

DISTINCTNESS ASSESSMENT ON YARDLONG BEAN (*Vigna sesquipedalis* (L.) Fruhw.) VARIETIES (CASE STUDY FOR FIVE YARDLONG BEAN VARIETIES IN PVP RIGHT APPLICATION)

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ABSTRACT

The distinctness assessment is one main aspect in DUS test. Five candidates of yardlong bean varieties namely Brawijaya 1, Brawijaya 3, Brawijaya 4, Bagong 2 and Bagong 3 were examined to propose PVP right. Those varieties belong to Kuswanto of Brawijaya University. The test was carried out into two planting seasons (April-June 2011 and September-December 2011). Randomized complete block design was used as test design with a total population of 60 plants per variety, divided into three replicates, and the sample size was 21 plants or plant parts per variety. Six varieties of common knowledge KP1, KP7, Putih Super, Hijau Super, Parade, and Pangeran were chosen as control varieties due to their similarity. The observations were recorded on 50 DUS characteristics as listed in the test guideline document of DUS yardlong bean (PPU/PVT/19/2). Appropriate statistical analysis (cluster analysis, RCBD Anova and Duncan Multiple Range Test) was used to strengthen the distinctness decision. The results showed all candidates had some clear and consistent difference from their similar varieties. Thus, all candidates should pass the distinctness assessment, which was confirmed by the results of statistical analysis.

Keywords: distinctness, DUS test, PVP right

INTRODUCTION

The distinctness assessment is one aspect of Distinct, Uniform, Stable (DUS) test which is a requirement for obtaining Plant Variety Protection (PVP). It is regulated in Indonesia PVP Law no. 29 year 2000. In some

countries, PVP is also called PBR (Plant Breeder Rights) due to the right provided by the authority to give the breeder legal certainty for their breeding efforts and investment in developing a novelty variety.

Yardlong bean has been chosen as a model plant in this research because this species is placed at the first rank in Indonesian PVP application. The chief sub division of registration at PVP office of Indonesia mentioned that 124 PVP certificates had been issued, where 16 were certificates for yardlong bean varieties, per April 30th 2011 (Priyono, 2011). It is very common because yardlong bean is the popular vegetable in Indonesia (Bambang, 2006) and its production continuously increased and reached 488.174 ton in 2010 (BPS, 2010). This condition has led local breeders to competing in developing new superior varieties for yardlong bean.

Kuswanto of Brawijaya University in Malang, has recently developed five superior yardlong bean varieties labeled Brawijaya 1, Brawijaya 3, Brawijaya 4, Bagong 2 and Bagong 3. These varieties were conventionally assembled through hybridization and some selection methods (Kuswanto, Pers.com 2012). Stability in some locations for high potential yield, good young pods quality (Zacky, 2010) and resistance to pest and disease are some of main characteristics possessed by those five varieties. Those superior characteristics lead the breeder to have protection for the material by applying PVP application for all five varieties.

The distinctness assessment is one aspect of DUS test that will lead to the conclusion whether each of five candidates deserve to be granted PVP right. All DUS tests were conducted referring to the test guidelines

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issued by PVP office. The document number PPU/PVT/19/2 issued in 2010 is the guidelines for DUS test of yardlong bean in Indonesia. It listed 50 characteristics which should be observed according to the type of characters and should be recorded in state of notation (PPVT, 2010).

In Article 7 of the 1991 Act of the UPOV Convention mentioned that a “variety shall be deemed to be distinct if it is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of the filing of the application.” To assess the distinctness notation of each characteristic between candidate and control varieties were compared. The distinctness will be granted for candidate which has one or more clear and consistent different notes from their control varieties (UPOV, 2002; PPVT, 2006) using notation scoring. In this study, statistical analysis was used for distinctness assessment in order to give high accuracy for the final result.

MATERIALS AND METHODS

The experimental material involved 11 varieties of yardlong beans, consists of five candidate varieties and six control varieties. Six varieties of common knowledge were chosen to be the control, namely KP 1, KP 7, Putih Super, Hijau Super, Parade, and Pangeran. The trials were conducted in two planting seasons (April-June 2011) and (September-December 2011) in randomized block design with three replications. Each replication comprised 11 rows, and each row contained 20 plants with 40 cm space between plants and 60 cm space between rows. The sample size comprised 21 plants or part of plants (seven samples per replication). For determining distinctness, all characteristics observation was recorded by photo imaging and measurement. For qualitative characteristics that were visually assessed, the difference of two varieties was considered definite if the expression of one or more characteristics lies into two different states per test guideline. For quantitative or measured characteristics, it should have at least two notes different (UPOV, 2011). Cluster analysis was used for similarity analysis on qualitative characteristics, while RCBD anova and Duncan test were used to analyze the difference on quantitative characteristics. These analysis were

used to evaluate the result of note scoring method in assessing distinctness.

RESULTS AND DISCUSSION

Complete calculation and observation results for all varieties are shown on Table 1. Notation was given under its observation result suitability with notation table characteristic listed on yardlong bean DUS, PVT/PPU/19/2 document. Notation for quantitative characters was calculated based on the notation range taken from the observed sample plants. Median value of KP7 variety performance was used as calibrating tool in notation marking for other varieties.

Table 2 shows different characteristic amount between candidate and control varieties as a resume for Table 1. It is proofing the distinctness of all candidate varieties. There were few clear characteristic different rather than the most similar control varieties. The characteristic number of unclear expression is indicated by gray colour (Table 2). According to PVP role, the distinctness was only given when the definite difference consistently existed. However, the gray-marked characteristics need further analysis using appropriate statistical analysis for distinctness.

Brawijaya 1 has proven to be distinct (Figure 1). It shows more than one definite characteristic difference from all of the control varieties. The most differences between Brawijaya 1 and the control varieties (Putih Super and Pangeran) were found in ten characters (see Table 1). KP1 had eight definite and consistent characteristic differences from Brawijaya 1. The remaining three varieties, KP 7, Hijau Super and Parade which made them the most similar varieties for Brawijaya 1, had six different characteristics.

The similarity between Brawijaya 1 and KP 7 was understandable. This was because KP 7 was the purification of genotype MLG 15151 which was the male parent of Brawijaya 1. Characteristic no 6 in Table 1 shows that Brawijaya 1 was a considerably high variety among all varieties involved. Brawijaya 1 had different pod color compared to KP 7 and hijau Super. The pod colour of Brawijaya 1 was light green whereas KP 7 and Hijau Super were green and dark green, respectively (Figure 1).

Table 1. Observation result based on visual observation and note scoring method

[illegible]

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Table 1 (Continuous)

Characteristics	Brawijaya 1	Brawijaya 3	Brawijaya 4	Bagong 2	Bagong 3	KP 1	KP 7	Hijau Super	Putih Super	Parade	Pangeran
24 (+) Ground color at mature stage, fresh pod	1 Green Group 139C	1 Yellow Green Group 144A	3 Green Group 137D	1 Green Group 138B	3 Green Group 137D	2 Green Group 137B	3 Green Group 137D	2 Green Group 137B	1 Green Group 139D	2 Green Group 137C	3 Green Group 143A
24 (+) Ground color at mature stage, fresh pod	1 Green Group 139C	1 Yellow Green Group 144A	3 Green Group 137D	1 Green Group 138B	3 Green Group 137D	2 Green Group 137B	3 Green Group 137D	2 Green Group 137B	1 Green Group 139D	2 Green Group 137C	3 Green Group 143A
25 (+) Wall fiber, taken at dry pod maturity	1 fleshy type fiber	1 fleshy type fiber	1 fleshy type fiber	3 excessive shattering	1 fleshy type fiber	1 fleshy type fiber	1 fleshy type fiber	1 fleshy type fiber	3 excessive shattering	1 fleshy type fiber	1 fleshy type fiber
26 (+) (*) Beak position	1 marginal	1 marginal	1 marginal	1 marginal	1 marginal	1 marginal	1 marginal	1 marginal	1 marginal	1 marginal	2 non marginal
27 (+) Beak orientation	3 downward	3 downward	3 downward	3 downward	3 downward	3 downward	3 downward	3 downward	3 downward	3 downward	3 Downward
28 (+) Pod color	1 green	1 green	1 green	1 green	1 green	1 green	1 green	1 green	1 green	1 green	1 Green
29. Secondary pod color	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 Absent
30. Hue of secondary seed color	-	-	-	-	-	-	-	-	-	-	-
31 (+) Stringiness	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 absent	1 Absent
32 (+) Shape of distal part	2 acute-truncate	2 acute-truncate	2 acute-truncate	2 acute-truncate	2 acute-truncate	2 acute-truncate	2 acute-truncate	2 acute-truncate	2 acute-truncate	2 acute-truncate	2 acute-truncate
33. Length of beak (cm)	3 (1.04)	2 (0.86)	2 (0.92)	7 (3.43)	3 (1.20)	3 (1.01)	3 (1.26)	2 (0.62)	5 (2.05)	4 (1.62)	9 (4.21)
34.(+) Surface texture of pod	3 smooth	3 smooth	3 smooth	7 rough	3 smooth	5 medium	5 medium	7 rough	7 rough	5 medium	7 Rough
35. Constrictions of pod, immature stage	3 absent or week	3 absent or week	3 absent or week	5 medium	3 absent or week	3 absent or week	3 absent or week	3 absent or week	5 medium	3 absent or week	3 absent or week
36. Shelf life	5 medium	5 medium	5 medium	5 medium	5 medium	5 medium	5 medium	5 medium	5 medium	5 medium	5 Medium
37. 100 seed Weight (g)	5 (18.80)	5 (20.30)	4 (15.07)	5 (19.93)	5 (20.60)	4 (15.97)	5 (18.65)	5 (20.14)	5 (18.49)	5 (21.65)	4 (15.99)
38 (+) Seed shape of median longitudinal section	2 circular to elliptical	3 elliptical	2 circular to elliptical	3 elliptical	2 circular to elliptical	3 elliptical	4 kidney shaped	4 kidney shaped	2 circular to elliptical	4 kidney shaped	2 circular to elliptical
39 (+) Seed shape of median cross section	3 elliptical	2 circular to elliptical	2 circular to elliptical	2 circular to elliptical	2 circular to elliptical	2 circular to elliptical	2 circular to elliptical	2 circular to elliptical	2 circular to elliptical	2 circular to elliptical	3 Elliptical
40 9+) Seed degree of curvature for kidney shaped only	-	-	-	-	-	-	5 medium	5 medium	-	5 medium	-
41. Seed width in cross section	5 (0.53)	5 (0.54)	5 (0.49)	5 (0.54)	5 (0.53)	5 (0.47)	5 (0.51)	5 (0.55)	5 (0.50)	6 (0.56)	5 (0.49)
42. Seed length median (*)	5 (1.11)	5 (1.14)	5 (1.03)	5 (1.12)	5 (1.20)	5 (1.11)	5 (1.11)	5 (1.15)	5 (1.10)	5 (1.17)	5 (1.02)
43. Seed number of colors	2 two	3 more than two	3 more than two	2 two	2 two	3 more than two	2 two	2 two	2 two	2 two	2 Two
44 (+) Seed main color	4 maroon	4 maroon	2 brown	4 maroon	4 maroon	4 maroon	4 maroon	4 maroon	4 maroon	4 maroon	2 Brown
45 (+) Seed predominant secondary color	2 brown	1 white	1 white	2 brown	2 brown	1 white	2 brown	2 brown	2 brown	2 brown	2 Brown

Table 1. (Continous)

Characteristics	Brawijaya 1	Brawijaya 3	Brawijaya 4	Bagong 2	Bagong 3	KP 1	KP 7	Hijau Super	Putih Super	Parade	Pangeran
46 (+) Seed distribution of predominant secondary color	2 in streak	4 in patches at tip	4 in patches at tip	5 random	2 in streak	4 in patches at tip	2 in streak	2 in streak	5 random	2 in streak	5 Random
47 (+) (*) Seed color of hilus ring	1 Same color of seed	1 Same color of seed	1 Same color of seed	1 Same color of seed	1 Same color of seed	1 Same color of seed	1 Same color of seed	1 Same color of seed	1 Same color of seed	1 Same color of seed	1 Same color of seed
48 (+) Seed veining	5 medium	5 medium	5 medium	3 weak	3 weak	3 weak	5 medium	3 weak	3 weak	3 weak	3 Weak
49. Seed surface texture	1 smooth	1 smooth	1 smooth	1 smooth	1 smooth	1 smooth	1 smooth	1 smooth	1 smooth	1 smooth	1 Smooth
50. Seed number per pod	5 (18.29)	5 (16.57)	5 (17.24)	5 (16.43)	5 (17.67)	6 (19.24)	5 (17.33)	5 (17.05)	6 (19.48)	5 (17.38)	5 (17.19)

Table 2. Number of different characteristics for candidates vs control varieties

Candidate varieties	Control varieties					
	KP 1	KP 7	Hijau Super	Putih super	Parade	Pangeran
Brawijaya 1	4	4	4	5	5	4
Brawijaya 3	8	6	6	10	6	10
Brawijaya 4	6	3	3	4	3	2
	5	8	10	13	12	16
Bagong 2	4	2	4	7	3	3
	5	7	9	11	11	11
Bagong 3	5	-	3	6	2	4
	9	10	8	2	9	8
	4	3	3	7	4	6
	6	4	4	8	4	9

Remark: ☒ Indefinite different characteristics ☐ Definite different characteristics



Figure 1. Pod comparison of Brawijaya 1 with Hijau Super and KP7

Another definite difference of Brawijaya 1 was in smooth texture pod surface, circular and elliptical seed shape while the others had medium to rough pod surface and kidney shape of seed.

Table 1 shows that **Brawijaya 3** had different morphologies description from the six

control varieties. Four control varieties with the most difference were Hijau Super, Parade, Putih Super and Pangeran (more than 10 characters different). Two control varieties KP 1 and KP 7 were the most similar varieties to Brawijaya 3; each had five and eight characteristic differences.

The obvious differences can be seen from the base of the leaf, as well as some characteristics of the pod. The results of field observation showed that Brawijaya 3 had unique yellow green pod color and smooth surface texture.

Brawijaya 4 shows similarity to Brawijaya 3 (see Table 1) where both of them had many characteristic differences from control varieties. Table 1 shows three control varieties in which Putih Super, Parade, and Pangeran had 11 different characteristics from Brawijaya 4. Hijau Super had nine characteristic differences from Brawijaya 4. On the other hand, the two control varieties with the most definite similarity were KP 1 and KP7. Brawijaya 4 and KP 7 had similar pod size and color, but were different in pod texture. Brawijaya 4 had smooth pod texture rather than KP 7. Brawijaya 4 and KP 1 were different in pod colour, in which the candidate had green pod colour while the control had dark green pod colour. The seeds of Brawijaya 4 had circular to elliptical shape, small size (1,03 x 0,49 cm) and three colors (brown, white and maroon). It, moreover, had similarity to the seed of KP1, but it was different in seed veining. On the other hand, KP 7 had kidney shape and maroon color.

Bagong 2 was one of the offspring from the hybridization of Putih Super vs MLG 15151 genotype. The crossing pedigree explained high similarity between Bagong 2 and Putih Super. Even so, the candidate was still considered distinct because it had two definite different characteristics from Putih Super. Control varieties out of Putih Super showed many differences from Bagong 2. Pangeran and Hijau Super were the next similar varieties after Putih Super, for they had eight different characteristics. However, the most different varieties from Bagong 2 were KP1, KP7 and Parade.

The distinctness of Bagong 2 from Putih Super lay into two characteristics, namely the length of beak and seed shape of median longitudinal section. There were other differences as well between the two varieties, but they were concluded indefinite as differences by scoring method due to only one note difference. Table 1 shows the height of the first fruiting node as one of the characteristics that possessed indefinite difference for those control varieties. On the other hand, Bagong 2 had many definite difference in

characters compared to Pangeran, particularly in height of plant, color of wing, pod color, pod waist, constrictions of pod, length of beak and main seed color.

In general, **Bagong 3** was the candidate which had similarity to all control varieties, Putih Super and Pangeran. This fact was based on Table 1 showing a few numbers of characteristic differences between Bagong 3 and other control varieties. It was different from Hijau Super, Parade and KP 7 in four characters, and from KP1 in five characters. The distinctness of Bagong 3 to Hijau Super and Parade occurred in three different characteristics, namely pod color, pod surface texture and seed longitudinal shape. The most definite difference of Bagong 3 from the control varieties (Hijau Super and Parade) was that the first had smooth pod texture and circular-elliptical seed shape while the latter had rough pod texture and kidney seed shape.

Statistical Distinctness Assessment

In this study, statistical analysis, which was divided into statistical analysis for qualitative and quantitative characters, was used to adjust the type of characters,. Cluster analysis is used for similarity analysis based on qualitative characteristics, because the distinctness among the varieties can be depicted through dendrogram (Gupta *et al.*, 2010). The usage of this analysis will provide a similarity between the varieties involved in testing. The distinctness of candidate varieties could be seen when it lay in different hierarchical line at dendrogram chart below.

Figure 2. shows each candidate had a close line with several control varieties, but separated in different cluster. The figure shows that Brawijaya 1 and Bagong 3 were categorized in the same similarity degree with KP7, and Hijau Super and Parade were in the same hierarchal group. Brawijaya 3 and Brawijaya 4 had the most proximity with KP1. The other proximity was also observed in the cluster group of KP7, Hijau Super and Parade. Meanwhile, Bagong 2 occurred in the very close cluster line to Putih Super which means that the two varieties were very similar. Pangeran represented the farthest of all varieties involved, it was shown from the cluster line which was separated from all cluster groups.

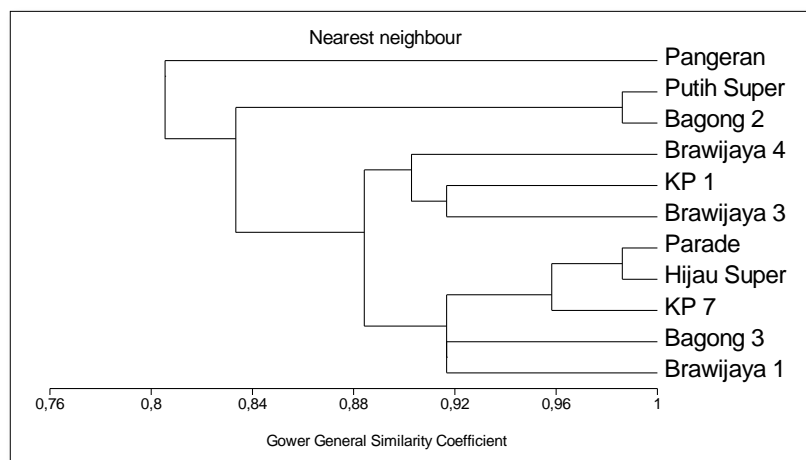


Figure 2. Dendrogram: distinctness based on qualitative characteristics

This dendrogram shows the same result in determining the most similar variety with the usual approach by counting the number of different characteristics among candidates with each control variety. Furthermore, we suggest that dendrogram can be used for similar varieties selection in determining the suitable control varieties before test implementation. The most importance of dendrogram is that it shows information that each candidate variety occurred in different cluster line, with value of similarity coefficient less than 1. It means Brawijaya 1, Brawijaya 3, Brawijaya 4, Bagong 2 and Bagong 3 was proven to be distinct.

The result of the distinctness shows great contradiction between result of Duncan analysis and range scoring notation (Table 1) such as number of days to 50% flowering (flowering age) character. All of the candidates were located in notation 5 (medium) as well as the control varieties, excluding Putih Super and Pangeran which were located in notation 4 (early maturing). Subsequently, one difference of notation mark means the differences are not clear enough (PPVT, 2006). However, the field visual observations obviously found that the flowering age of Putih Super and Pangeran had matured earlier than other varieties.

The result of Duncan test (Table 3) shows that flowering age character of Brawijaya 1 (38.23 dap), Brawijaya 3 (38.68 dap), Brawijaya 4 (38.30 dap) and Bagong 3 (39.34 dap) were significantly different to Putih Super (34.34 dap) and Pangeran (35.38 dap), whereas Bagong 2 (40.25 dap) was significantly different from all the control varieties.

For the distinctness assessment, it is not recommended to only use note scoring method because sometime this method is not accurate enough to find significant differences. Duncan test can be used to analyze the difference performance in these characteristics. This also suggests that Duncan test gave additional information which had made clear distinction among the candidates with the related comparison. This condition gave more reason to grant distinctness for all candidates.

For the quantitative characters that had a narrow range such as seed width character, PVP examiner should not consider making a distinctness decision based on statistical result only. It should be crosschecked with visual observation result. The distinctness assessment, related to DUS test must ensure that the differences between candidate and control varieties does not lie only in one character which has indefinite difference. If it happens, another test such as molecular test should be done for more accurate result of distinctness (Yim *et al.*, 2009).

Duncan test analysis can effectively be used to assess distinctness in DUS testing. The difference between scoring note method and Duncan test on pod length character showed similar results for Brawijaya 1. The results of the scoring showed that Brawijaya 1 was in the notation 8 (long to very long), considered clearly different from all the comparison. The remaining varieties were similar or one notation different (see table 1 character 21). Similar results were shown by Duncan test, in which pod length character in Brawijaya 1 was not significantly different from all control varieties.

Table 3. Duncan analysis for quantitative characteristics

Varieties		Quantitative Characteristics																			
		Petiole Length		Pod Length		Length of Beak		Seed Length		Seed Width		Seed Weight		Height of Plant		First Pod Bearing Node		Flowering Age		Number to 1 st Harvest	
candidates	Brawijaya 1	12.64	c	57.48	abc	1.04	ab	1.11	b	0.53	Bod	18.80	bc	114.55	od	104.37	f	38.23	c	42.27	c
	Brawijaya 3	11.12	abc	44.70	d	0.86	a	1.14	bc	0.54	od	20.30	bod	103.33	abc	83.67	od	38.68	od	42.73	c
	Brawijaya 4	10.90	ab	50.80	od	0.92	a	1.03	a	0.49	ab	15.07	a	104.54	abc	88.20	od	38.30	c	42.43	c
	Bagong 2	12.01	bc	55.37	abc	3.43	d	1.12	b	0.54	od	19.93	bod	98.31	a	79.23	bc	40.25	e	44.23	e
	Bagong 3	10.28	a	54.21	bc	1.20	ab	1.20	c	0.53	bod	20.60	od	114.04	bod	91.05	ode	39.34	d	43.43	d
comparisons	KP 1	11.50	abc	56.72	abc	1.01	ab	1.11	b	0.47	a	15.97	a	118.20	d	92.03	de	38.89	od	42.81	od
	KP 7	12.48	c	52.66	c	1.26	ab	1.11	b	0.51	abod	18.65	b	101.56	ab	84.63	od	38.90	od	42.85	od
	Hijau Super	10.94	ab	57.29	abc	0.62	a	1.15	bc	0.55	d	20.14	bod	111.65	bod	101.05	ef	38.75	od	42.78	od
	Putih Super	11.59	abc	60.74	ab	2.05	c	1.10	b	0.50	abc	18.49	b	98.33	a	71.47	ab	34.34	a	38.31	a
	Parade	10.71	ab	54.88	bc	1.62	bc	1.17	bc	0.56	d	21.65	d	123.40	d	110.74	f	38.76	od	42.72	c
	Pangeran	11.48	abc	61.61	a	4.21	e	1.02	a	0.49	ab	15.99	a	101.11	ab	65.06	a	35.38	b	39.36	b

Duncan test showed slightly different results on the length of pods of Brawijaya 3. It was significantly different from all the varieties of comparison. The scoring of Brawijaya 3 was included at note 6 (medium-long), which was considered to be indefinitely different from KP7 (52.66 cm) and Parade (54.88 cm) which lay in note 7.

The scoring note observation for length of beak character put varieties into various note. Most varieties had short beak on note 2 and 3, excluding Bagong 2 (note 7), Putih Super (note 5) and Pangeran (note 9). Duncan test analysis gave the same result as the scoring note method. This condition clarified the previous argument that Duncan test always gave different result with note scoring method in determining the clear significant differences.

Finally, the Duncan analysis showed more accurate result than scoring method. Duncan analysis showed more significantly different result than scoring notation method for nine characters, namely: number of days to 50% flowering, maturity (number of days to 1st harvesting or harvesting time), height of plant, first pod bearing node, height of plant, first fruiting node, petiole length, pod length, seed weight (for 100 seeds), seed width in cross section and seed length. Duncan analysis gave the same significant result with note scoring method only for length of pod beak character.

CONCLUSION AND SUGGESTIONS

CONCLUSION

The distinctness assessment result shows that all of five candidates of yardlong bean varieties had proven to be distinct.

The cluster analysis gave the same accurate result for similarity analysis based on qualitative character. In general, the statistical analysis and the note scoring method showed different comparison result for the distinctness assessment in quantitative characters. Overall, the Duncan analysis gives higher accuracy in determining distinctness in measured characteristics.

Despite all the arguments, all five candidates had proven to be distinct from all control varieties. Significant differences influenced the distinctness decision of each candidate variety. Candidates should meet the distinctness requirement in relation to the DUS test for having PVP right protection.

SUGGESTIONS

Significant differences influenced the distinctness decision of each candidate variety. Therefore, the authors suggest that the PVP examiner not decide distinctness in quantitative characters based on the note scoring method only. The results of scoring notation that gave indefinite differences (only showing one note different) on quantitative characters had to be followed by a statistical analysis to test significance.

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