok A.D.H. 2004.Growth, yield and rice quality of F5 genotypes from crossing of Mentikwangi X Poso for developing of aromatic upland rice.*J. of Rur.Development.*JendSoedirman Univ., **2**:122-128.

Totok A.D.H. and Utari, R.S. 2005. Yield trial of F6 pure lines from crossing of Mentikwangi×Poso in comparison with their parents. Res. Report. Fac.of Agric. Jend. SoedirmanUniv. 22-30.

Totok, A.D.H., Suwarto and Yoshida, T. 2008. Yield stability of aromatic upland rice with high yielding ability in Indonesia. *Plant Proc. Sci*., **11**: 96 – 103.

Watanabe, H., Futakuchi, K., Jones, M.P. and Sobambo, B.A. 2006. Grain protein content of interspecific progenies derived from cross of African Rice (Oryza glaberrima Steud.) and Asian rice (Oryza sativa L.). *Plant Prod. Sci.*, **9**(3):287-293.

Wilkinson, R.E.1994. Plant-Environment Interactions. Marcel Dekker, Inc. New York, Basel. Hongkong.

Zeiders, K.F. and Sherwood, T. 1985. Environmental interactions among reed canarygrass genotypes for nutritive value, height, and disease severity. *Agron. J.,***77**:94-98.

Table 1. Origin of upland rice genotypes used for genotype × location interaction study

Genotypes	Origin
UNRAM 1E UNRAM 4E UNRAM 9E UNRAM 17E	Improved breeding materials of Plant Breeding Laboratory, MataramUniversity, Mataram, Indonesia.
UNSOED G10 UNSOED G19 UNSOED G39 UNSOED G136	Improved breeding materials of Plant Breeding Laboratory, JenderalSoedirmanUniversity, Purwokerto, Central Java, Indonesia
B12498C-MR-1 B12644F-MR-2	Improved breeding materials of IndonesianCenter for Rice Research, Subang, West Java, Indonesia
SITU PATENGGANG	Reference cultivar for high yielding upland rice, 2002 released, (low protein content)
WAY RAREM	Reference cultivar for high yielding upland rice, 1994 released, (high protein content)

Table 2. Analysis of variance for protein content of genotype × location interaction effect in upland rice

Source of variation	d.f.	Sum of square	F predicted
Genotypes	11	126.1836	< 0.001
Locations	4	42.4221	< 0.001
Genotypes × Locations	44	61.5372	< 0.001
Residual	110	29.4374	

Table 3. Average of grain protein content in each genotype at five locations.

Genotypes	Locations				
	KPr	Cil	ELo	WSu	Bgl
UNRAM 1E	7.49 abc q	8.93 b p	7.76 e q	8.05 e q	7.82 e q
UNRAM 4E	7.01 de q	9.46 a p	9.34 b p	9.28 ab p	9.51 b p
UNRAM 9E	7.69 ab r	8.70 b q	9.31 b p	9.45 ab p	9.51 b p
UNRAM 17E	7.35 bcd q	7.86 cd q	8.90 c p	9.18 b p	9.25 b p
UNSOED G10	6.29 h s	7.86 cd pr	7.33 fg r	7.35 f r	8.23 d p
UNSOED G19	6.39 gh r	7.06 e q	8.39 d p	8.47 cd p	8.28 d p
UNSOED G39	7.11 cdepr	6.79 ef r	6.99 g pr	7.28 f pr	7.50 e p
UNSOED G136	6.53 fgh q	7.96 c p	8.26 d p	8.15 de p	8.28 d p
B12498C-MR-1	6.80 efgh r	9.03 b p	7.63 ef q	7.38 f q	7.63 e q
B12644F-MR-2	6.92 ef p	6.48 f p	5.43 h q	5.35 g q	5.40 f q
SITU.PATENGGANG	6.60 fgh r	7.15 e q	9.15 bc p	8.78 c p	8.82 c p
WAY RAREM	7.79 a q	7.54 d q	9.73 a p	9.64 a p	9.91 a p

Values with the same letter (abcdefgh) in a column and with the same letter (pqr) in a line do not differ significantly at p=0.05.

Table 4. Yield (t ha^{-1}) in each genotype at five locations

Genotypes	KPr	Cil	ELo	WSu	Bgl	Avg.
Unram 1E	5.87 a	5.38 bc	3.65 de	4.87 bc	4.49 cd	4.96 bc
Unram 4E	3.76 de	4.90 cd	3.14 e	4.38 cd	4.85 bcd	4.48 c
Unram 9E	4.59 c	4.46 de	3.98 cd	5.22 ab	4.96 bcd	4.70 c
Unram 17E	4.34 cd	4.93 cd	4.36 c	4.63 bcd	5.24 ab	4.88 bc
Unsoed G10	3.34 e	6.04 a	5.40 a	4.81 bc	5.85 a	5.11 ab
Unsoed G19	4.86 bc	4.27 de	3.55 de	4.73 bcd	4.79 bcd	4.57 bc
Unsoed G39	5.92 a	4.94 cd	4.57 bc	5.83 a	5.30 ab	5.14 ab
Unsoed G136	4.58c	5.29 bc	3.79 d	4.63 bcd	5.16 bc	4.51 c
B12498C-MR-1	3.23 e	4.85 cd	5.10 ab	5.24 ab	5.18 bc	4.90 bc
B12644F-MR-2	4.87 bc	3.90 e	3.75 de	4.73 bcd	4.34 d	4.63 c
Stp	3.62 e	5.71 ab	5.40 a	5.18 ab	4.47 cd	5.29 a
WAY RAREM	5.43 ab	5.18 bc	3.73 de	4.08 d	4.81 bcd	5.05 ab
Average	4.53	4.99	4.20	4.86	4.95	

Values with the same letter in a column do not differ significantly at p=0.05.

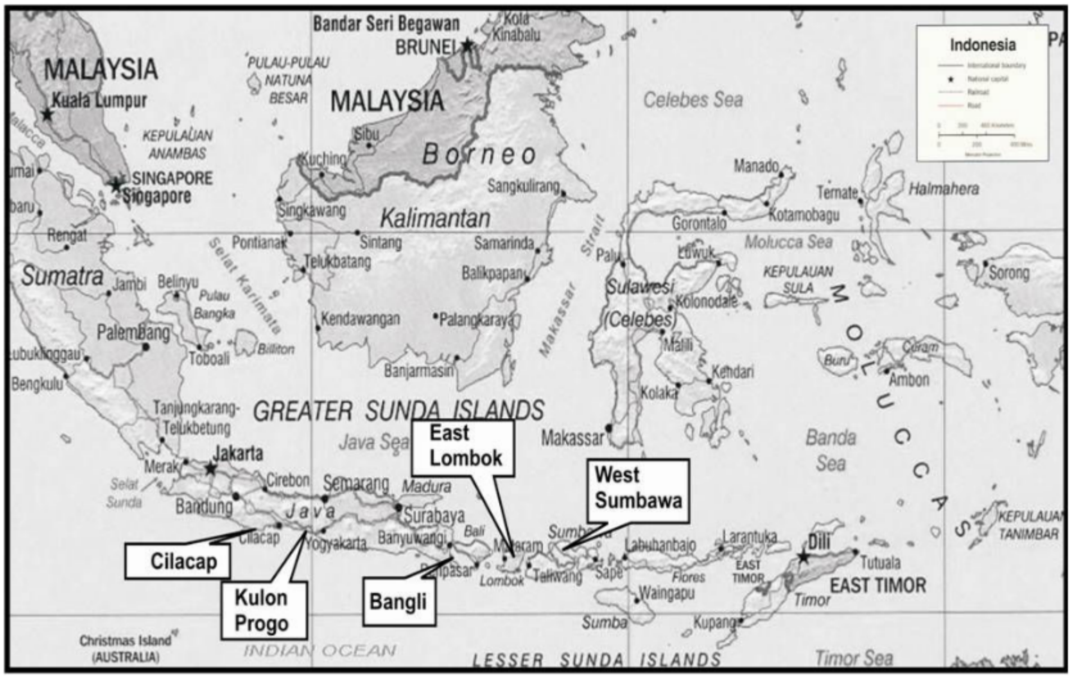

Fig 1. Map of Indonesia and locations where field experiment of varietal difference and genotype × location interaction of the protein content was conducted

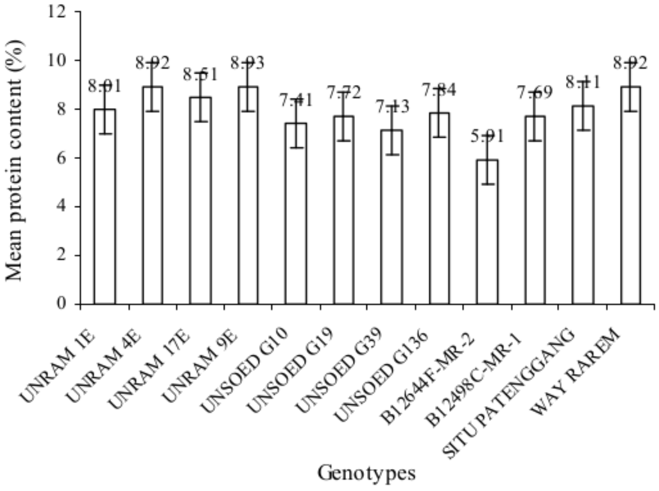

Fig. 2. Mean protein content in all locations for each upland rice genotype

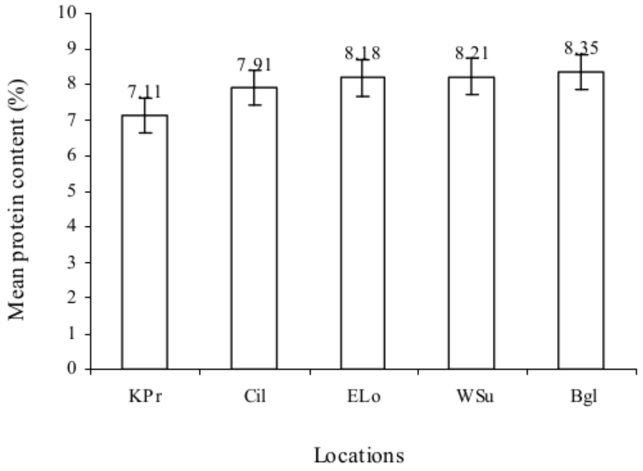

Fig. 3. Mean protein content of all upland rice genotype for each location

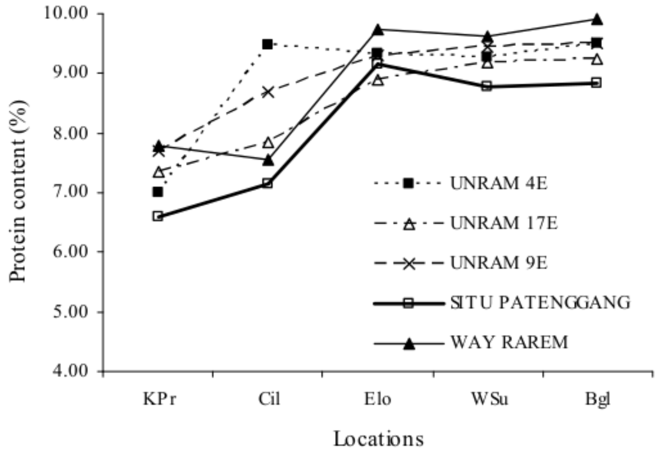

Fig.4. Genotypes having high protein content over all locations

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9