

# *Amaranthus* genetic resources in Indonesia: morphological and protein content assessment in comparison with worldwide amaranths

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**Abstract** The morphological variations (growth habit, leaf shape, leaf color, height, stem diameter, number of branches and internodes, leaf area, blade ratio, leaf thickness, number of leaves, and total leaf area) and protein content of 53 Indonesian amaranths (*Amaranthus* spp.), consisting of weedy-, vegetable-, and ornamental-types, were assessed. The extent of variation in the Indonesian *Amaranthus* accessions were compared with the worldwide collection (26 accessions from United States Department of Agriculture (USDA) and 5 ornamentals from Nepal) in the experimental field of the University of Tsukuba, Japan. The variation in average values of most morphological traits and protein content in the Indonesian accessions were similar to those of the worldwide germplasm, but the important parameters that influence vegetable yield (e.g., number of leaves and stem diameter) were superior in the Indonesian accessions. Protein content showed a positive correlation with the number of leaves, whereas a negative correlation was observed with leaf thickness. The Indonesian accession of *A. viridis* and *A. dubius* showed a great potential to be further selected as parental lines for high protein content and number of leaves. The relatively high protein content of the

leaves of *Celosia* may also potentially serve as an alternative protein source in the tropics.

**Keywords** *Amaranthus* · *Celosia* · Morphological diversity · Vegetable

## Introduction

The genus *Amaranthus* consists of up to 70 species (in the form of cosmopolitan weed or cultivated plant) and are widely spread in all tropical and subtropical regions of the world (Espitia-Rangel 1994). They are usually distinguished as grain, vegetable, ornamental, or weedy (i.e., uncultivated) (Brenner et al. 2000). The grain and the vegetable types, whose grain and leaves are highly appreciated for human consumption, are the two major cultivated amaranths. At the same time, the uncultivated amaranths are all weedy, including those that have not yet been cultivated or may escape out of cultivation (Pal 1972). The vivid color of the inflorescence of amaranths makes them attractive enough to be used as ornamental plants.

The utilization of *Amaranthus* as grain, vegetable or weedy depends on the regional preferences, as some grain species (*A. hypochondriacus* L., *A. caudatus* L., and *A. cruentus* L.) may also be utilized as vegetables in Asian and African countries (Hauptli and Jain 1983). *A. blitum* L. and *A. dubius* Mart. ex Thell. (also referred to as *A. dubius*) are associated with weedy

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species. Nevertheless, they are also valued as vegetables in most Asian and African countries (Costea et al. 2003; Grubben 2004). The prominent vegetable species is *A. tricolor* L., whose leaves are served as an affordable protein source in South-East Asia (Grubben 1994). The young leaves of the ornamental amaranths *Celosia* spp., which still belong to the family Amaranthaceae (Grubben 1976) are highly appreciated for human consumption in many African countries (Denton 2004; Olaniyi and Ojetayo 2012).

Vegetable amaranths are very popular in Indonesia (Grubben 1994). They are frequently traded in Indonesian traditional markets and are ranked as the third most-consumed leafy vegetables after ‘kangkong’ (*Ipomoea aquatica* Forsskal; further referred as ‘kangkong’) and cassava leaves (*Manihot esculenta*) (Maulana and Sayaka 2007). They are commonly sold in bundles of 10–20 plants and can be harvested within 3 weeks, with a yield ranging from 2 to 5 tonnes ha<sup>-1</sup> per harvest season (Hadisoeganda 1996). Approximately 65,723 and 152,334 tonnes of amaranths were produced in 2000 and 2010, respectively (Indonesian Central Statistic Bureau, cited in December 2011 at <http://www.bps.go.id>). Amaranths are considered as economically important in Indonesia, as they allow rapid income generation and support small farmers’ livelihood.

Despite their popularity in Indonesia, there has been no efforts to improve the nutritional value and productivity of *A. tricolor* L. as the major cultivated vegetable species. Its average protein content in general is approximately 12 % (Shukla et al. 2010). Moreover, the yield noted with leaf production per plant is quite low when compared with other vegetables available in Indonesia; such as cassava and “kangkong” leaves (Wargiono et al. 2002; Westphal 1994). Indonesia is recognized as a biodiversity hotspot in the tropics, and many varieties of amaranths (e.g., vegetable, weedy, and ornamentals) exist. Thus, weedy amaranths bear great potential as good resources for nutritional enhancement as some *A. blitum* L. was reported to have as much as 38 % protein (Grubben 2004). Moreover, many weedy types showed a great deal of variation with favorable crop morphology (high leaf number, many branches). In the remote highland areas of Takengon in Sumatra, local people have utilized some weedy types closely resemble with the vegetable amaranths as famine food, while the other types have been used as

traditional medicine (personal communication). Thus, taking into account the gaps in knowledge of the extent of the Indonesian resources, a thorough assessment of these with the rest of the accessions with regard to nutritional and agronomic features is urgently required.

Research on the extent of the diversity of amaranths would enable us to choose some of the weedy types as prospective genetic resources for useful traits. At the molecular level, Chan and Sun (1997) had assessed the diversity of 23 cultivated species and their relationship with wild *Amaranthus* by using isozyme and RAPD markers and they found that wild or weedy amaranths were found to be highly polymorphic. Coons (1982) tried to evaluate the yield related traits in grain amaranths (*A. caudatus* and *A. hybridus*) such as the flower’s component, seed diameters and color. She found that *A. hybridus* showed larger variability in these characters than *A. caudatus*. Other Asian researchers had tried to evaluate the field performance of *Amaranthus* in China and the diversity of vegetable amaranths, particularly *A. tricolor* L. (Wu et al. 2000; Shukla et al. 2010). However, information on the diversity of Indonesian amaranths and the potential of the weedy-types in breeding program has not been reported.

Thus, the aims of this study are to: (1) assess the variations in morphology and protein content of Indonesian amaranths and compare them with the worldwide variation; (2) evaluate the relative potential of Indonesian amaranths for vegetable improvement. These findings are expected to be a valuable contribution to amaranth breeding efforts in Indonesia.

## Materials and methods

### Plant materials

In this study, a total of 84 amaranth accessions consisting of grain-, ornamental-, vegetable-, and weedy-types were assessed (Table 1), of which 53 were collected from Sumatra and Java islands of Indonesia in 2008 and 2010 (Fig. 1). The Indonesian collection consisted of 2 accessions of *A. blitum* L., 2 accessions of *A. caudatus* L., 16 accessions of *A. dubius*, 3 accessions of *A. hybridus*, 5 accessions of *A. spinosus*, 20 accessions of *A. tricolor* L., 1 accession of *A. viridis*, and 4 accessions of *Celosia* spp. Seeds were collected from various sites such as

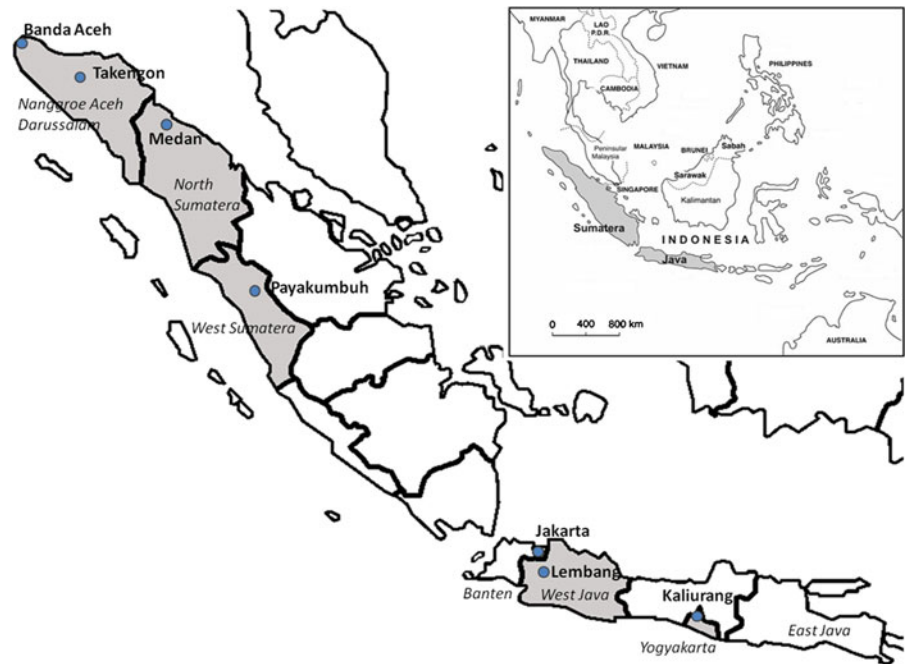
**Table 1** List of plant materials

Accession number	Accession name	Type	Species	Accession number	Accession name	Type	Species
	Worldwide			42	IDN 10/P.One-one	WD	<i>A. spinosus</i>
1	Ames 5315/IND	VG	<i>A. blitum</i> L.	43	IDN 11/Tn.Depet	WD	<i>A. spinosus</i>
2	PI 610262/IND	VG	<i>A. blitum</i> L.	44	IDN 13/Mandua	WD	<i>A. dubius</i>
3	PI 490298/KEN	VG	<i>A. blitum</i> L.	45	IDN 14/Mandua	WD	<i>A. dubius</i>
4	PI 606281/BGD	VG	<i>A. blitum</i> L.	46	IDN 15/Mandua	WD	<i>A. dubius</i>
5	PI 606282/BGD	VG	<i>A. blitum</i> L.	47	IDN 17/Medan	WD	<i>A. dubius</i>
6	PI 482049/ZWE	GR	<i>A. cruentus</i> L.	48	IDN 18/Medan	WD	<i>A. blitum</i> L.
7	PI 482051/ZWE	GR	<i>A. cruentus</i> L.	49	IDN 19/Mandua	WD	<i>A. blitum</i> L.
8	PI 490662/BEN	GR	<i>A. cruentus</i> L.	50	IDN 20/Ygy	WD	<i>A. dubius</i>
9	PI 494777/ZMB	GR	<i>A. cruentus</i> L.	51	IDN 22/Ygy	WD	<i>A. dubius</i>
10	PI 500267/ZMB	GR	<i>A. cruentus</i> L.	52	IDN 23/Ygy	WD	<i>A. dubius</i>
11	PI 538319/USA	GR	<i>A. cruentus</i> L.	53	IDN 24/JKT	WD	<i>A. dubius</i>
12	PI 566897/IND	GR	<i>A. cruentus</i> L.	54	IDN 25/JKT	WD	<i>A. dubius</i>
13	PI 604666/USA	GR	<i>A. cruentus</i> L.	55	IDN 26/JKT	WD	<i>A. dubius</i>
14	PI 605352/JAM	VG	<i>A. dubius</i>	56	IDN 27/JKT	WD	<i>A. dubius</i>
15	PI 642737/PRI	VG	<i>A. dubius</i>	57	IDN 29/K.Urang	VG	<i>A. tricolor</i> L.
16	PI 608661/IND	VG	<i>A. graecizans</i>	58	IDN 30/K.Urang	VG	<i>A. tricolor</i> L.
17	PI 500249/ZMB	GR	<i>A. hybridus</i>	59	IDN 33/Lembang	VG	<i>A. caudatus</i> L.
18	PI 605351/GRC	GR	<i>A. hybridus</i>	60	IDN 34/Lembang	VG	<i>A. caudatus</i> L.
19	PI 604577/MEX	GR	<i>A. hypochondriacus</i> L.	61	IDN 35/BNA	VG	<i>A. tricolor</i> L.
20	Ames 5134/USA	VG	<i>A. tricolor</i> L.	62	IDN 36/BNA	VG	<i>A. tricolor</i> L.
21	PI 349553/PNG	VG	<i>A. tricolor</i> L.	63	IDN 37/BNA	VG	<i>A. tricolor</i> L.
22	PI 566899/IND	VG	<i>A. tricolor</i> L.	64	IDN 38/BNA	VG	<i>A. tricolor</i> L.
23	PI 604669/TWN	VG	<i>A. tricolor</i> L.	65	IDN 39/BNA	OR	<i>Celosia</i> spp.
24	PI 586680/USA	OR	<i>C. argentea</i>	66	IDN 40/BNA	VG	<i>A. tricolor</i> L.
25	PI 482244/ZWE	OR	<i>C. trigyna</i>	67	IDN 41/Marelan	VG	<i>A. tricolor</i> L.
26	PI 608761/IND	VG	<i>A. tricolor</i> L.	68	IDN 42/Marelan	VG	<i>A. tricolor</i> L.
27	NPL 01/KTM	OR	<i>Celosia</i> spp.	69	IDN 43/Marelan	VG	<i>A. tricolor</i> L.
28	NPL 02/KTM	OR	<i>Celosia</i> spp.	70	IDN 44/Marelan	VG	<i>A. tricolor</i> L.
29	NPL 03/KTM	OR	<i>Celosia</i> spp.	71	IDN 45/Marelan	VG	<i>A. tricolor</i> L.
30	NPL 04/KTM	OR	<i>Celosia</i> spp.	72	IDN 46/Kresek	VG	<i>A. tricolor</i> L.
31	NPL 05/KTM	OR	<i>Celosia</i> spp.	73	IDN 47/Kresek	VG	<i>A. tricolor</i> L.
	Indonesia			74	IDN 48/Kresek	VG	<i>A. tricolor</i> L.
32	PI 540445/IDN/Java	WD	<i>A. viridis</i>	75	IDN 49/Yates	OR	<i>Celosia</i> spp.
33	IDN 01/Mongal	WD	<i>A. dubius</i>	76	IDN 50/P. Merah	VG	<i>A. tricolor</i> L.
34	IDN 02/Daling	WD	<i>A. dubius</i>	77	IDN 51/P. Merah	VG	<i>A. tricolor</i> L.
35	IDN 03/Daling	WD	<i>A. dubius</i>	78	IDN 52/SHS	VG	<i>A. tricolor</i> L.
36	IDN 04/Bur biah	WD	<i>A. spinosus</i>	79	IDN 53/SHS	VG	<i>A. tricolor</i> L.
37	IDN 05/Bur biah	OR	<i>Celosia</i> spp.	80	IDN 54/Tanindo	VG	<i>A. tricolor</i> L.
38	IDN 06/Bur biah	OR	<i>Celosia</i> spp.	81	IDN 55/P.Kb	WD	<i>A. hybridus</i>
39	IDN 07/Ulu Nuih	WD	<i>A. spinosus</i>	82	IDN 56/P.Kb	WD	<i>A. spinosus</i>
40	IDN 08/Asir-asir	WD	<i>A. dubius</i>	83	IDN 57G/P.Kb	WD	<i>A. hybridus</i>
41	IDN 09/Asir-asir	WD	<i>A. dubius</i>	84	IDN 57R/P.Kb	WD	<i>A. hybridus</i>

GR grain, OR ornamental, VG vegetable, WD weedy-type of amaranth

Country code: BEN Benin, BGD Bangladesh, GRC Greece, IDN Indonesia, IND India, JAM Jamaica, KEN Kenya, MEX Mexico, NPL Nepal, PNG Papua New Guinea, PRI Puerto Rico, TWN Taiwan, USA United States of America, ZMB Zambia, ZWE Zimbabwe. City code: BNA Banda Aceh, JKT Jakarta, KTM Kathmandu, P.Kb Payakumbuh, Ygy Yogyakarta. Seed producer code: P.Merah Panah Merah, SHS Sang Hyang Sri

**Fig. 1** Map of the sample collection regions (blue dots) for Indonesian amaranths



farmers' fields located in the villages and urban areas, home yards, and disturbed habitats such as roadsides, open or abandoned places, riverbanks, lake sides, and communal forests at the mountainous areas, with the elevation ranging from 100 to 1,200 m above sea-level.

A total of 31 worldwide accessions consisting of grain-, vegetable-, and ornamental-types were also assessed in this study. Seeds from 13 diverse origins (Africa, America, and Asia) representing the worldwide variation was provided by the United States Department of Agriculture–Agricultural Research Service (USDA-ARS), North Central Regional Plant Introduction Station (NCRPIS) in Ames (Iowa, USA). These consisted of 5 accessions of *A. blitum* L., 8 accessions of *A. cruentus* L., 2 accessions of *A. dubius*, 1 accession of *A. graecizans*, 2 accessions of *A. hybridus*, 1 accession of *A. hypochondriacus* L., 5 accessions of *A. tricolor* L., and 2 accessions of *Celosia*. In addition, 5 accessions of *Celosia* spp. were purchased in 2010 by one of the author in Kathmandu (Nepal). Species of the USDA accessions were identified by USDA personnel and available online (USDA 2011). The Indonesian materials were classified on the basis of their utilization (e.g., weedy-, vegetable-, or ornamental-types), as described by local farmers and according to amaranth's descriptors (Brenner 2002; Grubben 1976).

#### Experimental site

The experiment was conducted in vinyl houses located at the experimental field of the Agriculture and Forestry Research Centre, University of Tsukuba (Ibaraki, Japan), which is situated approximately 28 m above sea-level (36°07'01.71" latitude and 140°05'40.24" longitude) with a mean temperature of 20 °C during the growing season. Seeds from the 84 accessions were germinated on 6 August 2010 in plastic trays (6 × 6 holes; diameter, 4 cm; depth, 4.7 cm) containing a growing medium without fertilizer ("Metromix" from Sunagro). Three weeks after sowing, 3 or 4 plants per accession were selected and transplanted into clay pots (diameter, 18 cm; height, 16.5 cm) with ready soil "Sumirin" (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O = 120:1,000:50 mg/l, pH = 6.7). From each pot that contained 3 or 4 plants, only 1 plant was selected for the trait measurement. Five pots were prepared for each accession. The pots were arranged according to a completely randomized experimental design.

#### Morphological traits

Measurements of morphological traits according to guidelines provided in *Amaranthus*' descriptor (Brenner 2002) and Grubben (1976); were taken before flowering 2–3 months after sowing. The twelve

morphological traits included qualitative, plant architecture, leaf character, and vegetable production (Table 2). All quantitative traits presented were mean values of measurements on five plants. Data on quantitative and qualitative characteristics were compared between the Indonesian and worldwide accession on histogram.

Stem diameter and leaf thickness were measured with a slide caliper (Digimatic Solar DC-S15 m, Mitutoyo, Japan). Leaf area was measured by using VH-analyzer image analyses software (version 2.20, Keyence Co., Ltd., Osaka, Japan). Before measurement, the leaves were arranged on a white paper background and scanned on a GT-9800 F scanner (EPSON, Tokyo, Japan), with a 1-cm<sup>2</sup> color marker as the standard. Three different representative leaf sizes (small, middle, and large) per plant were randomly sampled for measurement of leaf thickness and area.

The values for leaf thickness and area represented average values of 15 measurements (5 plants per accession for each of the 3 different leaf sizes). Some weedy types had many very small leaves along the branches therefore, countable leaves were counted as number of leaves per plant. Total leaf area was calculated by multiplying the number of leaves per plant with the average leaf area. This was applied as an indirect measure of productivity in amaranths.

#### Protein analysis

Crude protein content was determined by the Kjeldahl method; protein content was determined as percentage of dry weight by multiplying the nitrogen content (N) by the conversion factor of 6.25 ( $N \times 6.25$ ) (AOAC 1980). Fresh leaves (10–25 g) were cut from 2 to 3-month-old plants. Leaves were ground in liquid

**Table 2** List of morphological traits analyzed

Number	Morphological trait	Method	Data-type
<b>Qualitative traits</b>			
1.	Growth habit <sup>a</sup>	Visual observation	Category
2.	Leaf shape <sup>b</sup>	Visual observation	Category
3.	Leaf color <sup>c</sup>	Visual observation	Category
<b>Plant architecture traits</b>			
4.	Plant height (cm)	Measurement of the height of a plant from the soil surface to the top of the inflorescence	Numerical
5.	Stem diameter (mm)	Measurement of the diameter of the middle part of the main stem	
6.	Number of branches on the main stem (no.)	Number of branches were counted along the main stem at the maturity growth stage	Numerical
7.	Number of internodes on the main stem (no.)	Number of internodes were counted along the main stem at the maturity growth stage	Numerical
<b>Leaf character traits</b>			
8.	Leaf area (cm <sup>2</sup> )	Measurement of the area of one leaf using VH-analyzer software	Numerical
9.	Blade ratio (length:width)	Measurement of the length and width of a blade	Numerical
10.	Leaf thickness (mm)	Measurement of the thickness at the centre of each blade	Numerical
<b>Vegetable production traits</b>			
11.	Number of leaves per plant (no.)	The main countable leaves were counted along main stem	Numerical
12.	Total leaf area (cm <sup>2</sup> )	Data from number of leaves per plant were multiplied with the average leaf area	Numerical

<sup>a</sup> 1 = erect or horizontally drooping at approximately 1–10°, 2 = semi-erect or horizontally drooping at approximately 11–20°, 3 = semi-erect or horizontally drooping at approximately 21–30°, 4 = horizontally drooping at approximately 31–40°, 5 = horizontally drooping at approximately 41–50°, 6 = horizontally drooping at approximately 51–60°, 7 = horizontally drooping at approximately 61–70°, 8 = horizontally drooping at approximately 71–80°, and 9 = prostrate or horizontally drooping at approximately 81–90°

<sup>b</sup> 1 = elliptic, 2 = ovate, 3 = lanceolate, 4 = obovate, 5 = cordate, 6 = obtuse, 7 = aristate, 8 = linear, 9 = not known

<sup>c</sup> 1 = dark green, 2 = green, 3 = green-variegated red or red-variegated green, 4 = red–purple–variegated green, 5 = red–purple

nitrogen and then freeze-dried (FD-1, EYELA, Rikakikai Co., Ltd., Tokyo, Japan). Samples (dry weight, 1 mg) were analyzed in duplicates.

### Data analysis

Data distribution of morphological traits was plotted using Sigma Plot version 10. All statistical analyses (ANOVA, *t* test, *F* test, and correlation and cluster analysis) were performed using the JMP version 7.0 (SAS Institute, Cary, NC, USA). Based on the field observation, the phenotypic performance was varied among the two groups (Indonesian and worldwide accession). Therefore, the *t* test was used to compare mean values, and the *F* test was used to compare the variances between the two groups. Correlation analysis was performed to elucidate the relationships among the investigated traits. Cluster analyses using Ward's method was employed to group the 53 Indonesian accessions and to bring out the patterns of similarity and dissimilarity based on 10 morphological characters. By applying the cluster methods, selection of parental lines with desirable traits would be achieved. Two-dimensional scatter plots were used to assess the relationship between protein content and selected vegetable traits such as number of leaves, total leaf area and leaf thickness.

## Results

### Morphological traits and protein content

The variation in morphological traits and leaf protein content in Indonesian and worldwide amaranths were presented in Fig. 2. The morphological traits were grouped into four categories: (1) qualitative (Fig. 2a–c), (2) plant architecture (Fig. 2d–g), (3) leaf character (Fig. 2h–j), and vegetable production traits (Fig. 2k–l).

### Qualitative traits

Most of the Indonesian accessions (83 %) possessed upright stature and the rest were prostrate (9 %) and semi-erect (6 %), whereas in the worldwide accessions, 87 % were upright and the rest were horizontally drooping with various deflection angles (10 %) and prostrate (3 %) (Fig. 2a). The predominant leaf shape in the Indonesian accessions was ovate (81 %), whereas

other leaf shapes observed were elliptic (9 %), aristate (6 %), and lanceolate (4 %). In the worldwide accessions, a majority of the leaves were ovate (65 %), whereas the rest were elliptic (10 %), lanceolate (10 %), aristate (7 %), obovate (3 %), or obtuse (3 %) (Fig. 2b). Both groups showed high variability in leaf color, with green as the predominant (70 %) color. In the Indonesian group, other leaf colors included red–purple (15 %), green-variegated red or vice versa (9 %), and very dark green (8 %). In the worldwide accessions, the other leaf colors observed were green-variegated red or vice versa (13 %), red–purple (13 %), and red–purple–variegated green (3 %) (Fig. 2c).

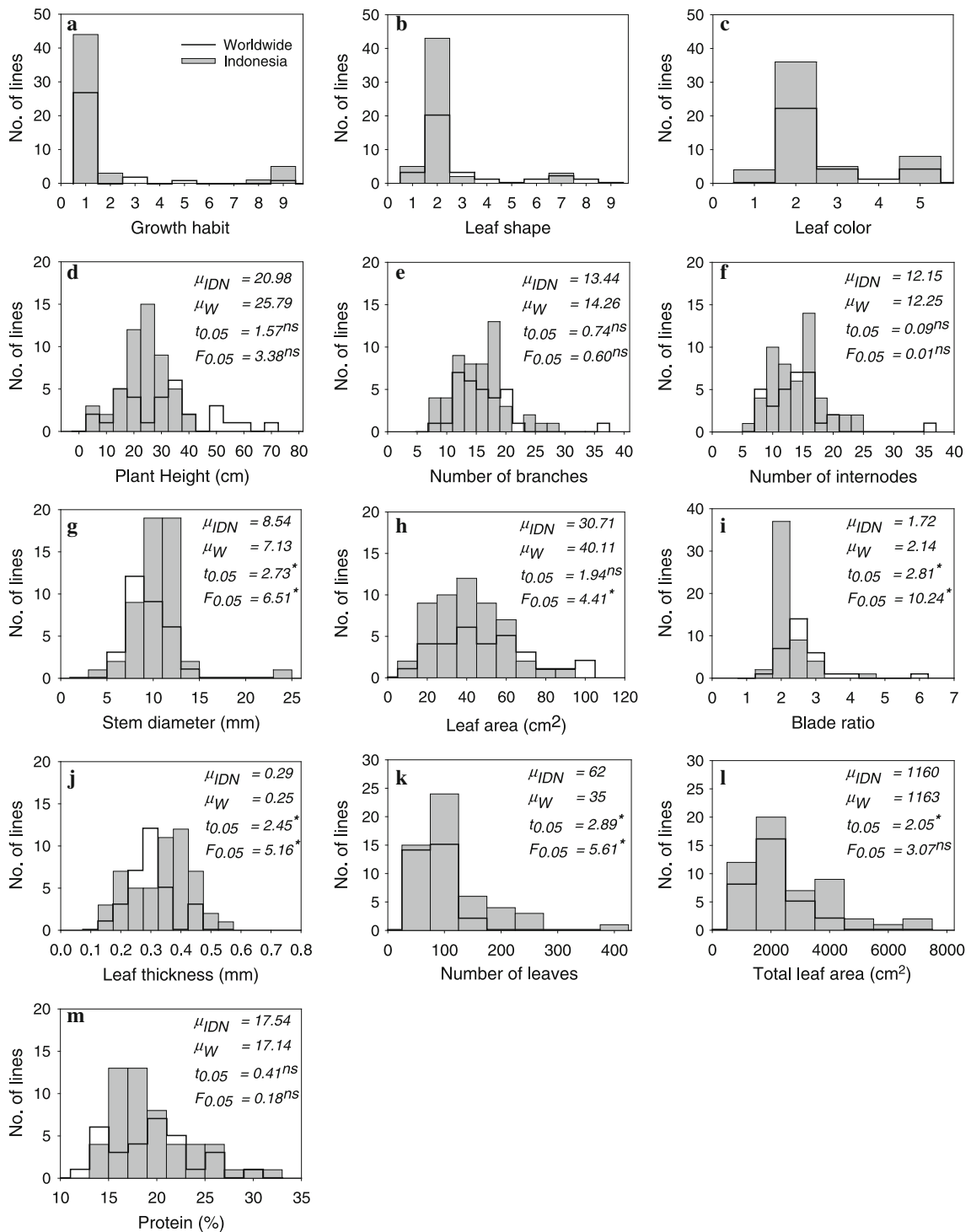
### Plant architecture traits

No statistically significant differences in terms of plant height and number of branches and internodes were observed between the Indonesian and worldwide groups (Figs. 2d–f). *C. argentea* (accession number or abbreviated as acc. nr. 24) was the shortest among all types but it had the highest number of branches and internodes. The second highest number of branches and internodes with an intermediate height were exhibited by *A. hybridus* (acc. nr. 81 and 84). Most of vegetable amaranths particularly those of *A. tricolor* L. had less branches and internodes, as shown by the Acehnese vegetable-type (acc. nr. 66).

A higher degree of variation in stem diameter was observed in the Indonesian accessions than in the worldwide accessions (Fig. 2g). Significant differences in the mean values and variations were also observed between the two groups. Within the Indonesian group, a large variation of stem diameter was observed, ranging from the thinnest to the thickest stems. Both were shown in *A. dubius*; namely acc. nr. 52 and acc. nr. 47, respectively.

### Leaf character traits

No statistically significant differences were observed in the mean leaf area between the Indonesian and the worldwide groups (Fig. 2h). In contrast, leaf area varied significantly between the two groups; this might be attributed to the large differences in leaf area among the grain amaranths and other amaranth-types. *A. cruentus* L. (acc. nr. 13) exhibited the largest leaf area (94 cm<sup>2</sup>), followed by *A. caudatus* L. (acc. nr. 60), which is actually classified as grain species but in



IDN = Indonesia; W = Worldwide

**Fig. 2** Distribution of morphological traits in *Amaranthus*. Data for the worldwide (N = 31) and Indonesian (N = 53) collections are shown as black line and grey shaded bars, respectively



Indonesia, it is utilized as a vegetable. A large variability in leaf area was found within the Indonesian group. The range of the variation was between 5 cm<sup>2</sup> to almost 70 cm<sup>2</sup> which were exhibited by *A. hybridus* (acc. nr. 83) and *A. dubius* (acc. nr. 33), respectively.

Blade ratio, which is useful in determining the form of a leaf, showed significantly higher variation in mean values in the worldwide collection than in the Indonesian collection (Fig. 2i). On the basis of our results, the blade ratios of the weedy and vegetable-types were typically between 1.5 and 2.0. The highest blade ratio (5.5) was observed in *A. cruentus* L. (acc. nr. 10) belongs to the grain species, whereas the lowest (1.2) was observed in *A. tricolor* L. (acc. nr. 23 and 78).

The Indonesian accessions showed significantly thicker leaves and higher variation than the worldwide collection (Fig. 2j). Our results implied that the most consumed Indonesian vegetable-types exhibited by *A. tricolor* L. (mean = 0.4 mm) were 21 and 40 % thicker than the average Indonesian and worldwide accessions, respectively. The Indonesian weedy-types showed higher variability in terms of leaf thickness (0.1–0.5 mm) and were approximately 30 % thinner than the most commonly consumed vegetable-types in Indonesia.

#### Vegetable production traits

The mean value and variation in the number of leaves among Indonesian amaranths were significantly higher than those in the worldwide amaranths (Fig. 2k). Most of *A. dubius* collected from the highland areas of Takengon in Sumatra such as acc. nr. 34, exhibited extraordinarily higher numbers of leaves than the cultivated types (*A. tricolor* L.). In regard to this trait, *Celosia* spp. showed the lowest number of leaves among all types of amaranths. The mean total leaf area of the Indonesian and worldwide accessions were significantly different (Fig. 2l). However, variation in the total leaf area did not differ significantly between the Indonesian and worldwide groups.

#### Protein content

The Indonesian and worldwide amaranth accessions did not show any significant differences in terms of leaf protein content (Fig. 2m). Most of weedy-types such as *A. dubius* generally showed the highest average protein content (20 %), whereas *Celosia*

spp. showed 17 % protein content. The cultivated species such as *A. cruentus* L. and *A. tricolor* L. exhibited an average protein content of 16 %.

#### Cluster analyses of morphological variation in Indonesian amaranths

The results of clustering (Fig. 3) revealed that the 53 Indonesian amaranth accessions could be broadly classified into four major groups at a distance scale of 9.5. The mean values of the morphological traits from each cluster are presented in Table 3.

Weedy-types were the most dispersed in each cluster. Cluster 1 consisted of 13 accessions, including 4 *Celosia* spp. and 8 weedy types such as *A. blitum* L., *A. dubius*, *A. hybridus*, *A. spinosus*, *A. viridis*. The plants were characterized with low plant height, few leaves, and low total leaf area but showed high leaf blade ratio and protein content (Table 3). One *A. tricolor* L. (acc. nr. 80) was also included in Cluster 1 on the basis of characteristics that were similar to those of other weedy-types.

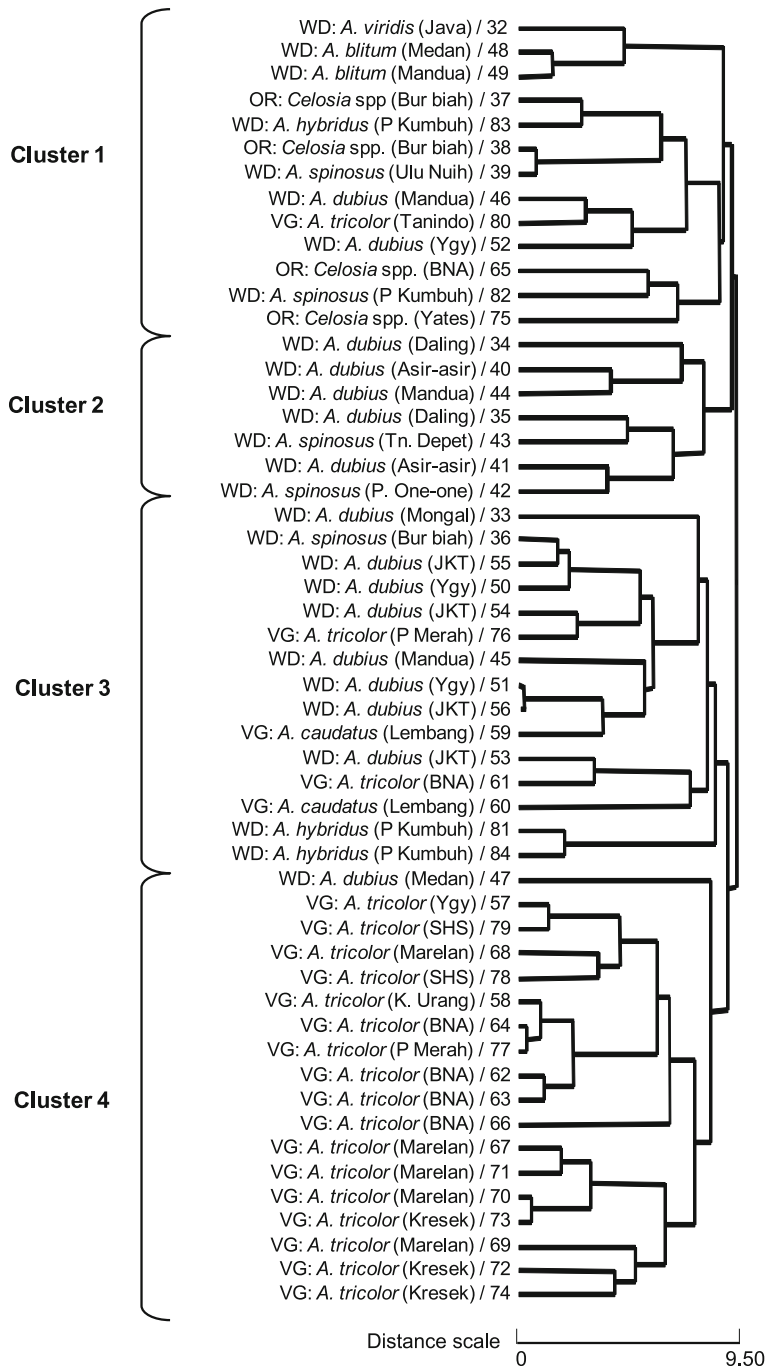
Seven weedy-types consisting of five *A. dubius* and two *A. spinosus* plants were distinctly grouped as Cluster 2. The morphological characteristics of the two *A. spinosus* plants closely resembled those of *A. dubius* during its early stages; however, axillary spines appeared at a later stage in *A. spinosus*. All weedy plants in Cluster 2 have originated from the highland areas of Takengon in Sumatra, and thus their morphology were similar to the highland adapted plants' features (e.g., tall plant with tender, small, and numerous leaves; highest number of branches; and large total leaf area) and notably high protein content (Table 3).

Cluster 3 comprised a mixture of weedy- (11 accessions); mostly represented by *A. dubius* and vegetable-types of *A. tricolor* L. and *A. caudatus* (4 accessions). These plants showed remarkably distinct morphotypes, such as the largest leaf area and the highest total leaf area, highest number of branches, and very thick leaves, but average protein content. Moreover, the weedy-types in cluster 3 showed phenotypes closely resembling the vegetable-types, except for *A. spinosus* (acc. nr. 36).

All vegetable accessions (*A. tricolor* L.) were distinctly grouped in Cluster 4. Only one weedy-type of *A. dubius*. (acc. nr. 47) was included in this cluster because of its shared morphological traits with common characteristics of vegetable plants, such as having



**Fig. 3** Dendrogram of Indonesian amaranths (N = 53) on the basis of 10 morphological characteristics, constructed using Ward's method. The origin of each amaranth is enclosed within parentheses; the number after the slash indicates the accession number. *OR* ornamental, *VG* vegetable, and *WD* weedy-type



a blade ratio of 1.5 and a very thick stem. The main phenotypes of cluster 4 were noted with thick stem and leaves, few leaves and branches, an average total leaf area, low protein content but an optimum blade ratio (1.5). The concerns about low content of protein and leaf number in the Indonesian vegetable-types were affirmed using these results.

#### Correlations among morphological and protein traits

Total leaf area was shown to have a positive correlation with number of leaves and leaf area, stem diameter, number of branches and internodes (Table 4). The strongest correlation was observed

**Table 3** Mean values of each morphological trait and protein content in the 4 clusters

Cluster	Morphological traits									
	H (cm)	SDM (mm)	NBR (no.)	NIN (no.)	NLV (no.)	LAR (cm <sup>2</sup> )	TLA (cm <sup>2</sup> )	RLW	LTK (mm)	PRT (%)
1	13.75 <sup>b</sup>	6.14 <sup>b</sup>	13.16 <sup>a</sup>	11.27 <sup>b</sup>	33.93 <sup>bc</sup>	17.16 <sup>c</sup>	525.36 <sup>b</sup>	2.07 <sup>a</sup>	0.25 <sup>b</sup>	18.44 <sup>ab</sup>
2	28.02 <sup>a</sup>	7.87 <sup>ab</sup>	16.91 <sup>a</sup>	16.16 <sup>a</sup>	188.38 <sup>a</sup>	13.21 <sup>c</sup>	2,552.61 <sup>a</sup>	1.75 <sup>ab</sup>	0.14 <sup>c</sup>	20.85 <sup>a</sup>
3	20.59 <sup>ab</sup>	8.94 <sup>a</sup>	16.43 <sup>a</sup>	15.11 <sup>a</sup>	67.93 <sup>b</sup>	46.37 <sup>a</sup>	2,987.37 <sup>a</sup>	1.64 <sup>b</sup>	0.35 <sup>a</sup>	17.28 <sup>ab</sup>
4	23.78 <sup>a</sup>	10.22 <sup>a</sup>	9.80 <sup>b</sup>	8.76 <sup>b</sup>	29.55 <sup>c</sup>	34.25 <sup>b</sup>	1,027.64 <sup>b</sup>	1.52 <sup>b</sup>	0.33 <sup>a</sup>	15.81 <sup>b</sup>

Same alphabets show no statistical difference (Tukey–Kramer test;  $p < 0.05$ )

H plant height, SDM stem diameter, NBR number of branches, NIN number of internodes, NLV number of leaves, LAR leaf area, TLA total leaf area, RLW blade ratio, LTK leaf thickness, PRT protein

**Table 4** Correlation matrices for the morphological traits and protein contents in the amaranths (N = 84)

Number	Traits	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1)	Plant height (cm)	0.175	0.172	0.258*	0.196	0.073	0.112	−0.052	0.104	−0.192
(2)	Stem diameter (mm)	–	0.085	0.045	0.030	0.364**	0.279*	−0.267*	0.333*	−0.114
(3)	Number of branches (unit)		–	0.939**	0.336**	0.168	0.366**	0.204	−0.184	−0.164
(4)	Number of internodes (unit)			–	0.417**	0.052	0.393**	0.146	−0.185	−0.175
(5)	Number of leaves (unit)				–	−0.299*	0.607**	−0.122	−0.321*	0.263*
(6)	Leaf area (cm <sup>2</sup> )					–	0.357**	−0.065	0.446**	−0.082
(7)	Total leaf area (cm <sup>2</sup> )						–	−0.208	0.088	0.129
(8)	Blade ratio							–	−0.048	−0.064
(9)	Leaf thickness (mm)								–	−0.221**
(10)	Protein (%)									–

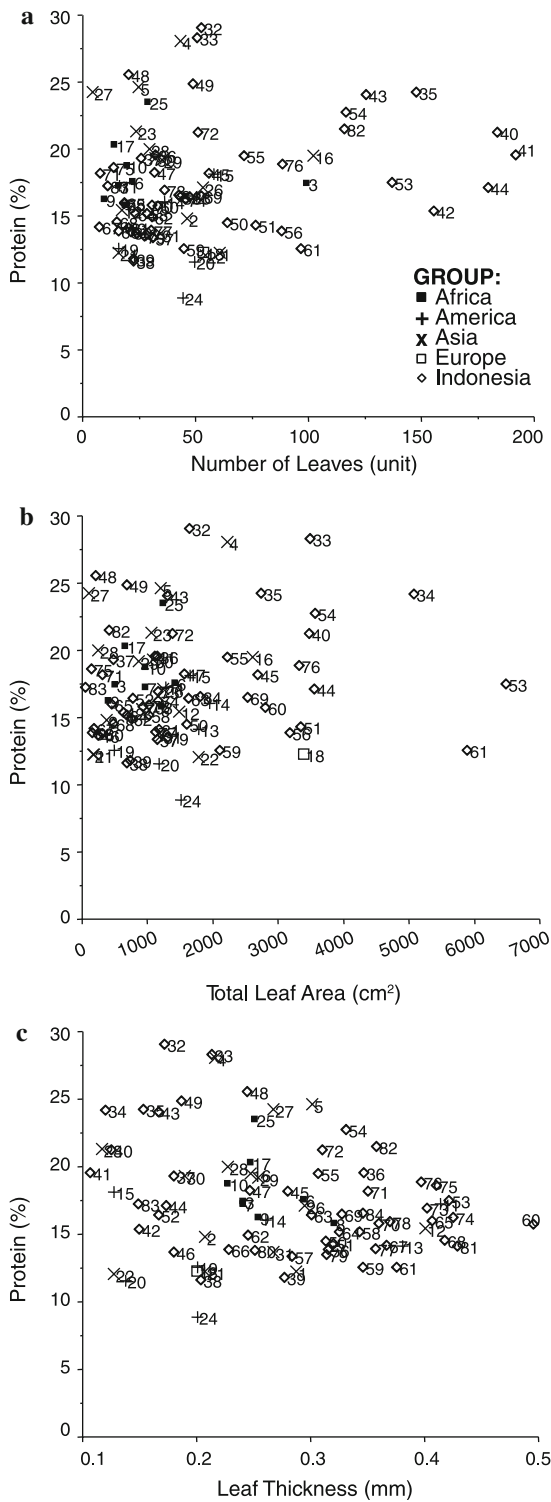
\* and \*\* represent significance at  $P < 0.05$  and  $P < 0.01$ , respectively

with number of leaves. Leaf number was shown to have a negative correlation with leaf area and thickness, indicating that the more leaves amaranths produced, the smaller and thinner the leaves were. No significant correlations were shown between blade ratio and any morphological trait, except for stem diameter. The height of the amaranths showed no correlation with stem diameter or number of leaves and branches, but taller amaranths significantly showed more internodes. Protein content showed a significant correlation with leaf number and thickness.

#### Relationship between protein content and selected vegetable traits

The relationship between protein content and selected vegetable traits such as number of leaves, total leaf area and leaf thickness were investigated (Fig. 4). Three *A. dubius* accessions (acc. nr. 40, 41, and 44) showed

remarkably high leaf numbers and protein content (Fig. 4a). Extraordinarily high protein content, albeit small to intermediate total leaf area, was identified in acc. nr. 4 (*A. blitum* L.), acc. nr. 32 (*A. viridis*), and acc. nr. 33 (*A. dubius*). In contrast, *A. dubius* (acc. nr. 34, 53) and *A. tricolor* L. (acc. nr. 61) showed remarkably large total leaf areas but very variable protein content (Fig. 4b). The relationship between protein content and leaf thickness is shown as a scatter plot diagram in Fig. 4c. The highest variation in protein content was observed in the amaranths with leaf thickness ranging from 0.15 to 0.30 mm, which was commonly observed in the weedy-types. In terms of the relationship between protein content and leaf thickness, the main Indonesian commercial vegetable amaranths mostly represented by *A. tricolor* L. showed variation in leaf thickness ranging from 0.31 to 0.50 mm but low to average protein content. These results validated the observations previously presented in Fig. 2j.



**Fig. 4** The relationship of protein content to leaf number (a), total leaf area (b), and leaf thickness (c). Numbers correspond to amaranth accession numbers (see Table 1)

### Discussion

The availability of genetic resources and their diversity assessment is a point for the success of breeding programs for any crop, including amaranths (Hoisington et al. 1999). Germplasm collections from underutilized crops can become essential resources for plant breeders to develop improved cultivars that will feed rapidly growing populations (Nelson 2011). Attempts have been made to use exotic germplasms obtained from weedy or wild relatives for crop improvement via gene introgression, cultivar selection, and conventional breeding (Hajjar 2005; Sagnard et al. 2011). To date, the genetic resources of Indonesian amaranths remain unexplored. Their screening at the morphological level will serve as an initial attempt to facilitate improvement of vegetable amaranths.

As there has been no available references, we have tried to measure the variation of Indonesian amaranths based on their phenotypic performance and then to compare them with the worldwide variation. In this study, the worldwide variation served as a preliminary reference which enabled us to value the potential of Indonesian resources for further breeding efforts. Based on our results, the average values and variations in most morphological traits and protein content in the Indonesian accessions were shown to be generally similar to those of the worldwide ones. Nevertheless, higher variation in the morphological appearance noted with higher standard deviation was shown by the Indonesian amaranths, especially in terms of stem diameter, leaf number and thickness in the weedy types. This finding is according to Pickersgill (1981) who stated that higher level of variability in morphological traits is maintained in many of weedy or wild relatives of the crops. Furthermore, higher variation in the morphological appearance might indicate higher variability at the genetic level (Jain et al. 1980). The higher variation in the morphological appearance observed in Indonesian amaranth accessions might be explained in three possible ways: (1) lack of selection pressure due to artificial or domestication processes (Chan and Sun 1997), as the variations have existed naturally and breeding-efforts have not yet been initiated in Indonesian amaranths; (2) mixed-mating system of amaranths may facilitate the natural introgression process (Kulakow and Hauptli 1994). Although self pollination is more likely to occur, amaranths may have combined their natural ability of

self and cross pollination through wind, with mean outcrossing rates ranging from 4 to 34 % (Brenner and Widrechner 1998; Kulakow and Hauptli 1994). Frequent outcrossing might have resulted in large numbers of varieties and a wide range of morphological diversity in amaranths (Das 2011). Natural introgression was also observed in other diverse plant species (Jarvis and Hodgkin 1999). However, Pal and Khoshoo (1972) argued that interspecific hybrids or natural introgression might not occur easily in amaranths due to the sterility and viability problems in the  $F_1$  seeds. Our data were consistent with the view that natural introgression among cultivated vegetables and weedy amaranths might have occurred in Indonesia, as shown by Cluster 3. In our work, cluster analysis was shown to be a helpful tool to group the Indonesian amaranths' resources based on the similarity of the ten morphological characteristics. Hence, the usefulness of cluster analysis in grouping germplasm collection based on similar morphological variations has been demonstrated in many crop plants, such as in sorghum (Ayana and Bekele 1999) and rice (Arietta-Espinoza et al. 2005). Such classification is simple, reliable and helpful to elucidate the distinct patterns of variation and evolutionary history; (3) Polyploidy, leading to gene combination, might have resulted in higher morphological variation. Most of the weedy-types assessed in our Indonesian accessions were *A. dubius*, which is the only known natural tetraploid amaranth species (Grubben 2004). Furthermore, they usually develop distinct morphological characters and undergo vigorous growth (Pal 1972). The above average performance of *A. dubius* was confirmed by our results. Further studies at the molecular level would be required to validate the occurrence of introgression and polyploidy in Indonesian amaranths.

Weedy resources are considered as rich sources of variation, and act as a reservoir of genetic diversity. Their assessment plays a vital role in crop improvement (Pickersgill 1981). Our study of Indonesian weedy amaranths also supports this notion. Moreover, this study has shown several morphological traits, such as leaf number and area, stem diameter, number of branches and internodes (except the height of the plant) that might influence the total leaf area, which is considered as an indirect parameter of yield in amaranths. Previously, Shukla et al. (2006) reported the positive contribution of several morphological (including stem diameter and leaf area) and nutritional

quality traits to the foliage yield in vegetable amaranths (*A. tricolor* L.). Furthermore, those two traits showed relatively high heritability values ( $h^2 > 0.95$ ), but Shukla et al. (2006) proposed that non-additive gene action might play a vital role in the transmission of these characters to the offspring.

A negative correlation between number of leaves and leaf area ( $r = -0.3^*$ ) might act as a challenge in the improvement of yield in amaranths. Breeding strategies should focus on increasing the area of each individual leaf or on producing more leaves in each main branch. Indonesian weedy resources might potentially serve as parental lines, particularly *A. dubius* accessions originating from the highlands of Takengon (acc. nr. 40, 41, and 44) that show a remarkably high number of leaves. In terms of improving the quality of vegetable amaranths, the results of this study suggest that leaf thickness is considered as a quality parameter, as acknowledged by the Asian Vegetable Research Development Centre (Acedo 2010). Leaf thickness plays a key role in palatability and is directly related to consumer acceptance. In general, Indonesian consumers prefer tender, thin, and middle-sized leaves with a blade ratio of 1.5–2.0 (personal observation and farmers' interview). Exclusively, the chip industries of fried amaranth's leaves in Western Java prefer thick leaves with large leaf areas and high blade ratios. For leaf quality improvement, Indonesian accessions of *A. dubius* (e.g., acc. nr. 33) may be selected as parental lines. If those positive characteristics from selected parental lines can be transferred via interspecific crossing, then new varieties with improved performance and desired traits can be developed. Interspecific crossing to produce hybrids in *Amaranthus* is possible to be obtained. However, this would not be so easily achieved due to reproductive barriers such as male sterility in the pollen grains (Gudu and Gupta 1988) and low pollen fertility (Gupta and Gudu 1991). Thus, both factors has made the production of  $F_1$  seeds difficult to be obtained. Moreover, the chromosome number difference of *A. dubius* ( $n = 32$ ) as the only known tetraploid species with the rest of *Amaranthus*' species ( $n = 16$  or  $17$ ), were believed to be the main barrier in the gene exchange between the weedy and the cultivated amaranths (Greizerstein and Poggio 1995; Pandey 1999). Therefore, conventional breeding such as backcrosses (Coons 1982) or modern breeding technologies via cytoplasmic male sterility

(Sodhi et al. 2006) may help us to overcome such constraints.

Leaf protein content and nutritional value, including that of carotenoids and minerals, are considered as the most important quality parameters in vegetables, particularly in amaranths (Shukla et al. 2010). Our result reaffirmed the nutritional potential of underutilized crops such as amaranths and *Celosia*. The high protein content in *Celosia*-leaves as compared to that in the common vegetable amaranths (*A. tricolor* L.), should be considered as an alternative crop to augment malnutrition, especially in tropic and famished regions worldwide (National Academy of Sciences 2006).

The observed similarity in the levels of variation in protein content among the Indonesian and worldwide amaranth accessions serves as an opportunity for breeders to increase protein content in Indonesian vegetable-types, especially those in Cluster 4. Cluster and scatter plot analyses have enabled us to identify specific weedy accessions that show potential for increase in protein content. It was mentioned in the sect. “Introduction” that *A. blitum* L. was found to be high in its protein content. Based on the result of this study, other weedy species such as *A. viridis* and *A. dubius* (acc. nr. 32, 33 and 34 grouped in Clusters 1, 2 and 3) were found to be good resources for the improvement of leaf protein content and total leaf area. By combining the superior nutritional characteristics of weedy-types with the good external appearance of vegetable amaranths via genetic recombination and selection, new vegetable varieties with high protein content can be obtained by breeding weedy types with superior nutritional characteristics with vegetable types producing better biomass. This will remain as the plant breeder’s task of transferring the useful trait from weedy to cultivated amaranth without adversely affecting other economically important traits.

## Conclusions

In this study, the Indonesian amaranth accessions were revealed to have high levels of genetic variability, with most morphological traits and protein content levels similar and in some cases, superior to those of the worldwide germplasm. The superior traits include leaf number, stem diameter, and leaf thickness—features that are essential parameters in vegetable improvement. Cluster and scatter plot analyses identified potential candidates for vegetable breeding programs.

Indonesian *A. viridis* (accession number 32) and *A. dubius* (accession numbers 33, 34, 41, and 44) accessions could serve as valuable parental lines for the improvement of protein content and yield, respectively. The relatively high protein content in *Celosia* leaves could potentially make this plant an alternative protein-rich vegetable.

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