Assessment of Genetic Diversity of Nutritional Values and Agronomic Traits in Indonesian Amaranths

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Rita ANDINI

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List of Abbreviations

AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
BMS	Biomass
BPS	Indonesian Statistics Bureau (Badan Pusat Statistik in *Indonesian*)
DM	Dry Matter (in %)
DN	Day neutral plants
DW	Dry Weight (in gram)
FAO	Food and Agriculture Organization
FW	Fresh Weight (in gram)
IDN	Indonesia (refers to the origin of accessions)
LD	Long day plants
NLV	Number of leaves or leaf number (number)
PC	Principal Components
PCA	Principal Component Analysis
RAPD	Random Amplified Polymorphic DNA
RDA	Recommended Dietary Allowance
SAS	Statistical Analyses Software
SD	Short day plants
SDM	Stem Diameter (in millimeter)
SSR	Simple Sequence Repeat
UNU	United Nations University
USDA	United States Department of Agriculture (refers to the origin of accessions)
USDA-ARS	United States Department of Agriculture-Agriculture Research Service
WHO	World Health Organization

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CHAPTER 1

General Introduction

Worldwide, almost 870 million people are suffering from hunger and the most greatly affected is Asia countries (FAO 2010 and 2012). So far, many efforts and strategies including agriculture improvements have been incorporated by the United Nations with an increase of 2.42% in the world food production (Huang *et al.* 2002, FAOSTAT 2012). Nevertheless, eradication of hunger still remains the main challenge for the future of mankind. In adjacent to hunger problem is so called as "hidden hunger" or malnutrition which has become not only a major public health but also a direct cause of about half of all deaths in young children in developing countries (Müller and Krawinkel 2005). Malnutrition is referred to Most (Lorch 2005). It is estimated that about 31% of all children under the age of 5 years in developing countries are protein-energy malnourished and under weight (Müller and Krawinkel 2005). Many factors have contributed such situation but the most underlying cause is poverty and economic crisis (Müller and Krawinkel 2005, Studdert *et al.* 2001).

Similar condition is also noted in Indonesia. It is estimated that 32% (~178 million) of children under the age of 5 years malnourished (Sari *et al.* 2010). To alleviate malnutrition cannot be done in a rapid way due to some remaining obstacles such as huge population number with more than 240 million people and 18% of poverty level. Many strategies have been applied by the Indonesian government such as improving policies to support agriculture productivity (Acosta and Fanzo 2012, Maulana and Sayaka 2007), promoting the utilization of fortifying food with high content of both protein and micro nutrients (Wargiono *et al.* 2002) and distributing vitamin supplements such as vitamin A in the poor urban areas at the beginning of 1980s (Berger *et al.* 2006, Persson 2001). However, relying solely on such approaches would not adequate to combat malnutrition in a huge area to cover especially in the rural, remote and marginal areas in Indonesia which have problematic coverage. Persson (2001) suggested that an increased food intake diversity particularly from dark green leafy vegetables as a promising solution to combat malnutrition particularly vitamin A in Indonesia. Consistent with Persson's view (2001), this thesis focuses on another approach to solve the protein-rich malnutrition in

Indonesia via assessment of genetic diversity of highly nutritious underutilized crops such as amaranths. The objectives of this thesis are further notified in Point 1.5.

1.1. Biodiversity and protein score value

Up to 12,000 edible plants were known, however, only 150-200 of them are used by human (FAO 2011). The inextricable link between biodiversity and nutrition security has been affirmed (Termote *et al.* 2012). Some types of underutilized leafy vegetables from wild edible plants had been identified to possess great potential for addressing nutritional security issues (Guil *et al.* 1997, Gupta *et al.* 2005). The proximate nutritional composition in underutilized plants such as amaranths was found to be excellent sources of protein and essential amino acids in comparison with the major cereals (Gorinstein *et al.* 2001). Plant genetic diversity interlinked with plant breeding such as the biofortification of several staple crops has brought a new strategy to combat malnutrition in a sustainable way (Pfeiffer and McClafferty 2007, FAO 2011).

There are two ways to evaluate protein quality in human nutrition: 1) bioassay method by using rat feeding experiments; 2) the protein digestibility-corrected amino acid score or usually referred as "protein score" (Seligson and Mackey 1984). The first assay has been criticized by many food scientists due to the differences by the requirements of essential amino acids and growth rates between rats and humans. Thus, the later one is the most widely adopted by the Food and Agriculture Organization (FAO), the National Academy of Sciences (NAS) and World Health Organization (WHO) (Seligson and Mackey 1984). The method applied by the evaluation of protein score is basically obtained by comparing the concentration of the first limiting essential amino acid in the test protein with the concentration of that amino acid in a reference (scoring pattern) (Schaafsma 2000).

1.2. Amaranth in general, evolution and history

The genus *Amaranthus* or amaranths encompasses about 70 species with worldwide distribution (Maughan *et al.* 2009). Amaranth in the ancient Greek means "*everlasting*". They occurred about 5,000 – 7,000 years ago in the New World and *A. hybridus* L. was believed as the putative progenitor of the current cultivated (grain) types (Kulakow and Hauptli 1994, Maughan *et al.* 2011).

The evolution of amaranths is presented on Figure 1.1 based on several references (Grubben, 1976, Hauptli and Jain 1984, Chan and Sun 1997). It is unclear exactly how amaranths were spread out to the other parts of the world (including to India) but the Columbian voyager was proposed as one possibility (Espitia-Rangel 1994, Brenner *et al.* 2000). Jain *et al.* (1980) and Hauptli and Jain (1984) found a high allozyme variation and high morphology by the Indian amaranths. Due to these evidences, India was proposed as the centre of diversity of vegetable amaranths.

The C_4-characteristics in amaranths enable them to grow rapidly. Thus, finally they are accounted as highly productive plants. Vegetable amaranths are cultivated in least 50 tropical countries in Asia and Africa due to their agricultural and economical advantageous characteristics (National Academy of Sciences 2006). The stems are also highly valued as animal fodder (Sleugh *et al.* 2001). Moreover, the leaves are also rich in minerals, such as iron, zinc, chromium and also vitamins (β-carotene, ascorbic acid and thiamine) (Prakash and Pal 1991, Gupta *et al.* 2005). Due to their nutritional superiority, amaranths have been suggested as alternative source of rich protein leafy vegetables feeding those overpopulated and undernourished areas (Gupta and Wagle 1988).

1.3. *Amaranthus*' genetic resources

A high genetic variability exists within *Amaranthus* which shows their potential for genetic improvement (Espitia-Rangel 1994). High morphological diversity is observed in amaranths (Das 2011). Fig. A.1 presents the diversity of *Amaranthus*' species. Basically, amaranths are classified due to their utilization as the grain, vegetable, ornamental and weedy types including the weeds (Brenner *et al.*2000). Different utilization in one species may occur as some leaves of grain or weedy species can be used as vegetable amaranths. Such overlap by the utilization and the high variability in the morphology in amaranths sometimes lead to confusion in its taxonomy (Brenner *et al.* 2000). Therefore, their characterization at the molecular level is sometimes required to reduce the taxonomic complexity and to confirm the phenotypic results. Molecular markers such as chloroplast and nuclear DNA (Lanoue *et al.* 1996), AFLP (Wassom and Tranel 2005), isozyme and RAPD (Chan and Sun 1997), microsatellite marker (Mallory *et al.* 2008) were applied to detect the genetic diversity of *Amaranthus.* The generated dendogram developed from each specified marker are not always uniform, however, such information

is useful for plant breeders to evaluate the possibility of inter or intra specific crossing or for study of evolutionary relationship.

1.4. Amaranth in Indonesia

Amaranth is very popular and ranked as the third mostly produced green leafy vegetables in Indonesia (Grubben 1994). The number of amaranth's production in Indonesia is presented on Fig. 1.2 (BPS-Statistics Indonesia, 2013). Amaranth is part of the Indonesian's daily dishes and its taste resemblance to that of spinach. It is mostly cultivated on small plots less than one hectare along the river side or open fields scattered at the periphery of sub urban areas and can be harvested within an interval of four weeks per harvest season (Andini 2009). Its yield is 2-5 tonnes·ha^{-1} per harvest season (Hadisoeganda 1996). Amaranths are available throughout the year and frequently traded in Indonesian traditional markets. They are sold in small bundles consist of 10–20 plants and counted as one of the economically important vegetables in Indonesia (Hadisoeganda 1996). Moreover, vegetable amaranths provide a high concentration of vitamin A and their nutritional benefit was incorporated by the eradication of children's dark blindness in 1973-1980 in Indonesia (Berger *et al.* 2006).

Siemonsma and Piluek (1994) estimated up to 225 primary use vegetables and more than 100 wild species are exist, including a large number of weedy companions. The high biodiversity in Indonesia may open opportunities for breeding new type of amaranths with desirable characters. Thus, screening of potential lines with novel characteristics in amaranths should be initiated and promoted for further breeding and conservation efforts.

1.5. Objectives of this thesis

The purposes of this thesis were to assess the diversity of Indonesian amaranth resources and to explore their potential for further exploitation for breeding efforts. Increasing the yield performance and the content of protein in amaranths has been determined as the main breeding objectives. The logical framework of this study is summarized on Fig. 1.3. The four main components of this research were compiled in each chapter. In Chapter 2, screening amaranths germplasm particularly those collected from Indonesia on the basis of their content of protein and morphological variation related to yield

improvement in vegetable (e.g. leaf number and biomass) were highlighted. Then, the exhibited variation by the Indonesian amaranths was compared with the worldwide amaranth resources to enable us to value the potential of Indonesian resources and to determine the extent of breeding target by relying on local resources. The possibilities that might lead to such higher variation by the Indonesian amaranths were being discussed. Finally, I tried to select the potential parental lines attributed with high protein content and leaf number. In Chapter 3, the photoperiodic flowering response of amaranths was evaluated under Japanese condition. The photoperiodic flowering response was applied as a tool to reveal the major environmental determinants affecting the process of flowering in amaranths. Moreover, studying the photoperiod response can be applied as a tool to characterize the different types in amaranths. We also tried to discuss the possibility of amaranths to be introduced as vegetable in a temperate and long day condition such as in Japan. In Chapter 4, the nutritional values (protein content and amino acids) by the leaves of the three types of amaranths were assessed. The potential of the leaves from the grain and edible weedy species to be further exploited as vegetables was also discussed. In Chapter 5, the level of polyploidy in amaranths was being assessed in order to validate the proposed hypothesis in the "Discussion" belongs to Chapter 2. We proposed that the higher morphology observed by the Indonesian amaranths was caused by many reasons and one of them was due to polyploidy. Chapter 6 presents general discussion based on the results described in Chapter 2 through 5. At the end, the most important findings of this research were summarized in the summary.

Figure 1.1. Evolution and distribution of *Amaranthus*' species worldwide.

This figure is developed based on three references (Grubben, 1976, Hauptli and Jain 1984, Chan and Sun 1997).

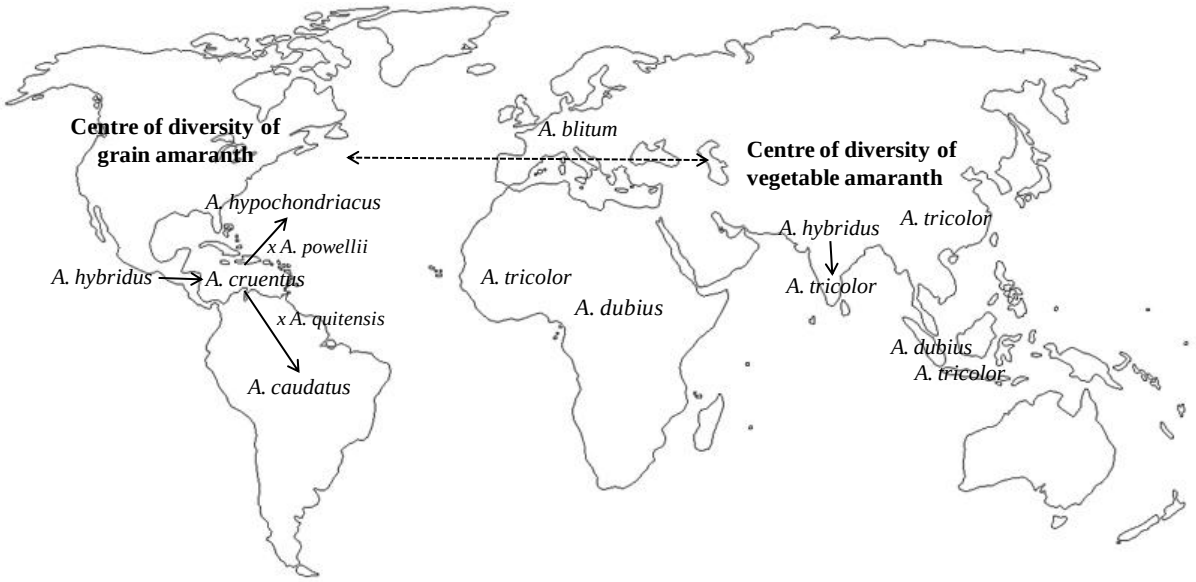
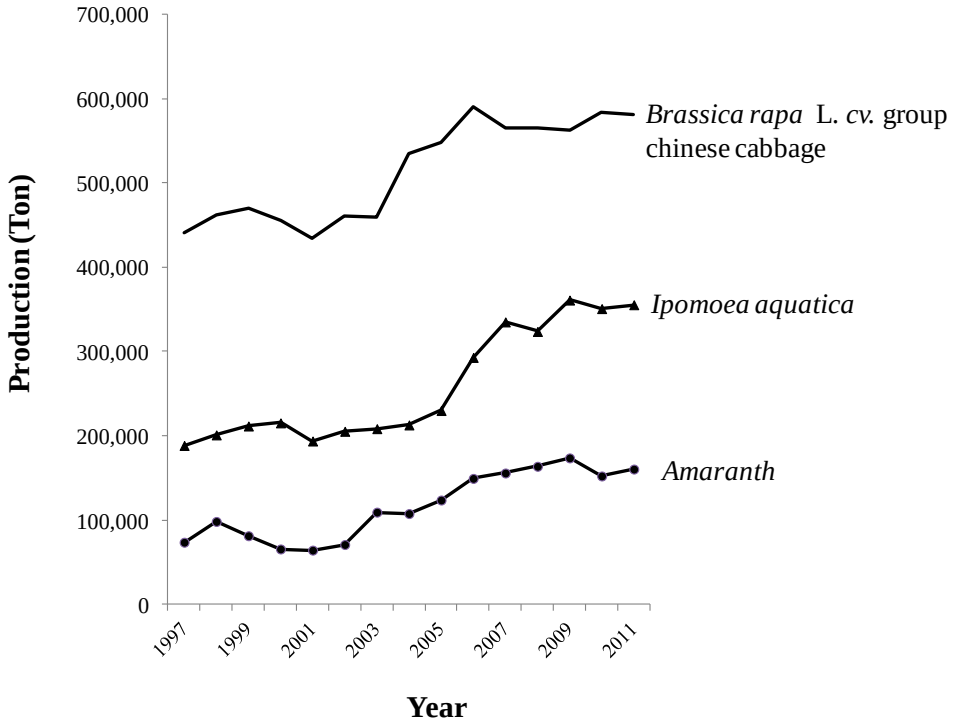
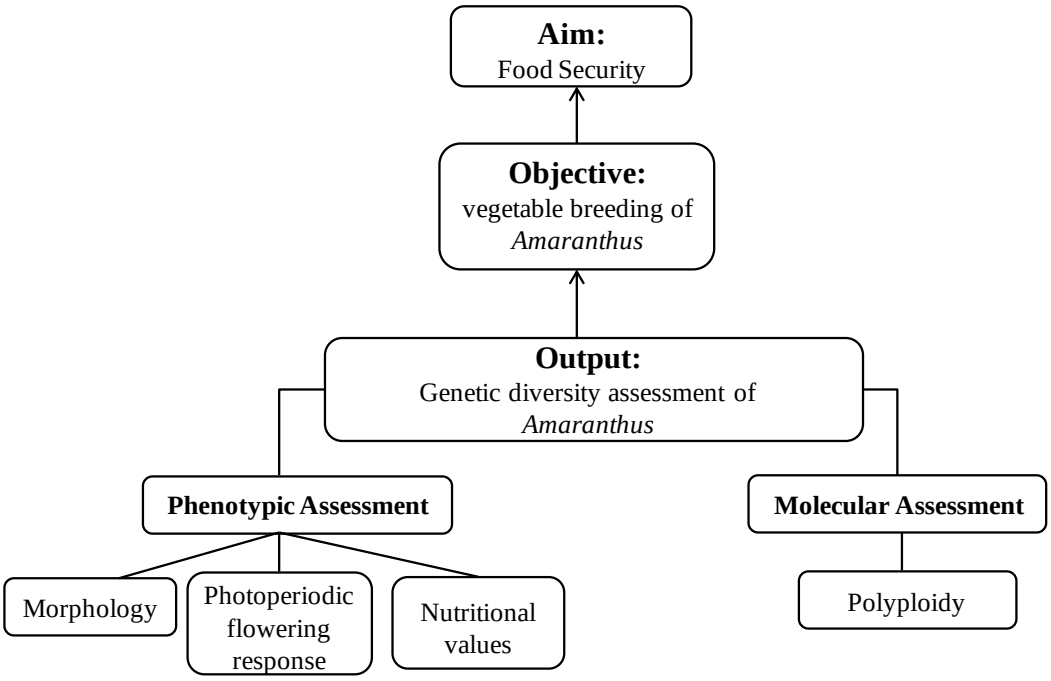


Figure 1.2. Production number of three major leafy vegetables in Indonesia.



(source: official website from BPS-Statistics Indonesia; www.bgps.go.id/ english; accessed on 2 February 2013).

Figure 1.3. Logical framework of this study.



CHAPTER 2

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

2.1. 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 (Espitia-Rangel 1994). 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). There are two perceptions of weedy types, first are those referred as "weeds" and not edible such as *A. powellii* S. Wats, *A. retroflexus* L. and *A. spinosus* L. which may escape out of cultivation (Pal 1972). Moreover, their antinutrional or toxic elements are relatively high (Grubben 1976, Siener *et al.* 2006). Second, the leaves of some amaranths' species are edible and highly nutritious but still remain underutilized such as *A. blitum* L. and *A. dubius* Mart. ex Thell. (Brenner *et al.* 2000, Costea *et al.* 2003, Grubben 2004).

The fascinated inflorescences' color of amaranths makes them attractive to be used as ornamental plants. In African countries such as Benin and Nigeria, the young leaves of the ornamental *Celosia* spp., which still belong to the family Amaranthaceae (Grubben 1976) are also used as leafy vegetable and serve as important source of protein, minerals and vitamin in marginal areas (Denton 2004, Olaniyi and Ojetayo 2012).

A. tricolor L. is the prominent vegetable species and serves as an affordable protein source for millions of people in South-East Asia countries, including Indonesia (Grubben 1994). Despite of its popularity, some lack points in *A. tricolor* as vegetable are pinpointed and their improvements should be initiated such as the relatively low protein content if comparing with cassava leaves (*Manihot esculenta*). The average protein content in *A. tricolor* was 12% DM on average, contrastingly, approximately 32-40% was reported in cassava leaves (Awoyinka *et al.* 1995, Shukla *et al.* 2010). Furthermore, yield component such as leaf number per plant in *A. tricolor* is still low if compared with other productive tropical vegetables such as "kangkong" (*Ipomoea aquatic* Forssk.) (Westphal

1994). These have been also acknowledged in an interview with the Indonesian Vegetable Research Institute in 2009.

Indonesia is recognized as a biodiversity hotspot in the tropics, and many varieties of amaranths (e.g., vegetable, weedy, and ornamentals) possess favorable crop morphology and high quality protein (Grubben 2004). Inspite of immense morphological diversity, not much work has been done for its genetic improvement including by the nutritional quality of *A. tricolor* (Shukla *et al.* 2006). Relying only on *A. tricolor* for long term utilization as the only vegetable species might lead to genetic vulnerability and finally, economical loss (Acquaah 2007). Some local people in the remote highland areas of Takengon in Sumatra, have utilized some weedy types closely resemble with the vegetable amaranths as famine food and as traditional medicine (personal communication). Takengon is a rural area located in the central mountainous areas of Aceh called as "*Gayo-upland*" (up to 2,800 – 3,000 m elevation) and still remain isolated. 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. The diversity of 23 cultivated and their relationship with wild *Amaranthus* had been assessed via molecular marker such as isozyme and RAPD (Chan and Sun 1997). They found that wild or weedy amaranths were highly polymorphic. At the phenotypic level, Coons (1982) tried to evaluate the yield related traits such as the flower's component, seed diameters and color in grain amaranths (*A. caudatus* and *A. hybridus*) and found the larger diversity in *A. hybridus* than *A. caudatus* in terms of that three characters. Moreover, the diversity of vegetable amaranths, particularly *A. tricolor* as well as the field performance of *Amaranthus* in China were reported by Shukla *et al.* (2010) and Wu *et al.*(2000), respectively. 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.

2.2. Materials and Methods

2.2.1. Plant materials

In this study, a total of 84 amaranth accessions consisting of grain-, ornamental-, vegetable-, and weedy-types were assessed (Table 2.1), of which 53 were collected from Sumatra and Java islands of Indonesia in 2008 and 2010 (Fig. 2.1). 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). The Indonesian collection consisted of 2 accessions of *A. blitum*, 2 accessions of *A. caudatus*, 16 accessions of *A. dubius*, 3 accessions of *A. hybridus*, 5 accessions of *A. spinosus*, 20 accessions of *A. tricolor*, 1 accession of *A. viridis* L., and 4 accessions of *Celosia* spp. Seeds were collected from various sites such as 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 originated from 13 diverse origins (Africa, America, and Asia) were assessed in this study. They are further classified as worldwide variation and were 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*, 8 accessions of *A. cruentus*, 2 accessions of *A. dubius*, 1 accession of *A. graecizans* L., 2 accessions of *A. hybridus*, 1 accession of *A. hypochondriacus*, 5 accessions of *A. tricolor*, and 2 accessions of *Celosia* spp.. In addition, 5 accessions of *Celosia* spp. were purchased by author in Kathmandu (Nepal) in 2010. Species of the USDA accessions were identified by USDA personnel and available online (USDA 2011).

2.2.1.1. 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 located on 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 350" from Sungro). 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" ($N:P_20_5:K_2O$ = 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.

2.2.2. Methods

2.2.2.1. 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 2.2). 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 histrogram.

Stem diameter and leaf thickness were measured with a slide caliper (Digimatic Solar DC-S15m, Mitutoyo, Japan). Leaf thickness was measured by hand calipers at the middle part of leaf. 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^2 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.

2.2.2.2. Protein analysis

Crude total 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 × 6.25) (AOAC 1980). Fresh leaves (10–25 g) were cut from 2–3-month-old plants. Leaves were ground in liquid nitrogen and then freeze-dried (FD-1, EYELA, Rikakikai Co., Ltd., Tokyo, Japan). Samples (dry weight, 1 mg) were analyzed in duplicates.

2.2.3. Statistical 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.

2.3. Results

2.3.1. Variation of morphological traits and protein content in *Amaranthus* spp.

The variation in morphological traits and leaf protein content in Indonesian and worldwide amaranths were presented in Fig. 2.2. The morphological traits were grouped into four categories: (i) qualitative (Figs. 2.2a–c), (ii) plant architecture (Figs. 2.2d–g), (iii) leaf character (Figs. 2.2h–j), and vegetable production traits (Figs. 2.2k–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. 2.2a). 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. 2.2b). 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. 2.2c).

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. 2.2d-f). *Celosia argentea* L. (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* 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. 2.2g). 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. 2.2h). 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* (acc. nr. 13) exhibited the largest leaf area (94 cm^2), followed by *A. caudatus* (acc. nr. 60), which is actually classified as grain species but in 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^2 to almost 70 cm^2 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. 2.2i). 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* (acc. nr. 10) belongs to the grain species, whereas the lowest (1.2) was observed in *A. tricolor* (acc. nr. 23 and 78).

The Indonesian accessions showed significantly thicker leaves and higher variation than the worldwide collection (Fig. 2.2j). Our results implied that the most consumed Indonesian vegetable-types exhibited by *A. tricolor* (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 (Figs. 2.2k). Most of *A. dubius* collected from Takengon in Sumatra such as acc. nr. 34, exhibited extraordinarily higher numbers of leaves than the cultivated types (*A. tricolor*). 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. 2.2l). 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. 2.2m). 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* and *A. tricolor* exhibited an average protein content of 16%.

2.3.2. Cluster analyses of morphological variation in Indonesian amaranths

The results of clustering (Fig. 2.3) 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 2.3. Mean values of each morphological trait and protein content in the four clusters.

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*, *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 2.3). One *A. tricolor* (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* Mart. ex Thell. during its early stages; however, axillary spines appeared at a later stage in *A. spinosus*. All weedy plants in Cluster 2 have originated from 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 2. 3).

Cluster 3 comprised a mixture of weedy- (11 accessions); mostly represented by *A. dubius* and vegetable-types of *A. tricolor* 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*) 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 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.

2.3.3. Correlations among morphological and protein traits

Total leaf area is determined as an indirect measure of productivity or yield performance indicator in amaranths. Therefore, we would like to reveal the relationship of total leaf area with other morphological traits. Number of leaves and leaf area, stem diameter, number of branches and internodes were positively correlated with total leaf area. Number of leaves had the strongest correlation with total leaf area (Table 2.4). 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 was not strongly correlated with most of the morphological traits.This result implied that protein content by the leaves is not influenced by any morphological character. A weak correlation between protein content with leaf number and thickness was observed but these values of correlation do not imply the biological relationship tendency such as by the case of plant height and total dry matter yield (Mayo 1987).

2.3.4. Relationship between protein content and selected vegetable traits

Based on the results of correlation, there was significant but very weak correlation between protein and leaf number and thickness. This result may suggest us that leaf number and leaf thickness might be applied as indirect selected parameters by the improvement of protein. Therefore, we would like to study more deeply the relationship of protein content and with other selected vegetable traits such as number of leaves, total leaf area and leaf thickness (Fig. 2.4). The main objective of producing these scatter plots are intended for breeder as a *practical purpose* by the selection of potential parental lines.

The relationship between protein content and leaf number is presented on Fig. 2.4a. The majority of amaranths produces leaf number up to 50 leaves per plant but large variability in their protein (less than 10 up to almost 30 %). A relatively low protein content (12-20%) was observed by those accession with higher leaf number (50-100 per plant) and leaf area ranging from 10-61 cm^2. There is a tendency that the more leaves produced in amaranths, protein content is not so high, and vice versa. Three accessions of *A. dubius* (acc. nr. 40, 41, and 44) were shown to have high protein and leaf number but relatively small leaf area (6-20 cm^2). Nevertheless, these three accessions are worth to be selected for the improvement of leaf number in amaranths.

Leaf number and leaf size or leaf area are the two important parameter in vegetable amaranths (Shukla *et al.* 2005). Similar with this view, we tried to select the potential lines having leaf area at least 20 cm^2 with high leaf number or referred as the total leaf area. The total leaf area can be applied

as an indirect parameter to measure the productivity in amaranths. The relationship between total leaf area and protein is presented on Fig. 2.4b. Based on this figure, we can select four potential parental lines with their yield related characteristics. The first potential group are acc. nr. 32 (*A. viridis*) and acc. nr. 4 (*A. blitum*), and acc. nr. 33 (*A. dubius*). They are noted with specific characteristics such as leaf area varies from 20-50 cm^2, very high protein content but small to intermediate total leaf area. The second ones showed very high total leaf area, middle to high protein content but very small leaves (less than 20 cm^2). These are presented by *A. dubius* (acc. nr. 34, 53). The third one is presented by *A. tricolor* (acc. nr. 61) which showed remarkably very large leaf area (84 cm^2) and total leaf areas but protein is relatively low (Fig. 2.4b). Depending on the breeding target, one of these lines can be prospected as parental lines by the improvement of leaf area and leaf number.

The scatter plot diagram presented on Fig. 2.4c validated the previous result (Fig. 2.2j). The highest variation in protein content was observed in the weedy-types of amaranths with leaf thickness ranging from 0.15–0.30 mm. In contrast, the main Indonesian commercial vegetable amaranths (*A. tricolor*) had lower protein content but thicker leaves (0.31 mm to 0.50 mm). This result confirms the richness of weedy resources for leaf quality improvement.

2.4. 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 Widrlechner 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 (discussed in Chapter 5).

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.

Several morphological traits can be applied as indirect selected parameters for improving yield in amaranths. In our result, total leaf area was applied as an indirect measure for yield biomass in amaranths. Leaf number and area, stem diameter, number of branches and internodes (except the height of the plant) might influence the yield biomass. Our results are in similar view with Shukla *et al.* (2006) who investigated the positive contribution of several morphological traits (including stem diameter and leaf area) and protein content to the of yield biomass in amaranths (*A. tricolor*). Shukla *et al.* (2006) proposed that relatively high heritability values ($h^2 > 0.95$) of leaf size and stem diameter characters. Thus, these charactes can be transmited to the offspring.

Increase in biomass is the main target of vegetable breedings including in amaranths (Brenner *et al.* 2000). Nevertheless, yield biomass was affected strongly by the variation of environments, genotype and genotype environment interaction (Wu *et al.* 2000, Svirskis 2003). Higher temperature might enhance the growth, and thus finally the yield biomass of *A. tricolor* (Khandaker *et al.* 2009). Moreover, management practices such as nitrogen fertilizer application and clipping might also enhance the yield biomass (Gélinas and Seguin 2008, Moreno *et al.* 1999).

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 dor 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 be 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*), 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 "Introduction" that *A. blitum* 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 and 2) 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.

2.5. 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* L. (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.

Table 2.1. List of plant materials applied for morphological assessment and protein content.

Acc. Nr.	Accession Name	Type	Species	Acc.Nr.	Accession Name	Type	Species
	WORLDWIDE			42	IDN 10/P.One-one	WD	*A. spinosus*
1	Ames 5315/IND	VG	*A. blitum*	43	IDN 11/Tn.Depet	WD	*A. spinosus*
2	PI 610262/IND	VG	*A. blitum*	44	IDN 13/Mandua	WD	*A. dubius*
3	PI 490298/KEN	VG	*A. blitum*	45	IDN 14/Mandua	WD	*A. dubius*
4	PI 606281/BGD	VG	*A. blitum*	46	IDN 15/Mandua	WD	*A. dubius*
5	PI 606282/BGD	VG	*A. blitum*	47	IDN 17/Medan	WD	*A. dubius*
6	PI 482049/ZWE	GR	*A. cruentus*	48	IDN 18/Medan	WD	*A. blitum*
7	PI 482051/ZWE	GR	*A. cruentus*	49	IDN 19/Mandua	WD	*A. blitum*
8	PI 490662/BEN	GR	*A. cruentus*	50	IDN 20/Ygy	WD	*A. dubius*
9	PI 494777/ZMB	GR	*A. cruentus*	51	IDN 22/Ygy	WD	*A. dubius*
10	PI 500267/ZMB	GR	*A. cruentus*	52	IDN 23/Ygy	WD	*A. dubius*
11	PI 538319/USA	GR	*A. cruentus*	53	IDN 24/JKT	WD	*A. dubius*
12	PI 566897/IND	GR	*A. cruentus*	54	IDN 25/JKT	WD	*A. dubius*
13	PI 604666/USA	GR	*A. cruentus*	55	IDN 26/JKT	WD	*A. dubius*
14	PI 605352/JAM	VG	*A. dubius*	56	IDN 27/JKT	WD	*A. dubius*
15	PI 642737/PRI	VG	*A. dubius*	57	IDN 29/K.Urang	VG	*A. tricolor*
16	PI 608661/IND	VG	*A. graecizans*	58	IDN 30/K.Urang	VG	*A. tricolor*
17	PI 500249/ZMB	GR	*A. hybridus*	59	IDN 33/Lembang	VG	*A. caudatus*
18	PI 605351/GRC	GR	*A. hybridus*	60	IDN 34/Lembang	VG	*A. caudatus*
19	PI 604577/MEX	GR	*A. hypochondriacus*	61	IDN 35/BNA	VG	*A. tricolor*
20	Ames 5134/USA	VG	*A. tricolor*	62	IDN 36/BNA	VG	*A. tricolor*
21	PI 349553/PNG	VG	*A. tricolor*	63	IDN 37/BNA	VG	*A. tricolor*
22	PI 566899/IND	VG	*A. tricolor*	64	IDN 38/BNA	VG	*A. tricolor*
23	PI 604669/TWN	VG	*A. tricolor*	65	IDN 39/BNA	OR	*Celosia* spp.
24	PI 586680/USA	OR	*C. argentea*	66	IDN 40/BNA	VG	*A. tricolor*
25	PI 482244/ZWE	OR	*C. trigyna*	67	IDN 41/Marelan	VG	*A. tricolor*
26	PI 608761/IND	VG	*A. tricolor*	68	IDN 42/Marelan	VG	*A. tricolor*
27	NPL 01/KTM	OR	*Celosia* spp.	69	IDN 43/Marelan	VG	*A. tricolor*
28	NPL 02/KTM	OR	*Celosia* spp.	70	IDN 44/Marelan	VG	*A. tricolor*
29	NPL 03/KTM	OR	*Celosia* spp.	71	IDN 45/Marelan	VG	*A. tricolor*
30	NPL 04/KTM	OR	*Celosia* spp.	72	IDN 46/Kresek	VG	*A.tricolor*
31	NPL 05/KTM	OR	*Celosia* spp.	73	IDN 47/Kresek	VG	*A. tricolor*
	INDONESIA			74	IDN 48/Kresek	VG	*A. tricolor*
32	PI 540445/IDN/Java	WD	*A. viridis*	75	IDN 49/Yates	OR	*Celosia* spp.
33	IDN 01/Mongal	WD	*A. dubius*	76	IDN 50/P. Merah	VG	*A. tricolor*
34	IDN02/Daling	WD	*A. dubius*	77	IDN 51/P. Merah	VG	*A. tricolor*
35	IDN 03/Daling	WD	*A. dubius*	78	IDN 52/SHS	VG	*A. tricolor*
36	IDN 04/Bur biah	WD	*A. spinous*	79	IDN 53/SHS	VG	*A. tricolor*
37	IDN 05/Bur biah	OR	*Celosia* spp.	80	IDN 54/Tanindo	VG	*A. tricolor*
38	IDN 06/Bur biah	OR	*Celosia* spp.	81	IDN 55/P.Kb	WD	*A. hybridus*
39	IDN 07/Ulu Nuih	WD	*A. spinosus*	82	IDN 56/P.Kb	WD	*A. spinosus*
40	IDN 08/Asir-asir	WD	*A. dubius*	83	IDN 57G/P.Kb	WD	*A. hybridus*
41	IDN 09/Asir-asir	WD	*A. dubius*	84	IDN 57R/P.Kb	WD	*A. hybridus*

Acc. Nr., Accession Number; GR, grain; OR, ornamental; VG, vegetable; and 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.

Table 2.2. List of morphological traits analyzed.

Number	Morphological trait	Method	Data-type
	Qualitative traits		
1.	Growth habit[1)]	Visual observation	Category
2.	Leaf shape[2)]	Visual observation	Category
3.	Leaf color[3)]	Visual observation	Category
	Plant architecture traits		
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
	Leaf character traits		
8.	Leaf area (cm^2)	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
	Vegetable production traits		
11.	Number of leaves per plant (no.)	The main countable leaves were counted along main stem	Numerical
12.	Total leaf area (cm^2)	Data from number of leaves per plant were multiplied with the average leaf area	Numerical

[1)] 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°

[2)] 1 = elliptic, 2 = ovate, 3 = lanceolate, 4 = obovate, 5 = cordate, 6 = obtuse, 7 = aristate, 8= linear, 9 = not known

[3)] 1 = dark green, 2 = green, 3 = green-variegated red or red-variegated green, 4 = red-purple–variegated green, 5 = red-purple

Table 2.3. 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^2)**	**TLA (cm^2)**	**RLW**	**LTK (mm)**	**PRT (%)**
1	13.75^{b}	6.14^{b}	13.16^{a}	11.27^{b}	33.93^{bc}	17.16^{c}	525.36^{b}	2.07^{a}	0.25^{b}	18.44^{ab}
2	28.02^{a}	7.87^{ab}	16.91^{a}	16.16^{a}	188.38^{a}	13.21^{c}	$2,552.61^{a}$	1.75^{ab}	0.14^{c}	20.85^{a}
3	20.59^{ab}	8.94^{a}	16.43^{a}	15.11^{a}	67.93^{b}	46.37^{a}	$2,987.37^{a}$	1.64^{b}	0.35^{a}	17.28^{ab}
4	23.78^{a}	10.22^{a}	9.80^{b}	8.76^{b}	29.55^{c}	34.25^{b}	$1,027.64^{b}$	1.52^{b}	0.33^{a}	15.81^{b}

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

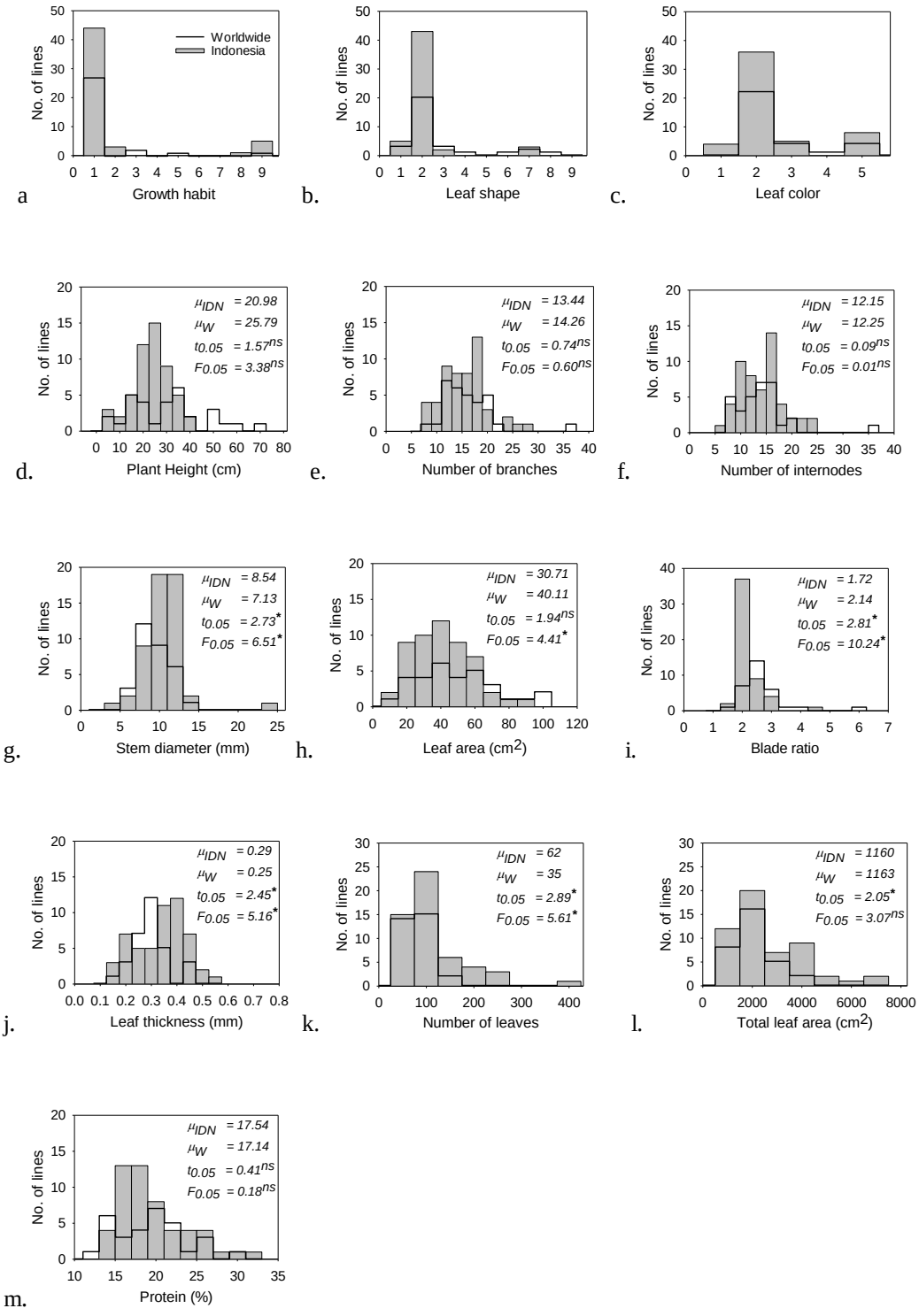
H, plant height; SDM, stem diameter; NBR, number of branches; NIN, number of internodes; NLV, number of leaves; LAR, leaf area; TLA, total leaf area; RLW, blade ratio; LTK, leaf thickness; and PRT, protein

Table 2.4. Correlation matrices for the morphological traits and protein contents in 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^2)					-	**0.357****	-0.065	**0.446****	-0.082
(7)	Total leaf area (cm^2)						-	-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

Figure 2.1. Map of the sample collection regions (dots) for Indonesian amaranths.



IDN = Indonesia; W = Worldwide

Figure 2.2. Distribution of morphological traits in *Amaranthus*. Data for the worldwide (N = 31) and Indonesian collections (N=53) are shown as black line and grey shaded bars, respectively.

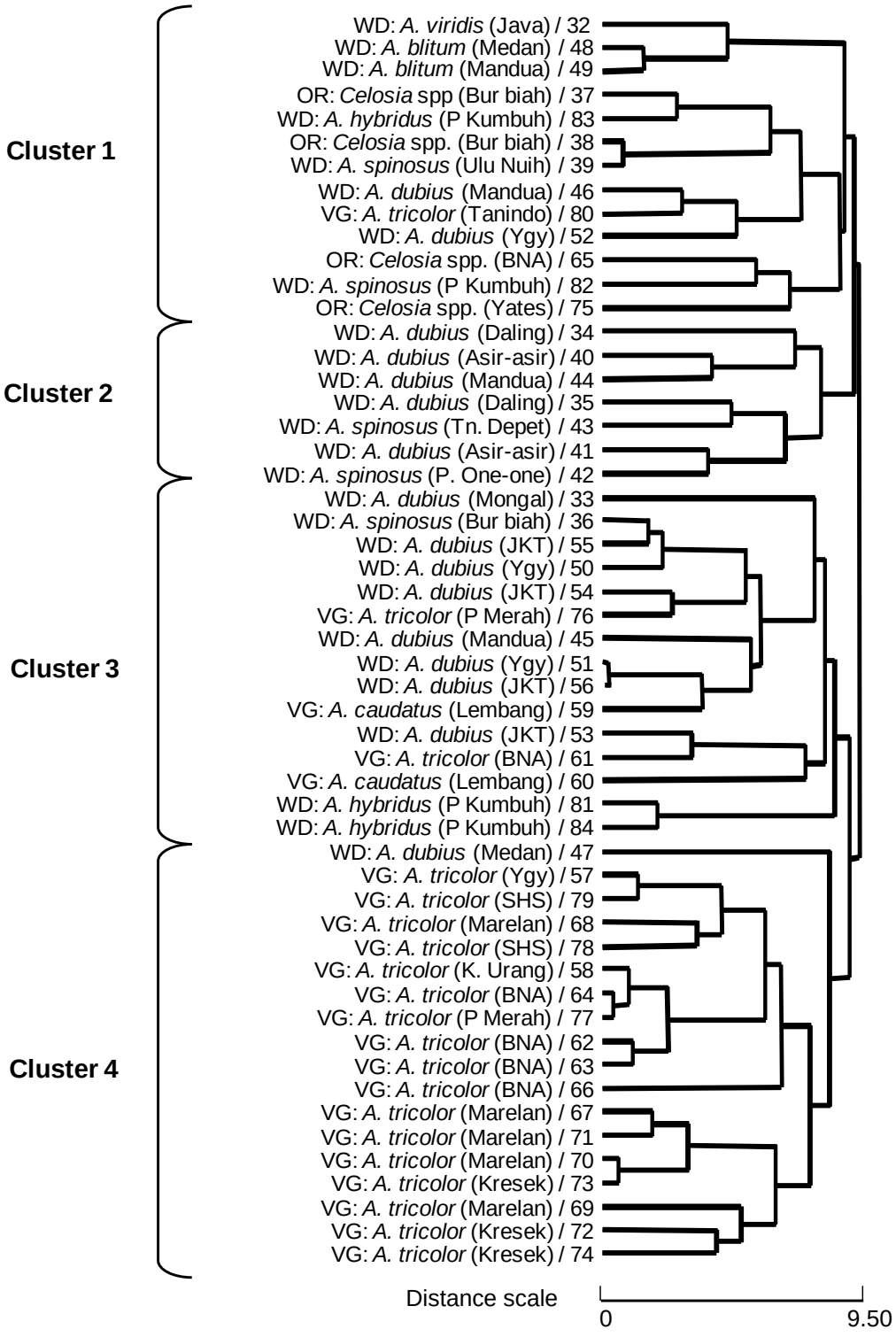


Figure 2.3. Dendrogram of Indonesian amaranths (N = 53) on the basis of 10 morphological characteristics, constructed using Ward's method. The origin of each amaranth is enclosed within parentheses; the number after the slash indicates the accession number. OR, ornamental; VG, vegetable; and WD, weedy-type.

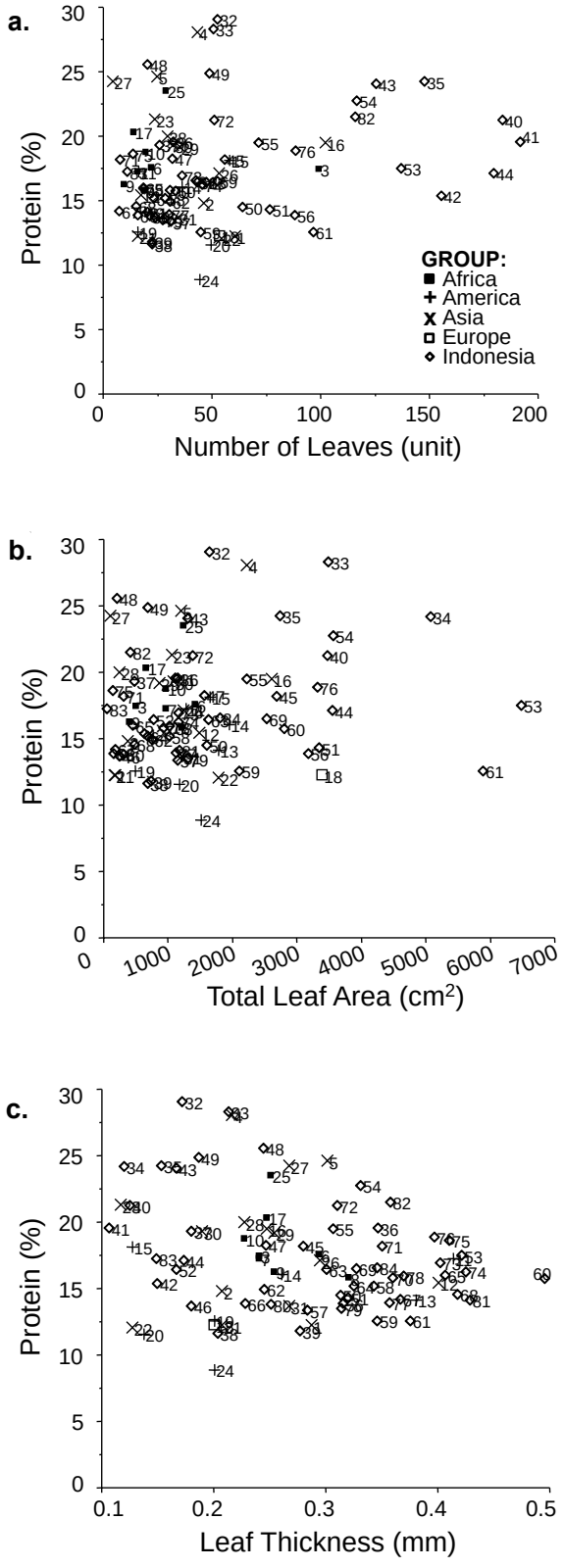


Figure 2.4. The relationship of protein content to leaf number (a), total leaf area (b), and leaf thickness (c). Numbers correspond to amaranth accession numbers (see Table 1).

CHAPTER 3

Photoperiodic flowering response and growth characteristics of *Amaranthus* spp.

3.1. Introduction

Flowering is an important life decision in plant development (Jaeger *et al*. 2006). In many plants, flowering is regulated by environmental signals such as photoperiod (day length), temperature, light signals, and seasonal changes such as sowing dates (Benzioni *et al*. 1991, Kikuchi and Handa 2009). Plants are generally classified as long-day (LD), short-day (SD) and day-neutral (DN) plants based on their day length requirement (Kikuchi and Handa 2009).

Amaranth (*Amaranthus* spp.) possesses diverse latitudinal ecotypes ranging from short day up to day neutral types (Deutsch 1977, Kulakow and Jain 1985). This unique adaptive characteristics enable them to be globally distributed (Grubben 1976). Amaranths are generally classified as short day plants. This means that flowering occurs when day length lasts at least 8 hours per day with a critical day length between 12 to 14 hours and 15 – 16 hours for *A. retroflexus* and for amaranths in general, respectively (Kigel 1994, Ahmed 2005). The grain and vegetable types are the two major cultivated types which show distinct biological characteristics in terms of biomass allocation in one plant (Mapes *et al*. 1996). Different growing environment is also noted between them. The grain species are mostly cultivated in temperate or in the high elevated regions of the tropics such as in the Himalayan foothills of Asia (Grubben 1976, Kulakow and Hauptli 1994). In contrast, the vegetable amaranths are better adapted to hot and humid climates such as in the tropics. The weedy types including weeds have no specific environmental requirement and they remarkably possess a large geographical distribution (Grubben 1976).

Time of flowering and time of plant maturity are important adaptive traits in amaranths (Kulakow and Hauptli 1994). In temperate growing regions, the grain types with early flowering tendencies and short day requirement are more preferable (Kulakow and Hauptli 1994). In opposite to

that, in the tropics, a delay flowering in vegetable amaranths is favored as this may increase the possibility to accumulate plants' vegetative growth which leads finally to higher biomass production (Siemonsma and Piluek 1994). Biomass accumulation, growth characteristics and plant morphology in amaranths are known to be majorly affected by environmental condition such as temperature and light intensity (Kigel 1994, Khandaker *et al.* 2009).

Depends on which type, different environmental requirement might be applied for promoting the flowering in amaranths. Light signals, different sowing dates and photoperiod condition were proposed as the main factors highlighted in photoperiodic flowering response (Kikuchi and Handa 2009, Bhosale *et al.* 2012). In this study, 69 accessions of amaranths originated from various ecogeographical regions; mainly from the tropical Indonesia were assessed in their photoperiodic flowering response. Study the flowering response can lead to important advances in crop improvement strategies in many commercial crops in their relationship with yield increase (Kulakow and Jain 1985, Jung and Müller 2009). We hypothesized that, the tropical Indonesian amaranths would show photoperiodic flowering response under the changing of day length such as in Japan where day length is longer than 12 hours in summer, and vice versa in winter. In Indonesia, such response in amaranths is masked as no differentiation of day length occurs at the tropical equator. Therefore, study of the photoperiodic flowering response in amaranths based on their day length and temperature requirement is prerequisite by the screening of amaranth germplasm for any further breeding efforts including in plant introduction process.

According to our best knowledge, so far, there has been no previous report dealth with the photoperiodic flowering response of various types (grain, vegetable and weedy) of amaranths under different day length condition. So far, reports had dealt only with the determination of critical day length in grain amaranths (Ahmed 2005). Therefore, in this study, we would like to: 1) study the photoperiodic flowering response in amaranths under Japanese condition 2) study the major factors affecting the flowering response in amaranths; 3) differentiate the three types based on their photoperiodic flowering response; 4) compare the growth characteristics of amaranths under different day length condition.

3.2. Materials & Methods

3.2.1. Plant materials

This study involved 69 accessions of *Amaranthus* consist of grain, vegetable and weedy types including 27 accessions representing the worldwide variation originated from 13 countries in Africa, America and Asia (Table 3.1). The ornamental types were excluded in this study. Detailed information regarding the worldwide and Indonesian collection: please refer to Point

3.2.1.1. Experimental site

Please refer to **2.2.1.1. Experimental Site**.

3.2.2. Methods

3.2.2.1. Germination

Seeds were sown in 4 different sowing times from 2010 to 2012 (Table 3.2). The seeds were germinated in plastic trays (6 x 6 holes with 4 cm diameter and 4.7 cm depth) containing no fertilizer ready soil ("Metromix 350" from Sungro). Three weeks after sowing, 3 or 4 plants per accession were selected and transplanted into clay pots (18 cm diameter and 16.5 cm height) with ready soil "Sumirin" ($N:P_20_5:K_2O$= 120:1,000:50 mg/l, pH= 6.7). Pots were arranged according to a completely randomized experimental design. Out of 4 plants, only 2 plants were left in one pot and grown until their maturity. Each accession was prepared in duplicate. Amaranth plants were watered daily and applied with insecticide when necessary.

3.2.2.2. Flowering time

In total, 2 plants were served for the flowering trait per accession. Thus, values presented were means from two plants grown in different pots. Number of days from sowing to flowering were noted as flowering time (abbreviated as d.a.s). Time of flowering in amaranths was recognized by the first emergence of the terminal inflorescence (Fig. 3.1). The emergence of the terminal inflorescence

is being emphasized here as in some vegetable amaranths (*A. blitum* and *A. graecizans*) the auxiliary inflorescences emerged at the commencement of the flowering period and prior to the terminal ones' emergence (personal observation).

3.2.2.3. Temperature and photoperiod data

The average air temperature was determined in grad Celsius (^{o}C) and recorded daily during the experiment with Thermo recorder RS 11 (Tabai Espec Corp. Osaka, Japan). The accumulative temperature (^{o}C) was obtained via the addition of daily mean temperature from sowing until the date of flowering in each accession (Fig. 3.4A). The photoperiod data was obtained from a japanese website for the information of Mito city on: http://www1.kaiho.mlit.go.jp/KOHO/automail/sun_form3.htm (accessed on 16 July 2012). The accumulative day length was obtained via the addition of day length from sowing until the date of flowering in each accession (Fig. 3.4B) and via the accumulation of average monthly day length (Fig. 3.5).

3.2.2.4. Growth characteristics

Plant height (H), stem diameter (SDM) and number of leaves (NLV) were determined as growth characteristics. Data were taken at flowering time obtained from two growing seasons with two different day length condition: i) under long day (season IV) and ii) changing from long to short day (season II). Values of H, SDM, NLV presented were means of measurements on four individual plants for each accession. Plant height (cm) was measured with a ruler from the soil surface to the top of the inflorescence. The diameter at the middle part of the main stem was measured manually with a slide caliper (PAT.No 946933, Kanon, Tokyo, Japan) and determined as stem diameter (mm). Main countable leaves along main stem were calculated as number of leaves per plant.

3.2.3. Statistical analysis

Average flowering time obtained from four different condition (season I up to IV) and the mean of growth characteristics from two different condition were subjected in the data analysis. Data distribution of each trait was plotted using Sigma Plot version 11. All statistical analysis (one-way

ANOVA, t-test, F-test and principal component) was performed by using JMP version 7.0 (SAS Institute, Cary, NC, USA). The t-test was applied to compare means and the F-test to compare the variances of two different groups. Two-way ANOVA for analyzing the amount of variance that is contributed to a sample by different factors was calculated based on SAS 9.0 (SAS Institute, Cary, NC, USA). Principal Component Analysis (PCA) based on correlation was applied to summarize the variation from the data of flowering and separate groups based on their major characteristics based on their scores of principal components (PCs) (Syafaruddin *et al*. 2006).

3.3. Results

3.3.1. Photoperiodic flowering response in *Amaranthus*

In Japan, with natural air temperature ranging from 5 to 38.1 ^{o}C and day length ranging from 9.75 to 14.60 hours from season I to IV, amaranths showed photoperiodic flowering response (Fig. 3.2). The total number of amaranths flowered in four different sowing groups was presented in Fig. 3.2. Based on flowering, three main groups were formed consisting of early (0-60 d.a.s), medium (61-80 d.a.s) and late flowering accessions (more than 80 days). Different sowing dates which lead to the variations of the temperature and day length had affected very highly significant the variation of flowering time in amaranths according to two-way ANOVA (Table 3.3). The variations of materials applied in this study (referred as "accessions"), the seasonal changes and the interaction of both factors were shown to be the major factors affecting the variation of flowering. Under long day condition such as season I and IV, amaranths required 60-90 d.a.s on average to flower. Meanwhile, flowering was the shortest (50-60 d.a.s) when day length changes from long (14.83 hours) to short day (10.65 hours) such as in season II. Similar phenomenon was also observed in sorghum where their flowering was accelerated only when day length became shorter (Gupta and Saha 1950). In contrast, the longest flowering period ranging from 80 to 105 d.a.s on average was occurred in winter under short day condition (season III) (Table 3.4 and Fig. 3.2). Moreover, the flowering time among the three types of amaranths (grain, vegetable and weedy) was significantly different in season III and

season IV, however, no significant differences was observed in season I and II (Table 3.4). The results exhibited on Figure 3.2 is re-affirmed in Table 3.4.

According to the results of PCA, the weedy types are the most widely dispersed in each cluster (Fig. 3.3.). This means that many flowering variation was observed within the weedy types. The second ones were followed by the vegetable and later with the grain ones. The PCA coordinate can be divided into two major categories, the upper (the positive values of PC2) and the below part of the y-axis (the negative values of PC2) which indicated the stability of their flowering. Those accessions which were not greatly affected due to the changes of day length from season I to IV are grouped on the upper part. Those were mostly represented by the grain and vegetable types belong to USDA accessions or might have been acclimatized with the northern latitude regions such as acc. nr. 6,7, 11,17 and acc. nr. 3,4,5, 22, 23, 24, respectively. There are two weedy types from Indonesia grouped the upper part (acc. nr. 33 and 67) and two tropical Indonesian vegetable types belong to *A. tricolor* (acc. nr. 55 and 64). Almost 95% of those accessions whose flowering time was strongly affected by the day length changes was observed in the Indonesian amaranths, mostly from the weedy and the vegetable types, such as acc. nr. 29, 30, 34, 37. Only two grain species *A. hybridus* (acc. nr. 18) and *A. hypochondriacus* (acc. nr. 20) classified as affected by the day length changes (Fig. 3.3). The scores of the eigenvalue and cum percent which contributed to PC1 to PC4 are presented on Table 3.5.

3.3.2. Temperature and day length as the major effects of flowering in *Amaranths*

The two environmental factors, namely (accumulative) temperature and day length were shown to majorly affect the response of flowering in amaranths.

Table 3.5 presents the accumulative temperature in ^{o}C, day length at the time of flowering and mean flowering time of each accession of amaranths. There were 6 species listed in the vegetable types. Meanwhile, the grain and weedy types were represented by three and four species, respectively. The overall mean of accumulative temperature was between 1100-1600 ^{o}C from season I to IV during the growth of amaranths. This result is in accordance with Henderson *et al*. (2000) who classified

amaranths as warm loving plants. A higher values by the overall mean of accumulative temperature and longer day length (14 hours) were observed (1563 ^{o}C) in season IV. Nevertheless, the overall mean values of flowering time (mean= 67 d.a.s ± 10 days) was not significantly different between season IV and I (Table 3.5 and Fig. 3.1). The flowering time of the three types of amaranths was between 56 to 83; except by *A. retroflexus*.

A. retroflexus (USDA 23/ PI 607447/ JAM) showed unique early flowering tendency almost in all seasons; between 31-42 days after sowing. This early flowering tendency in *A. retroflexus* was in accordance with previous result (Kulakow and Hauptli 1985).

Only one accessions of *A. graecizans* showed no sensitivity to flower in season I (Table 3.5).

The grain species such *A. cruentus*, *A. hypochondriacus* and the dual type of *A. caudatus* required 11-13 hours of day length to initiate their flowers with mean flowering time in the range of 69-80 d.a.s to flower.

Moreover, the vegetable and (edible) weedy types required similar day length between 12-13 hours to induce their flowering; with flowering time ranging from 58 to 80 after sowing date.

Moreover, most of grain amaranths required about 9–10 hours of day length to flower; with timing of flowering between 74-80 days in season I and II.

There are two important findings from the flowering response in this winter condition (season III). First, the range of 9 – 10 hours of day length was seemly enough to induce flowering by the grain species but it was not enough for those tropical origin vegetable species such as *A. graecizans*, *A. tricolor*, and *A. blitum*. This result shows us that by decreasing day length (less than 12 hours per day), a tremendous delay of flowering was observed within those vegetable species. The longest delay was observed in *A. graecizans* which flowered after 124 days. This is a very great delay as it flowered only after 40 days in season II. The second great delay of flowering was observed in *A. tricolor*, which required 114 days to flower. This period was almost twice longer than its flowering

time in season II. Second, there is a contrast difference between the flowering of the cultivated and the weedy types of *A. blitum* under 10 hours of day length. As vegetable or the already domesticated *A. blitum*, it flowered after 86 days. Meanwhile, weedy *A. blitum* required longer time to flower (112 d.a.s) under 10-11 hours of day length.

3.3.3. Growth characteristics of *Amaranthus*

The growth characteristics from amaranths grown under two different day length condition are presented in Figure 3.3. The mean values in most of the morphological traits were not strongly affected due to the day length differences; except by the plant height (Figure 3.3). The long day and warm summer condition of season IV had affected amaranths to be 35% taller than those grown in season II. The effect of accession, season and interaction of both effect had significantly affected the variation in their morphology, particularly by the plant height in amaranths according to two-way ANOVA (Table 3.7).

3.4. Discussion

Previous studies suggested that variability of flowering is exist in amaranths as a result of different day length requirement depending on types or species (Kulakow and Jain 1985).and unique characteristics such as early flowering observed in *A. retroflexus* (Kulakow and Jain 1985, Espitia-Rangel 1994).

These results showed us that PCA was shown to be an effective and practical tool to group the accessions based on their photoperiod flowering response. The selection of potential line for further development can be achieved by applying this method.

Many of *Brassicaceae* family, especially *Brassica rapa* is counted as one of the most important vegetables especially in East Asia, such as Japan (Kubo *et al*. 2010). Usually, they are better adapted in low temperature condition such as in autumn or in early spring and required

vernalization to induce their flowering (Tanaka *et al*. 2008). Moreover, most of them (e.g. lettuce and Chinese cabbage) can not thrive their growth well under summer heat condition (Edie and Ho 1968). In contrast, many green leafy vegetables adapated in the tropical regions are known to possess an ability to endure summer heat or when temperature is above 25 ^{o}C and still can perform their growth satisfactorily on the field. *Amaranthus* and *Ipomoea aquatica* were counted as the major leafy vegetables source that can be obtained during the summer months in India and Hongkong, respectively (Edie and Ho 1968, Shukla *et al*. 2006, Shukla *et al.* 2010). Based on these experiences outside Japan, therefore, further development of new vegetable variety with improved heat tolerance brings a new market for summer vegetable types in Japan.

Amaranths showed a wide range of photoperiodic flowering response ranging from 30 to 140 days of flowering time under Japanese condition. In general, the flowering in amaranths was greatly varied from 70 to 240; depending on the variety and environmental factors (Espitia-Rangel 1994). The different dates of sowing in this experiment had resulted the variation of temperature and day length during the growing seasons. The flowering in amaranths was getting longer during the cold seasons. Moreover, by the decreasing day length, amaranths flowered rapidly. Similar phenomena was also observed in sorghum by Gupta and Saha (1950) where sorghum flowered more rapid by the decreasing day length. However, we could not clearly differentiate whether day length or the temperature might play a bigger role by such variation of flowering.

Similar condition and conclusion were reported by particularly in *A. tricolor* under natural and artificial condition was first reported by Deutsch (1977). However, he could not clarify whether which factors applied might affect the variability of flowering in amaranths. Light intensity in its conjunction with day length exposure and temperature are the two major environmental stimuli promoting flowering (Franklin 2009, Kobayashi and Weigel 2012).

Kulakow and Hauptli (1994) suggested that there will be always variation in photoperiod response within several population in amarnaths as they are greatly affected by the growing condition.

We proposed that further evaluation under several environments would might help us to determine the major factor of flowering in amaranths in the future study.

Flowering or no flowering is an important decision in one plant's life (Jaeger *et al*. 2006). The decision to flower is majorly affected by the environmental stimuli (Franklin 2009). Despite a long day condition with more than 12 hours in season I, we observed that the tropical Indian origin of *A. graecizans* showed no sensitivity to flower. The thermorecorder noticed an extreme heat up to 49 ^{o}C in summer in 2010 which was very rare happened in Japan (Fig. 2). We proposed that such unusual high temperature might affect the no flowering condition in *A. graecizans* according to Heggie and Halliday (2005).

Many biophysiological-, genes-interaction and-expression are integrated in the process of flowering (Putterill *et al*. 2004). The unique characteristic of the early flowering *A. retroflexus* was being confirmed in our study. *A. retroflexus* shows early flowering under both short (8 hour) and long day condition (16 hour day length). The early flowering condition was proposed by Kulakow and Jain (1985) to be controlled by at least one single gene (*Ea*) which is dominant over the crop-weed hybrids and backcrosses generation between *A. cruentus* x *A. retroflexus*. Kulakow and Jain (1985) proposed as many as to three genes plus linked gene complexes were needed to clarify the flowering mechanism. This *Ea* gene was proposed to be a pleiotropic gene as it should not only control the (early) flowering but also in other plant morphology such as development of inflorescence and number of internodes. Further studies at the molecular work (such as major quantitative trait loci or QTL) in terms of the day length response should be conducted to uncover other major genes involved in determination of flowering.

Zheleznov *et al*. (1997) and Grubben (1976) proposed that growth characteristics in amaranths were affected significantly due to environmental condition. Both authors suggested that the vigorous growth in amaranths was exhibited under warm condition. Based on our results, warmer temperature had significantly affected the elongation of amaranths but not in other morphological traits (stem diameter and leaf number). This result confirmed to the previous theory proposed by

Franklin (2009) in the model plant of *Arabidopsis.* Warm temperature in the range of 22 to 28^{o}C facilitates the elongation in plant. Meanwhile, cooler temperature such as 16 ^{o}C promote the dwarf performance in *Arabidopsis*.

Ecotypical variation in the materials studied here seemed to suffice to detect their response according to their geographical origin. Moreover, we revealed the usefulness of studying the photoperiodic flowering response to distinguish amaranth based on their types in relationship with their plant geographical adaptation. From the result of PCA, many weeds or weedy plants showed a greater variation of photoperiodic flowering response if compared with vegetable and grain types. A high level of morphotype variation including in their flowering phenotype should be exist in many wild or weedy plants in order to support wider adaptation and distribution (Kassa *et al.* 2012). In amaranths, such large diversity of photoperiodic flowering response equipped with their ability to produce great quantities of small seeds is very advantageous adaptive character for their rapid widespread all around the globes (Grubben 1976).

We confirmed from this result that amaranths are short day plants (Kohli and Sawhney 1979). A day length less than 12 hours was enough to promote the flowering in most of the grain species. Such early flowering under less than 12 hours might be resulted due to human selection process (Mapest *et al.* 1996). In contrast, a day length less than 12 hours was not sufficed to promote flowering in vegetable amaranths based on our results. Thus, they showed a slightly tendency to behave such as long day plants. This evidence was supported from our finding by the flowering response in winter (season III). During winter and day length less than 12 hours, amaraths were grown inside the house and despite cool temperature outside, the overall mean accumulative temperature inside the house was ~1400 ^{o}C. If only temperature factor, this value should be enough to promote flowering of amaranths as in season I (overall mean accumulative temperature = 1100 ^{o}C). However, amaranths delayed their flowering a lot under day length less than 12 hours. This means that, for vegetable amaranths, a range of 12-13 hours of day length would be required to induce flowering. A longer day length requirement for vegetable amaranths might be resulted from their plant adaptation process in a particular region as by the case in maize (Ducrocq *et al*. 2008). One gene which is called

Vgt1 was highly correlated with geographical origins of maize collection. The *Vgt1* allele was found to be more abundance in the maize collection from warmer regions than those ones grown under temperate climates. Similar condition might be applied to amaranths.

Based on their assessment of photoperidic flowering and growth response, we can conclude that amaranth bears a great potential as a promising summer vegetable type to be introduced and developed in Japan. Its wide adaptability in many continents, good endurance to heat and other environmental constraints, have made them as durable vegetable crops that deserved to be further exploited.

3.5. Conclusions

Amaranths were shown to exhibit photoperiodic flowering response under 11 to almost 15 hours of day length. Under Japanese condition, amaranths can be classified into three groups according to their flowering time: early flowering (0-60 d.a.s), middle flowering (61-80 d.a.s) and late flowering type (more than 80 days). A wide flowering phenotype was observed in most of weedy types. Based on the requirement of day length, the grain species were classified as short day plants with 8-12 hours of day length enough to induce flowering. In contrast, vegetable amaranths required a minimum day length in the range of 12 hours per day. Growth characteristics were affected due to the variation of accession, season and the interaction of both. Nevertheless, the differences by the mean values of growth characteristics of amaranths grown under two condition of day length were not significantly different; except in plant height.

Table 3.1. List of plant materials of the 69 accessions of *Amaranthus*.

Acc. No.	Accession Name	Type	Species
	WORLDWIDE		
1	USDA 01/Ames 5315/ IND	VG	*A.blitum*
2	USDA 02/ PI 610262/ IND	VG	*A.blitum*
3	USDA 03/ PI 490298/ KEN	VG	*A.blitum*
4	USDA 04/ PI 606281/ BGD	VG	*A.blitum*
5	USDA 05/ PI 606282/ BGD	VG	*A.blitum*
6	USDA 06/ PI 482049/ ZWE	GR	*A.cruentus*
7	USDA 07/ PI 482051/ ZWE	GR	*A.cruentus*
8	USDA 08/ PI 490662/ BEN	GR	*A.cruentus*
9	USDA 09/ PI 494777/ ZMB	GR	*A.cruentus*
10	USDA 10/ PI 500267/ ZMB	GR	*A.cruentus*
11	USDA 11/ PI 538319/ USA	GR	*A.cruentus*
12	USDA 12/ PI 566897/ IND	GR	*A.cruentus*
13	USDA 13/ PI 604666/ USA	GR	*A.cruentus*
14	USDA 15/ PI 605352/ JAM	VG	*A. dubius*
15	USDA 16/ PI 642737/ PRI	VG	*A. dubius*
16	USDA 17/ PI 608661/ IND	VG	*A.graecizans*
17	USDA 19/ PI 500249/ ZMB	GR	*A.hybridus*
18	USDA 20/ PI 605351/ GRC	GR	*A.hybridus*
19	USDA 21/ PI 604577/ MEX	GR	*A.hypochondriacus*
20	USDA 22/ 604796/ N.A	GR	*A.hypochondriacus.*
21	USDA 23/ PI 607447/ JAM	WD	*A.retroflexus*
22	USDA 24/ Ames 5134/ USA	VG	*A.tricolor*
23	USDA 25/ PI 349553/ PNG	VG	*A.tricolor*
24	USDA 26/ PI 477918/ N.A	VG	*A.tricolor*
25	USDA 27/ PI 566899/ IND	VG	*A.tricolor*
26	USDA 28/ PI 604669/ TWN	VG	*A.tricolor*
27	USDA 29/ PI 608761/ IND	VG	*A.tricolor*
	INDONESIA		
28	USDA 30/ PI 540445/ IDN/Java	WD	*A.viridis*
29	IDN 01/ Mongal	WD	*A. dubius*
30	IDN02/ Daling	WD	*A. dubius*
31	IDN 04/ Bur biah	WD	*A.spinosus*
32	IDN 07/ Ulu Nuih	WD	*A.spinosus*
33	IDN 08/ Asir-asir	WD	*A. dubius*
34	IDN 09/ Asir-asir	WD	*A. dubius*
35	IDN 10/P.One-one	WD	*A.spinosus*
36	IDN 11/ Tn.Depet	WD	*A.spinosus*
37	IDN 14/ Mandua	WD	*A. dubius*
38	IDN 15/ Mandua	WD	*A. dubius*
39	IDN 17/ Medan	WD	*A.dubius*
40	IDN 18/ Medan	WD	*A.blitum*
41	IDN 19/ Mandua	WD	*A.blitum*
42	IDN 20/ Yogya	WD	*A.dubius*
43	IDN 22/ Yogya	WD	*A. dubius*
44	IDN 23/ Yogya	WD	*A. dubius*
45	IDN 24/ JKT	WD	*A. dubius*
46	IDN 25/ JKT	WD	*A. dubius*
47	IDN 26/ JKT	WD	*A. dubius*
48	IDN 27/ JKT	WD	*A. dubius*
49	IDN 30/ Kl.Urang	VG	*A.tricolor*
50	IDN 33/ Lembang	VG	*A.caudatus*
51	IDN 34/ Lembang	VG	*A.caudatus*
52	IDN 35/ BNA	VG	*A.tricolor*
53	IDN 36/ BNA	VG	*A.tricolor*
54	IDN 37/ BNA	VG	*A.tricolor*
55	IDN 38/ BNA	VG	*A.tricolor*
56	IDN 43/ Marelan	VG	*A.tricolor*
57	IDN 44/ Marelan	VG	*A.tricolor*
58	IDN 45/ Marelan	VG	*A.tricolor*
59	IDN 46/ Kresek	VG	*A.tricolor*
60	IDN 47/ Kresek	VG	*A.tricolor*
61	IDN 48/ Kresek	VG	*A.tricolor*
62	IDN 50/ P. Merah	VG	*A.tricolor*
63	IDN 51/ P. Merah	VG	*A.tricolor*
64	IDN 52/ SHS	VG	*A.tricolor*
65	IDN 53/ SHS	VG	*A.tricolor*
66	IDN 54/ Tanindo	VG	*A.tricolor*
67	IDN 55/ P.kumbuh	WD	*A.hybridus*
68	IDN 56/ P.kumbuh	WD	*A.spinosus*
69	IDN 57G/P.kumbuh	WD	*A.hybridus*

GR, grain; VG, vegetable; and WD, weedy type

Country code: BEN, Benin; BGD, Bangladesh; GRC, Greece; IDN, Indonesia; IND, India; JAM, Jamaica; KEN, Kenya; MEX, Mexico; 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.; P.kumbuh, Payakumbuh. Seed producer code: P.Merah, Panah Merah; SHS, Sang Hyang Sri

Table 3.2. Four different sowing dates by the growing of *Amaranthus*.

Season	Sowing dates	Growing Period	Day length
I	25 January 2010	January - June	LD
II	6 August 2010	August - November	LD and SD
III	29 October 2011	October - May	SD
IV	18 March 2012	March - August	LD

LD= long day; SD= short day

Table 3.3. Source of variation affecting the variation of flowering in *Amaranthus* (N= 69) based on two-way ANOVA.

Source	DF	Sum of Square	Mean Square	F Value	P. > F
Accession	68	127, 664	1, 877	48	***
Season	3	109, 681	36, 560	935	***
Accession x Season	204	198, 795	975	25	***

Table 3.4. Mean flowering of the three types of amaranths (N= 69) from season I to IV.

Type	N	Season I				Season II				Season III				Season IV			
		Mean‡	S.D.	F Ratio	P. > F	Mean‡	S.D.	F Ratio	P. >F	Mean‡	S.D.	F Ratio	P. >F	Mean‡	S.D.	F Ratio	P. >F
GR	12	71[a]	14	0.94	n.s.	61[a]	10	3.02	n.s.	78[b]	14	9.67	***	94[a]	52	5.47	**
VG	33	82[a]	36			56[a]	14			105[a]	21			81[ab]	25		
WD	24	74[a]	15			49[a]	15			86[b]	24			64[b]	9		

‡ a, b, c Means with the same letter are not significantly different according to Tukey-Kramer Test at $P < 0.05$ level. GR, Grain; VG, Vegetable; OR, Ornamental; WD, Weed

*, ** and *** represent significant level at $P < 0.05$, very significant at $P < 0.01$ and very highly significant at $P < 0.0001$, respectively. n.s. means not significant

Table 3.5. Accumulative temperature (°C), mean flowering time and day length at the time of flowering represented by 13 species of amaranths

TYPE	Species	N	Season I (LD)						Season II (LD to SD)					
			Acc. T (°C)	S.D. Acc. T (°C)	DL at FT	S.D. of DL	Mean FT	S.D. of FT	Acc. T (°C)	S.D. Acc. T (°C)	DL at FT	S.D. of DL.	Mean FT	S.D. of FT
GR	*A. cruentus*	8	1208.66	228.10	12.87	0.53	74.66	14.88	1663.12	76.76	12.53	0.13	65.25	3.77
	A. hybridus	4	992.60	10.44	11.22	0.07	57.13	1.03	1634.55	366.95	12.33	0.18	64.13	17.63
	A. hypochondriacus	2	1104.25	n.a.	12.68	n.a.	69.13	0.18	1336.58	146.54	12.64	0.44	49.00	7.07
VG	*A. blitum*	5	1031.71	57.49	12.36	0.22	61.05	5.70	1268.76	360.01	12.80	0.05	47.20	15.72
	A. dubius	2	1006.00	n.a.	12.27	n.a.	58.50	n.a.	1117.53	n.a.	12.52	n.a.	39.00	n.a.
	A. graecizans subsp. silvestris	1		n.a.		n.a.	no flowering	n.a.	1142.61	n.a.	12.48	n.a.	40.00	n.a.
	A. viridis	1	1082.20	n.a.	12.60	n.a.	67.00	n.a.	1656.24	n.a.	12.53	n.a.	65.00	n.a.
	A. caudatus	2	1258.30	n.a.	13.08	n.a.	80.00	n.a.	1554.80	348.81	12.24	0.06	59.50	17.68
	A. tricolor	21	1267.32	147.25	12.05	0.69	80.11	11.20	1530.20	247.40	12.35	0.25	58.52	12.50
WD	*A. retroflexus*	1	714.00	n.a.	11.50	n.a.	38.00	n.a.	933.35	n.a.	12.82	n.a.	31.50	n.a.
	A. dubius	14	1249.83	173.51	13.02	0.42	78.45	11.89	1204.62	120.32	12.44	0.18	42.86	5.49
	A. spinosus	4	1160.89	258.51	12.72	0.68	70.63	19.01	1283.52	331.99	12.48	0.08	46.88	15.75
	A. blitum	3	1223.72	39.34	13.01	0.11	77.67	3.06	1790.17	125.72	12.28	0.25	72.00	7.00
	Overall mean		1108.29		12.45		67.69	8.37	1393.54		12.50		52.37	11.40

LD, Long Day; SD, Short Day; GR, Grain; VG, Vegetable; WD, Weedy; Acc.T, Accumulative Temperature; S.D., Standard Deviation; DL, Day Length; FT, Flowering Time

Table 3.5. (continued)

TYPE	Species	N	Season III						Season IV					
			Acc. T (°C)	S.D. Acc. T (°C)	DL at FT	S.D. of DL	Mean FT	S.D. of FT	Acc. T (°C)	S.D. Acc. T (°C)	DL at FT	S.D. of DL	Mean FT	S.D. of FT
GR	*A. cruentus*	8	1189.40	311.02	10.03	0.38	79.31	16.29	1544.14	297.16	14.20	0.23	66.79	12.41
	A. hybridus	4	1086.77	139.43	9.88	0.10	74.00	7.39	1822.90	557.02	14.32	0.19	78.00	22.28
	A. hypochondriacus	2	1180.03	n.a.	9.95	n.a.	79.00	n.a.	1761.50	n.a.	14.42	n.a.	75.50	n.a.
VG	*A. blitum*	5	1330.85	15.72	10.22	0.55	86.60	21.33	1448.68	195.52	14.14	0.20	62.90	8.22
	A. dubius	2	1092.90	n.a.	9.88	0.11	74.50	6.36	1483.23	248.65	14.17	0.24	64.25	10.25
	A. graecizans subsp. silvestris	1	2088.93	n.a.	11.37	n.a.	124.00	n.a.	1810.80	n.a.	14.47	n.a.	78.00	n.a.
	A. viridis	1	1321.68	n.a.	10.12	n.a.	86.50	n.a.	1307.40	n.a.	13.98	n.a.	56.50	n.a.
	A. caudatus	2	1456.68	17.68	10.37	0.57	93.50	19.09	1849.65	124.66	14.48	0.07	79.50	4.95
	A. tricolor	21	1882.04	12.50	11.02	0.62	113.93	17.60	1925.68	254.58	14.48	0.07	82.48	10.22
WD	*A. retroflexus*	1	498.83	n.a.	9.80	n.a.	41.50	n.a.	838.40	n.a.	13.30	n.a.	35.00	n.a.
	A. dubius	14	1236.74	414.56	10.18	0.39	81.18	23.05	1477.61	170.73	14.17	0.19	64.11	7.34
	A. spinosus	4	1545.19	476.98	10.52	0.72	97.13	23.92	1518.15	198.02	14.20	0.22	65.63	8.68
	A. blitum	3	1843.85	103.12	10.96	0.17	112.83	4.65	1534.47	66.11	14.24	0.05	66.50	2.60
	Overall mean		1365.68		10.33		88.00	15.52	1563.28		14.20		67.32	9.66

LD, Long Day; SD, Short Day; GR, Grain; VG, Vegetable; WD, Weedy; Acc.T, Accumulative Temperature; S.D., Standard Deviation; DL, Day Length; FT, Flowering Time

Table 3.6. Eigenvalue and cum percent for the principal component analysis from the three types of amaranths observed in the 69 accessions based on covariance.

Eigenvectors	PC1	PC2	PC3	PC4
Season I	0.487	-0.275	0.827	-0.058
Season II	0.349	0.912	0.111	0.186
Season III	0.556	-0.304	-0.382	0.674
Season IV	0.576	-0.026	-0.398	-0.713

Table 3.7. The effect of accession, season and interaction of both factors on the variation of morphological traits in amaranths (N=69) growing in two different condition of day length based on two-way ANOVA.

Nr.	Growth Characteristics	Two-way ANOVA P. > F		
		Accession Effect	Season Effect	Interaction of Both Effect
1	Height (cm)	***	***	***
2	Stem Diameter (mm)	***	**	***
3	Leaf Number (unit)	***	**	***

*, ** and *** represent a significant level at $P < 0.05$, very significant at $P < 0.01$ and very highly significant at $P < 0.0001$, respectively. n.s. means not significant

Figure 3.1. Terminal inflorescence of red amaranth (left) and green one (right) at flowering time .

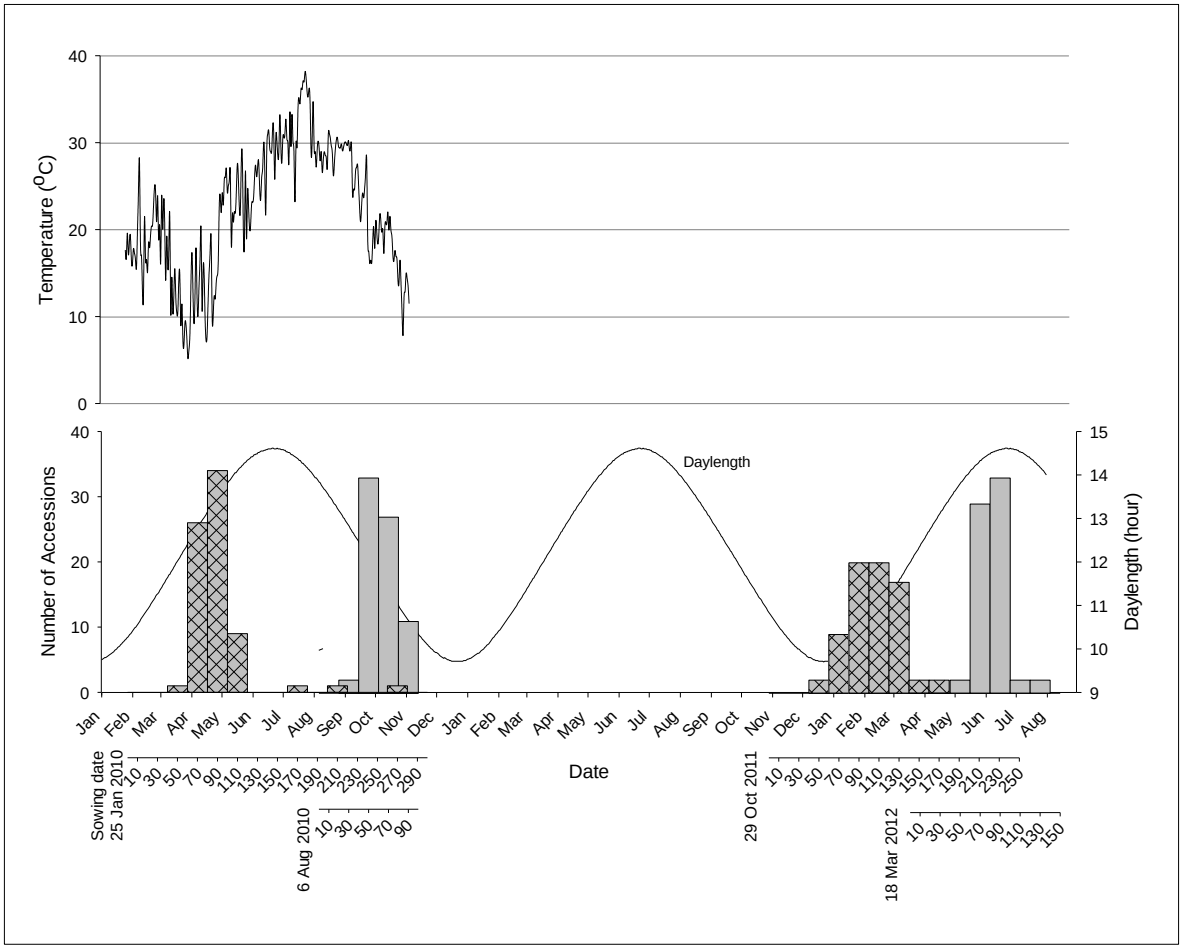


Figure 3.2. Number of flowered *Amaranthus* accessions (N= 69) which was grown under different photoperiod condition and seasonal temperature due to different sowing dates.

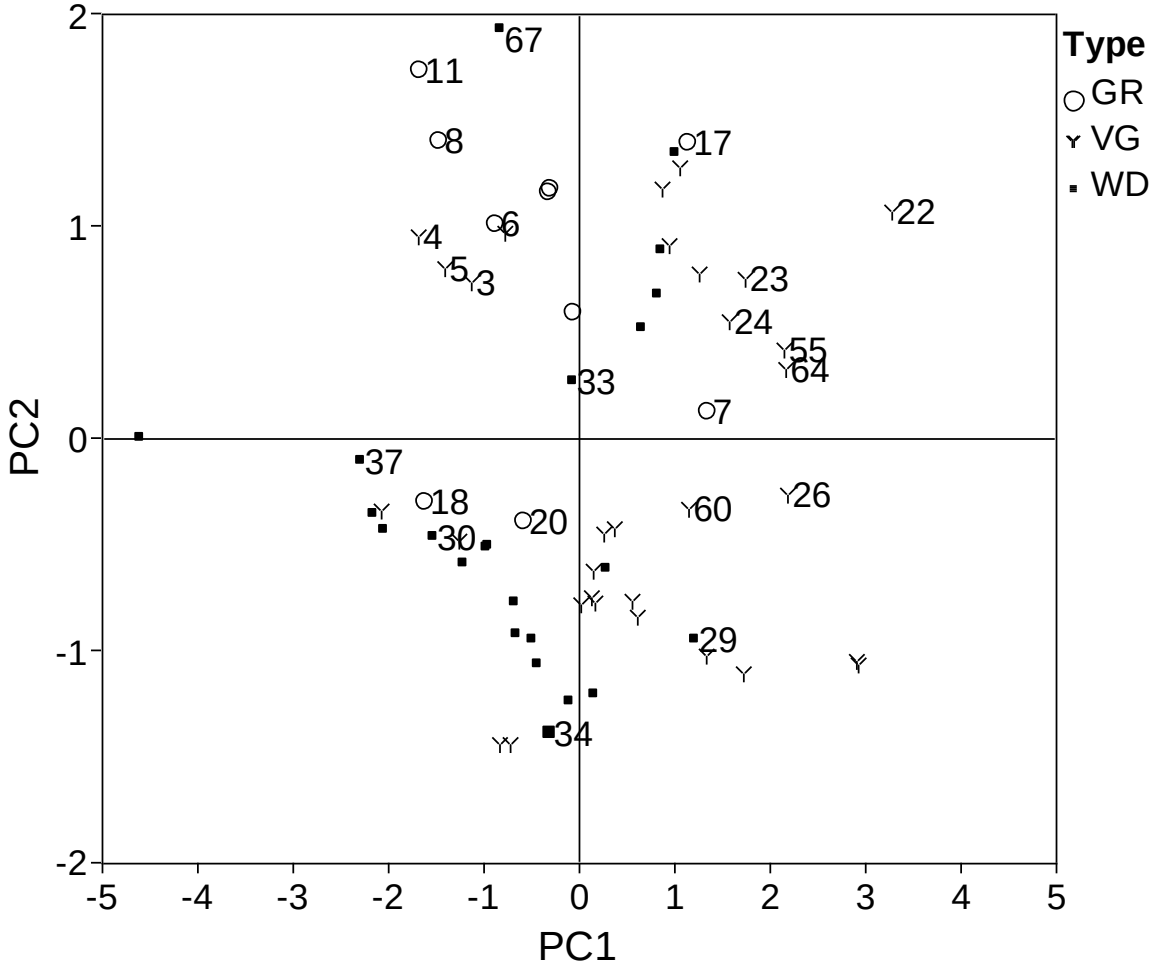


Figure 3.3. Scatter plot of the two principal components for the 69 *Amaranthus*' accession summarized from four flowering traits by the principal component analysis based on correlation.

The coordinates are divided based on the opposite to the clock counter-wise; coordinate I: top right; coordinate II: top left; coordinate III: bottom left; coordinate IV: bottom right.

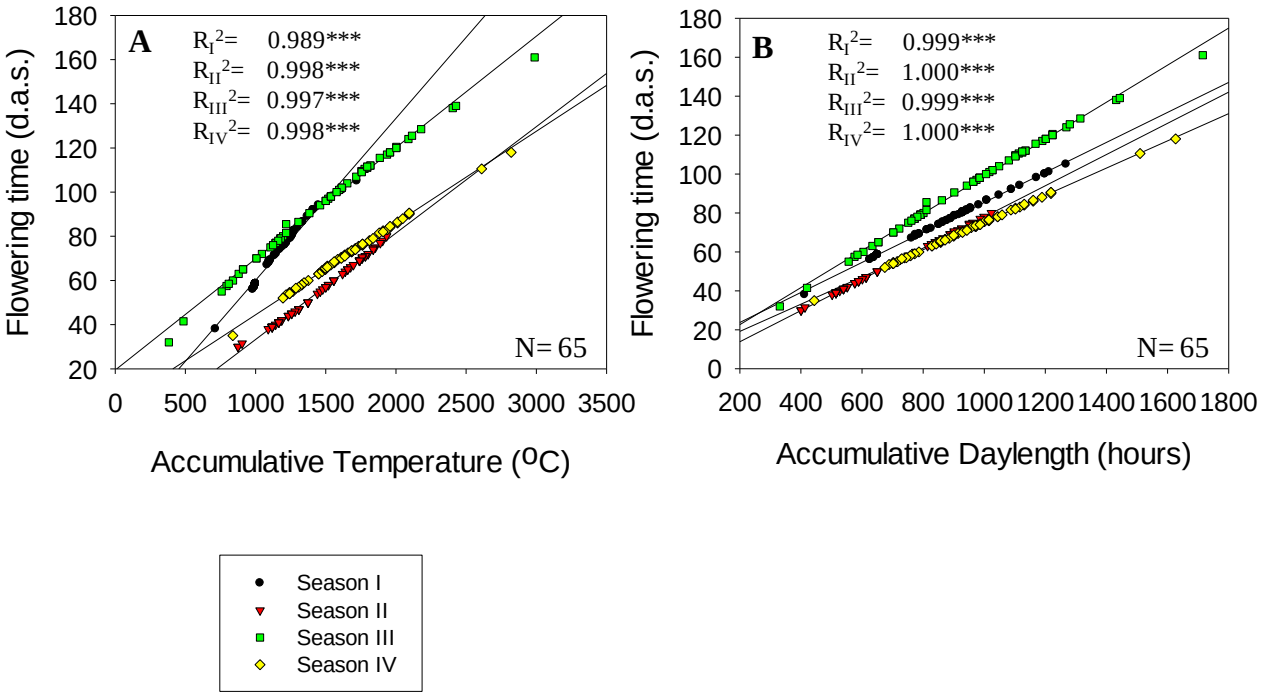


Figure 3.4. Linear relationship between flowering time in amaranths (total= 13 species) and accumulative temperature (°C) (A); accumulative day length (hours) (B).

*, ** and *** represent a significant level at $P < 0.05$, very significant at $P < 0.01$ and very highly significant at $P < 0.0001$, respectively.

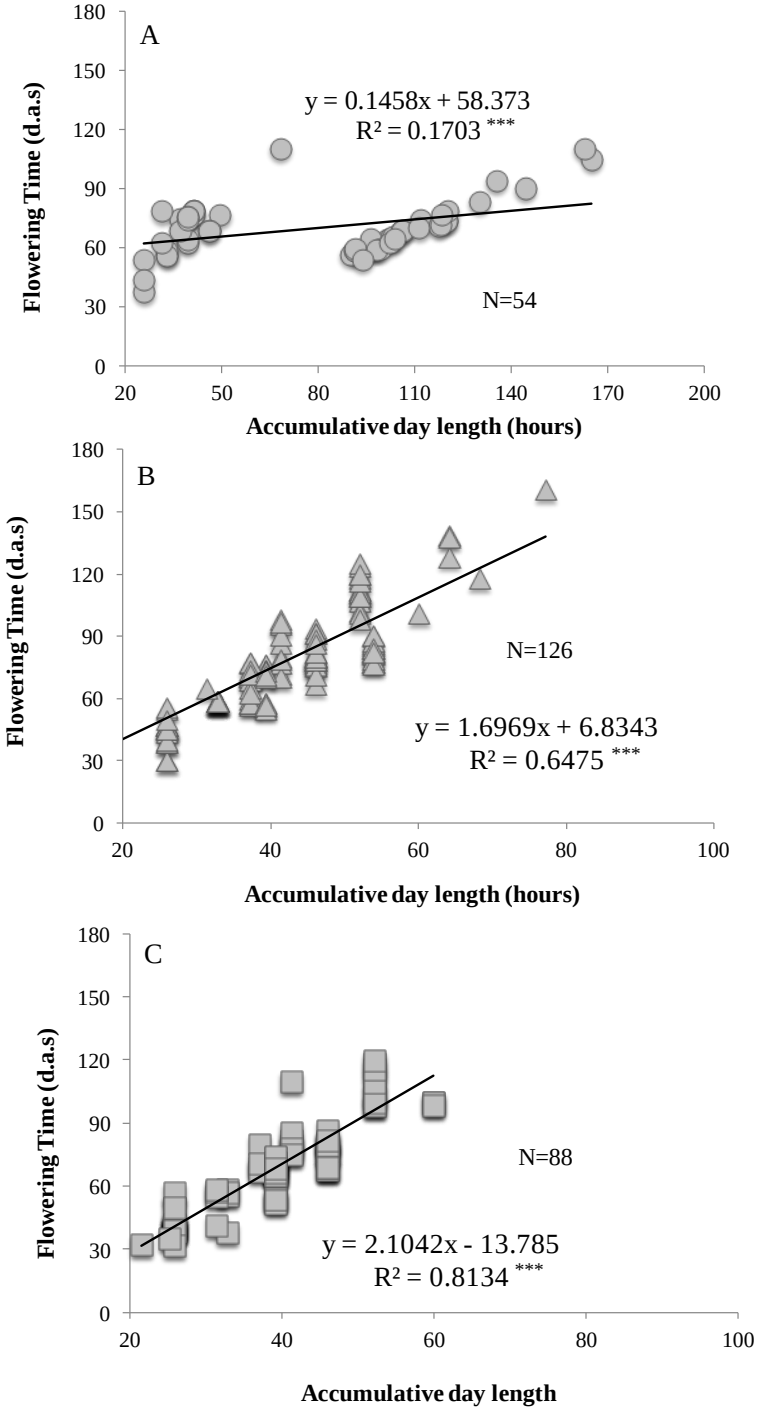


Figure 3.5. Linear relationship between flowering time and accumulative day length (hours) from the grain-(A); vegetable-(B); weedy-amaranths (C).

*, ** and *** represent a significant level at $P < 0.05$, very significant at $P < 0.01$ and very highly significant at $P < 0.0001$, respectively.

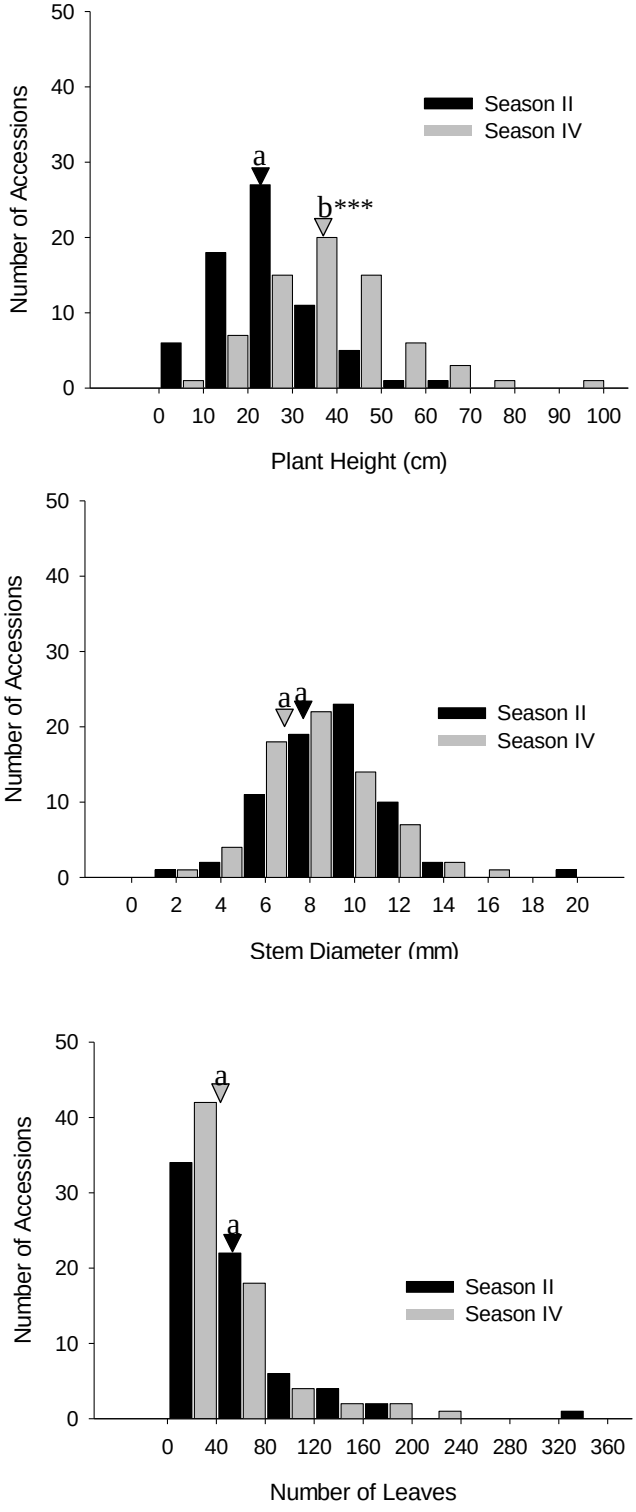


Figure 3.6. Growth characteristics of *Amaranthus* cultivated under two environmental condition.

CHAPTER 4

Variation of protein and amino acids in the leaves of grain, vegetable and weedy types of *Amaranthus*

4.1. Introduction

Global food production is still a main challenge for the future of mankind. Feeding the population will require a considerable increase in food production, particularly in Asian countries (Hoisington *et al.* 1999). The rapidly growing population demands not only an increase in quantity but also in terms of highly nutritious food with good protein quality (Drzewiecki *et al.* 2003). New strategies such as the exploration and utilization of underutilized crops as well as their genetic assessment are mentioned as some of the promising fields to meet world food needs (FAO 2011).

The family of *chenopodium* including amaranths is known as a good source of protein rich green leafy vegetables (Gupta and Wagle 1988). Amaranths, of which 17 species classified as edible, are counted as the most important leafy vegetables with excellent nutrition for the lowland tropics of Africa and Asia (Grubben 2004, National Academy of Sciences 2006). They are also acknowledged as rich and inexpensive source of dietary fiber, mineral, vitamins, protein and antioxidant (Gupta *et al.* 2005, Hill and Rawate 1982, Steffensen *et al.* 2011). Protein contents in the grain and in the leaves are found to be 15-18% DM and 12-38% DM, respectively (Barba de la Rosa *et al.* 1992, Grubben 1994). Furthermore, the composition of well balanced essential amino acid in food sources especially the limiting amino acids such as lysine, methionine and trypthopan are counted as an important aspect by the assessment of protein quality (Millward *et al.* 2008). Amaranths contain high lysine concentration which is found to be very limited in cereal plants. The lysine content in the seeds of amaranths exceeds 2 times that of wheat and 3 times that of maize (Zheleznov *et al.* 1997). This fact supports the high nutritional quality of amaranths (Caselato Sousa and Amaya-Farfán 2012).

The classification of species in *Amaranthus* was already explained in Chapter 1 and 2. Some grain species such as *A. cruentus* and *A. caudatus* are known as the “dual purpose” types. Their leaves are appreciated as leafy vegetables in some of African and Asian countries (Coons 1982).

In Indonesia, vegetable amaranth has been part of daily diets since olden times with *A. tricolor* as the main vegetable species (Grubben 1976, Ugas *et al.* 2008). The limitation of *A. tricolor* has been highlighted in the previous Chapter 2 (Paragraph 3). So far, no breeding efforts have been attempted. Alternatively, there is a possibility to breed new varieties of amaranths with high protein content as a wealth of genetic resources of *Amaranthus* in Indonesia is exist (Andini *et al.* 2013). Andini *et al.* (2013) reported the high potential of some uncultivated members of amaranths (e.g. *A. dubius* and *A. blitum*) that can be selected as parental lines. Some reports have indicated the success in different crops by the search for and utilization of high protein materials for nutritional enhancement by the utilization of wild types or weedy relatives which are usually rich in several nutrients (Guil *et al.* 1997, Guzmán-Maldonado *et al.* 2000). However, the scant information about the nutritional value of weedy plants has made them remain untapped.

The changing of the protein content during the growth period or plant age is another quality issue for further exploitation in amaranths' nutrition (Prakash and Pal 1991). Most of farmers in Africa and also in Indonesia tend to harvest them at the young age (about 21-28 days after sowing) due to their best morphological performance at this stage; such as soft stem, fresh and tender leaves (Grubben 1976, personal observation 2009). In contrast, harvesting amaranths in a later time (35-42 days after sowing) is more advantageous from nutritional quality point of view (Grubben 1976). So far, there has been no report dealing with the determination of the "right timing" harvesting amaranths in regard to its protein quality. Therefore, in this study, we would like to: 1) investigate the right timing of harvesting amaranths in relation to the leaf protein content; 2) assess the protein and amino acid variation of the leaves in the grain, vegetable and weedy type amaranths for further utilization of amaranths' germplasm.

4.2. Materials & Methods

Two kinds of experiments were conducted. First, the protein and amino acid determination in the three types of amaranths. This experiment was conducted in summer 2010. Second, study of the effect of growth stage on protein content occurred in spring 2011. The screening of 12 materials (10 vegetable- and 2 weedy accessions) was based from the previous result. However, in the presentation

of "Materials & Methods" and "Results", the two are presented reversely.

4.2.1. Plant materials

Effect of growth stage on protein content: twelve accessions with protein content ranging from low to high were selected (Table 4.1). Seeds were germinated on 26 May 2011. Further preparation of growing media is similar to that one described in Point **2.2.1.1.**

Protein and amino acids variation in three types of amaranths: the leaves of the grain, vegetable and weedy types were assessed (Figure 4.1). In total, 76 accessions comprised of 27 worldwide- and 49 Indonesian accessions were studied (Table A3 in Appendix). Further information of materials and experimental sites were pointed out in Point **2.2.1.** and Point **2.2.1.1.**, respectively.

4.2.2. Experimental design

Effect of growth stage on protein content: In total, 72 pots were arranged in a split-split plot design with three main blocks. Each block represented the growth stage (Table 4.2). Twelve accessions prepared in duplicate (total= 24 pots) were planted in each block and arranged according to Table 4.1. Fresh leaves (10-25 g) were harvested from each stage. Further step is described in Point 4.2.3. Methods.

Protein and amino acids variation in three types of amaranths: growing media preparation is already described in Point **2.2.1.1. Experimental site**. After two months from its sowing date, 10-25 g fresh leaves were cut from each accession and packed in a container containing ice until the samples were transported to the laboratory.

4.2.3. Methods

Leaves' preparation: Leaves were ground in liquid nitrogen and then freeze dried (EYELA, Tokyo Rikakikai Co Ltd, FD-1). The freeze dried samples were filtered with metal sieves (100- 125 mesh size) and the fine powder was employed for protein analysis and amino acids' determination.

4.2.3.1. Protein analysis

This section was described in Point **2.2.2.2**. **Protein analysis** (Page 14). The protein content was calculated as g 100 g^{-1} DW.

4.2.3.2. Amino acids determination

Sample preparation: One milligram powdered samples with 1 ml 6N HCl were put in

cylinder glass tube and oxidized under (cold-) vacuum condition. The vacuum sealed glass tube was hydrolyzed for 24 hours at 110^{o}C (PIERCE Reacti Therm 18870). The vacuum-dried hydrolysate was then added with 5 ml distilled water and then centrifuged at 3,000 rpm (LC06 SP Tommy-Seiko Co. Ltd, Tokyo, Japan) for 10 minutes. The supernatant was withdrawn with a pipette, filtered through a 0.45-µm filter and placed into an Erlenmeyer flask ($V_{max.}$= 20 ml). Five milliliters distilled water was added in the flask. Rotary Vapory Evapometer (RE 300 LC06 SP Tomy-Seiko Co. Ltd, Tokyo, Japan) was applied to remove HCl in the solution. After dried up the solution, 5 ml sodium acetate buffer was added to dissolve the solution. The solution was then filtered through a 0.22-µm filter. About 300 µl of the filtrate was ready to be delivered to the analytical centre of University of Tsukuba.

Amino acid determination: sample (50 nmol/ ml) was applied on an automatic amino acid analyzer JEOL (JLC 500/V). The amino acids were calculated as g in 100 g^{-1} DW protein.

4.2.3.3. Biomass (BMS)

At flowering time, the plant's whole part except the roots and five leaves was cut and put in an A4- paper bag (further will be referred as "bag"). The sample (bag containing one plant) was freshly weighted on a scale (CPA 4202S, Sartorius Weighing Technology GmbH, Göttingen, Germany) and noted as total fresh weight (FW_{sample}). Prior to that, each empty bag was freshly weighed and noted as FW_{bag}. Four paper bags were prepared for each accessions and dried at 65 ^{o}C in an oven (MOV- 2 1 2(U), Sanyo, Made in U.K.). The weight was weekly checked and final dry weight of samples (DW_{sample}) was recorded after their weight was constant (approximately after 2 weeks). Along with the samples, three paper bags with similar sizes were dried in the same oven. After drying, the dried weight of paper was averaged and noted as DW_{bag}. All calculation was determined in grams (g). Biomass (BMS) was calculated following this formula:

$$BMS\ (\%) = [(DW_{sample} - DW_{bag}) / (FW_{sample} - FW_{bag})] * 100$$

BMS-values presented were means of measurements on four plants for each accession and determined in % DM.

4.2.3.4. Leaves' dry matter (DM)

At flowering time, five leaves were cut from each plant per pot and put in a small paper bag (dimension: 18 x 7 cm). The drying procedure applied as well and how to calculate the end DM of the

leaves follow the method and formula applied follow the section in **Point 4.2.3.3**. All calculation was determined in grams (g). Leaves' Dry Matter (DM) values presented were means of duplicates per accession number and determined in % DM.

4.2.4. Statistical analysis

The effect of growth stage on variation of protein content was plotted with Sigma Plot version 10. Analysis of variance (ANOVA) was applied to each of the accessions and differences among traits ($P < 0.05$) were determined by t-test means comparison by using JMP version 7.0 (SAS Institute, Cary, NC, USA). Two-way ANOVA for analyzing the amount of variance that was contributed to a sample by different factors, was calculated based on Sigma Plot version 10 and SAS 9.0 (SAS Institute, Cary, NC, USA), respectively.

4.3. Results

4.3.1. Effect of growth stage on protein content

Growth stage had a highly significant effect on the variation of protein, followed by accession applied in this study based on the analysis of variance (Table 4.3). No significant interaction was observed between accession and growth stage. There was a wide variability of protein content by the selected materials of this study (Table 4.1). The low protein group (vegetable accessions of A-F) showed an increased level of protein at young stage and their values were almost similar to those classified as high content group (accession G-L) (Figure 4.2). Mean overall value of 30 g in 100 g^{-1} DW in protein content was recorded in the young stage. The vegetable amaranths showed the highest level of protein content at their optimum stage; except the weedy types I (IDN 18/ Medan) and K (IDN 01/ Mongal) (Figure 4.2). Mean overall value of protein content up to 32 g 100 g^{-1} DW was noted at the optimum stage. At the late stage, the protein content decreased up to 18 g in 100 g^{-1} DW in all accessions as the quality of protein was mostly deteriorated in the late stage. Up to 44% decreased value was noted than that one at the optimum. These were shown by the vegetable *A. tricolor* such as observed in G (IDN 46/ Kresek), B (USDA 27/ PI 566899/ IND) and C (IDN 43/ Marelan). From this study, we affirmed the changes of protein during the growth stage of amaranths as previously stated by Prakash and Pal (1991). The right timing of harvesting amaranths in regards to

their protein content should be conducted at their optimum stage, between 30 to 40 days after sowing.

4.3.2. Protein and amino acids variation in three types of *Amaranths*

The data set containing the results of biomass, leaves' dry matter, the values of 15 amino acids, total amino acids and non amino acids from 76 accessions was presented in Table A3 in Appendix. Table 4.4 presents the range of the variation of nutritional values based on the three types of amaranths. Out of 20, 12 of the nutritional traits assessed showed significant to very high significant differences among the three types.

In general, weedy amaranths showed the valuable criteria in most of the nutritional traits if compared with the other two types. The highest leaves' dry matter (~ 38%) and protein content (~ 30% DM) were observed in *A. dubius* (IDN 15/ Mandua) and *A. viridis* (USDA 30/ PI 540445/ IDN/ Java), respectively. These accessions are also noted with good characteristics in protein and total amino acids by their leaves such as: *A. dubius* (IDN 01/ Mongal, IDN 02/ Daling) and *A.blitum* (USDA 01/ Ames 5315/ IND, IDN 18/ Medan and IDN 19/Mandua) (Table 4.4. and Table A3). In contrast, the real weed species such as *A. spinosus* (IDN 07/ Ulu Nuih and IDN 56/ P.Kb) were found to be very low in leaves' dry matter (11-17%) but an adequate in the content of protein, total amino and lysine.

The second highest nutritional values were observed in the vegetable types. A high leaves' dry matter (~ 29% found in USDA 26/ PI 477918/ N.A) was the most distinct character in vegetables if compared with the other two types. This result is in accordance with Mapes *et al.* (1996) who stated that vegetable types allocate a higher proportion of biomass if compared with other types in amaranths. Moreover, high variability in biomass and high total amino acids were also observed in most of vegetable amaranths. In contrast, the leaves of many grain species such as *A. hypochondriacus* (USDA 22/ PI 604796/ N.A) were found to be less inferior in dry matter, biomass values and leaf protein if compared with the other two types. On the other hand, other grain species called as the dual purpose types such as *A. cruentus*, *A. caudatus* had a slightly higher leaf protein if compared with common vegetable amaranth *A. tricolor* (12 g 100 g^{-1} DW) (Shukla *et al.* 2010). Their values were in the range from 12 up to 18 g 100 g^{-1} DW of protein.

Lysine, the major target of this study was found to be in the range of 5 to 7 g 100 g^{-1} DW

protein and was not significantly different among the three types (Table 4.4 and A3). A value of 6.0 g 100 g^{-1} DW protein was observed in most of vegetable and weedy types such as *A. tricolor*, *A. dubius* and also in the leaves of grain species such as *A. cruentus* (USDA 07/ PI 482051/ ZWE, USDA 13/ PI 604666/ USDA), *A. hypochondriacus* (USDA 21/ PI 604577/ MEX, USDA 22/ PI 604796/N.A) and *A. hybridus* (IDN 55/ P.kb, IDN 57R/ P.kb). Cysteine and methionine were detected in very small amount as both might have been (partially) denaturized during the acid hydrolysis process. The total non protein amino acids (abbreviated as total NPAA) were found in relatively small amounts in the three types (~2%) and they also showed no significant differences among the three types.

4.4. Discussion

A strong link between biodiversity and nutrition security plays a fundamental role for many researchers to fulfill the gap between world's rapidly rising population and food security. Assessment of underutilized crop in their nutritional values may provide good opportunity to feed the world population with high quality nutrition and good composition of macro-and micronutrients (Termote *et al.* 2012).

The nutritional values (e.g. amino acids, protein and biomass) of the three types (grain, vegetables and weedy) are integrated in one extensive study. Usually, the information of the three types is being presented in separate reports. These results complement the previous assessment by the nutritional composition in amaranths' seeds (Becker *et al.* 1981, Gorinstein and Moshe 1991, Gorinstein *et al.* 2001). Such extensive information is useful for breeder to provide good determinants of high quality vegetables. Moreover, the extent by the nutritional variation shown in the three types of amaranths also enable breeder to determine the limits of selection for improving *A. tricolor* as the major vegetable resource (Shukla *et al.* 2005).

The nutritional characteristics in amaranths vary considerably depend on types and species. Moreover, protein content was affected greatly both by environmental factors and the genotype of amaranths. Similar observation was also reported by Schnetzler and Breene (1994). Protein and the composition of amino acids as well as plant morphology of amaranths were greatly affected due to age of plant, growth temperature and harvesting time.

The weedy species *A. dubius, A. blitum, A. viridis* may serve as an alternative high quality protein food source for many developing nations (Shukla *et al.* 2005). The quality of protein including the total essential amino acids in the leaves was found to be high. Protein content was up to 19 g 100 g^{-1} DW of protein on average in the leaves of weedy. Meanwhile, total amino acids content were found to be 2.2 higher in the leaves than in the seeds of amaranths (Gorinstein *et al.* 2001).

The Recommended Dietary Allowances (RDA) is the average daily dietary intake level sufficient to meet the nutrient requirements of healthy individuals. Thus, the RDA values of protein is 0.66 g/ kg per day (WHO/FAO/UNU 2007). Thus, an average consumption of 100 g per day of (fresh) vegetable amaranths already satisfies the RDA requirement (Grubben 1976). The superiority of protein quality by the leaves of vegetable amaranths compared with the leaves of grain species was acknowledged (Fomsgaard *et al.* 2009). Our results are in similar view with Fomsgaard *et al.* (2009). This condition implies that "no intense" human selection by the leaves of grain amaranths. Interestingly, the leaves of some of dual purpose amaranths (*A. cruentus* and *A. caudatus*) showed a slightly higher content of protein than the leaves of *A. tricolor* which is categorized as the uprooting type. In Indonesia, two types of vegetable amaranths based on the way they are harvested are known: 1) the uprooting type; 2) the picking type; with the common type is the uprooting one (Hadisoeganda 1996). These dual types may be incorporated in improving the performance of "picking types" vegetable amaranths in Indonesia due to their morphological characteristics such as taller plant, bigger size of leaves but high content of protein. Another utility for the dual type is their potential as fodder crop (Pedersen *et al.* 1987).

According to the protein reference pattern defined by FAO/ WHO, amaranth's content of protein is close to the optimum human requirements (Coultate 2002). Moreover, the values of lysine which is close to an ideal protein score have made amaranths to be highly recommended by many food nutritionists (National Academy of Sciences 1996). Egg and human milk proteins are considered as reference standard for high biological value. A value of 7 g 100 g^{-1} DW protein of lysine has been adopted by FAO/ WHO as a reference (Coultate 2002). Moreover, the daily requirement of lysine for infants and adults, including children in the school age is 7 and 5 g 100 g^{-1} DW protein, respectively. A lysine value of 6.0-6.5 g 100 g^{-1} DW protein was exhibited in this study which is in agreement with

Grubben (1976). The protein scores in valine and histidine which are close to the reference values (Seligson and Mackey 1984) were also noted in our results. These confirm the highly appreciable content by the leaves of amaranths.

There are several weak points that are acknowledged in amaranths. First, the limiting amino acids such as leucine, valine and threonine were reported in the seeds of amaranths (Pedersen *et al.*1987). However, we found relatively high values of leucine, valine and threonine by the leaves of three types of amaranths. In contrast, lack of cysteine and particularly methionine were found in our results. These relatively low values in cysteine and methionine in our study might be explained from two possibilities: they might have been denaturized during analysis or their values are very limited (2 g 100 g^{-1} DW protein) which is in accordance with previous studies (Grubben 1976, Hill and Rawate 1982, Bressani and García-Vela 1990). To compensate this lack points by the cysteine and methionine, a highly diverse food intake in the dishes via additional consumption of plant-/ non plant-origin proteins such as milk and animal proteins might overcome this situation (National Academy of Sciences 2006, Schaafsma 2000). Beans, soybean and soybean derivate products can also augment the cysteine and methionine requirement (Sarwar 1997, Gorinstein *et al.* 2001). Second, the relatively high oxalate content as an anti nutritive element should be taken as precautious by the consumption of amaranths. Moreover, content of oxalate is counted as one of the major challenges by the exploitation of weedy types (Noonan and Savage 1999). A range of 300-500 g consumption of fresh leaves was determined as the limit of the toxicity level (Brenner *et al.* 2000, Grubben 1976). Good cooking preparation might promote the safe consumption of amaranths via discarding the cooking water (Grubben 1976).

Malnutrition, such as anemia, vitamin A and protein deficiencies have affected one hundred million, nine million and twelve million population in Indonesia, respectively. Such huge amount of malnourished people is mainly occurring in children of school age and mostly in rural and isolated parts of Indonesia (Wargiono *et al.* 2002). Despite its inferiority as a so called “underutilized crop”, the high nutritional quality of vegetable amaranths has been recognized (National Academy of Sciences 2006). Its high content of vitamin A and other micro nutrients (iron, calcium and magnesium) (Gupta *et al.* 2005) have increased the attractiveness of amaranths as potential plants to

combat the malnutrition problem in densely populated and huge areas to cover such as in Indonesia, at low cost, via the promotion of green leafy vegetables consumption such as amaranths (Brenner *et al.* 2000).

4.5. Conclusions

It is concluded from the present study that the three types of amaranths (grain, vegetable and weedy types) show a wide array of highly nutritional characteristics; particularly the edible weedy species such as *A. dubius*, *A. blitum* and *A. viridis*. Their content of protein may reach up to 29 g 100 g^{-1} DW. Moreover, the values of lysine which is close to an ideal protein score. A consumption of at least 100 grams of vegetable amaranths is sufficed to fulfill the Recommended Dietary Allowance. The dual type species such as *A. caudatus* and *A. cruentus* contain an acceptable high content of protein (about 18 g 100 g^{-1} DW) and they can be prospected as alternative protein rich vegetable or as fodder. The quality of protein was significantly affected by the growth stage in amaranths. Therefore, good timing of harvesting amaranth (31-39 d.a.s) should be considered as quality control for consumer.

Table 4.1. The selected amaranth species with various content of protein.

Symbol	Accession Name	Origin	Protein content (g 100 g^{-1} DW)
A	USDA 24/ Ames 5134/ USA	USA	11.59
B	USDA 27/ PI 566899 / IND	India	12.03
C	IDN 43/ Marelan	Indonesia	16.53
D	USDA 03/ PI 490298/ KEN	Kenya	17.44
E	USDA 10/ PI 500267/ ZMB	Zambia	18.78
F	USDA 17/ PI 608661/ IND	India	19.47
G	IDN 46/ Kresek	Indonesia	21.28
H	USDA 05/ PI 606282/ BGD	Bangladesh	24.59
I	IDN 18/ Medan	Indonesia	25.59
J	USDA 04/ PI 606281/ BGD	Bangladesh	28.03
K	IDN 01/ Mongal	Indonesia	28.34
L	USDA 30/ PI 540445/ IDN/ Java	Indonesia	29.06

Table 4.2. The definition of stages and the condition of the harvested amaranths in each stage.

Growth Stage	Description
Young	From the two leaves' emergence until 30 d.a.s. In this juvenile stage, the growth's speed was varied and not uniform among the accessions. Some had only two leaves but others had already 3 – 7 leaves per plant. The average height was 4 – 15 cm and an average stem diameter of 0.2 – 2 mm. Leaves were small and very tender. After 20 - 25 d.a.s, amaranths showed a very rapid growth. The shifting of from young to optimum stages occurred in a very rapid time.
Optimum	A quite short period of about 31 - 39 d.a.s. Plant produced many leaves (10- 70 leaves) actively during this period at this stage. The average plant height was about 30 cm. Some remarkable changes by their phenotypes were observed such as: 1) a very intense and bright leaf color was performed; 2) bigger sizes of the leaves if compared with the young stage; 3) the leaves showed an optimum tenderness between crispy and soft feeling. Nevertheless, some parts of the plants started to thicken such as in stem diameter. Stem diameter was in the range of 5 – 14 mm.
Late	About 40 - 60 d.a.s. The average plant height was 50 – 75 cm. Some amaranths were still producing leaves actively with leaf number varied from 14 to 96 per plant. Despite of this, the decrease by the leaf quality was observed such as: 1) leaf color was fading and it was not so bright green or shiny red anymore until finally, some leaves had started to be yellow; 2) many leaves started to wilt and fall from the branches; 3) leaf and stem diameter thickening had been thickened. Stem diameter was in the range of 6 – 16 mm.

d.a.s= days after sowing

Table 4.3. Two way ANOVA of the effect of growth stage on protein content in amaranth.

Source of Variation	DF	SS	MS	F	P. > F
Accession (A)	11	225.27	20.48	3.06	*
Growth Stage (GS)	2	2,699.27	1,349.63	201.58	***
A x GS	22	252.44	11.48	1.71	n.s.
Residual	36	241.04	6.69		
Total	71	3,418.02	48.14		

* and *** represent significance at $P < 0.05$ and $P < 0.001$, respectively

Table 4.4. Variation of biomass (BMS, %), leaves' dry matter (in %), protein (g 100 g^{-1} DW) and amino acids of amaranths (g 100 g^{-1} DW protein) of the three types of amaranths.

		GRAIN (N=12)				VEGETABLE (N= 37)				WEEDY (N=27)					
Nr.	**Traits**	Min.	Mean	Max.	S.D.	Min.	Mean	Max.	S.D.	Min.	Mean	Max.	S.D.	**F-value**	**P. > F**
1	BMS	13.20	15.55	17.81	1.68	7.57	14.83	27.57	4.64	5.85	17.94	25.42	4.33	4.13	*
2	Leaf DM	12.64	19.69	24.37	3.10	10.97	19.16	29.22	5.31	11.55	23.23	37.70	6.34	4.37	**
3	Protein	10.78	15.71	20.31	2.82	11.59	16.22	28.03	3.56	11.84	19.40	29.06	4.71	6.29	**
4	Leu 3)	6.71	7.46	8.19	0.53	6.82	7.81	8.48	0.37	5.44	7.87	8.40	0.55	3.34	*
5	Ala	5.59	5.97	6.55	0.34	5.21	6.07	1.72	0.28	5.37	6.59	7.69	0.60	14.42	***
6	Lys	5.50	5.92	6.42	0.30	5.18	6.05	6.63	0.36	5.63	6.13	6.54	0.25	1.77	n.s.
7	Gly	5.59	6.15	6.96	0.38	5.42	5.88	6.34	0.22	5.26	5.85	6.70	0.36	4.51	**
8	Ser	4.55	5.40	5.69	0.30	2.63	5.33	6.08	0.52	5.02	5.54	6.11	0.32	1.89	n.s.
9	Arg	4.27	4.73	5.27	0.35	4.50	5.15	6.01	0.27	4.66	5.21	5.80	0.26	13.35	***
10	Thr	4.41	4.86	5.28	0.28	4.67	5.00	5.22	0.13	4.68	5.03	5.39	0.19	3.88	*
11	Phe	4.12	4.49	4.92	0.28	4.04	4.63	5.12	0.25	4.38	4.83	5.36	0.20	9.93	***
12	Pro	4.08	4.53	5.09	0.28	3.89	4.72	5.67	0.36	3.99	4.68	5.53	0.35	1.31	n.s.
13	Val	3.67	4.43	5.18	0.44	4.03	4.76	5.25	0.32	3.25	4.45	5.32	0.65	4.01	**
14	Ile	3.18	3.78	4.33	0.37	3.48	4.04	4.76	0.32	2.67	3.86	6.25	0.75	1.60	n.s.
15	Tyr	2.74	3.65	4.19	0.40	2.87	3.62	4.15	0.32	3.30	3.73	4.46	0.24	1.10	n.s.
16	His	1.87	1.99	2.10	0.07	1.41	1.78	2.10	0.17	1.68	1.84	2.22	0.11	9.65	***
17	Cys	0.45	0.53	0.62	0.05	0.00	0.42	0.83	0.22	0.00	0.50	1.06	0.22	1.92	n.s.
18	Met	0.00	0.27	0.75	0.25	0.00	0.32	1.22	0.32	0.00	0.52	2.39	0.48	2.90	n.s.
19	Total AA	80.35	85.37	91.24	3.48	79.77	88.60	91.39	2.23	84.40	90.42	92.96	1.68	20.15	***
20	Total NPAA	1.05	2.04	3.10	0.63	0.93	2.03	3.32	0.65	0.89	1.78	3.63	0.76	1.19	n.s.

Unit: Nr.1 and 2 (%), Nr.3(g 100 g^{-1} DW), Nr.4-20 (g 100 g^{-1} DW Protein)

Min., minimum; Max. maximum; S.D., Standard Deviation; N, number of samples; AA, Amino Acids; NPAA, Non Protein Amino Acids.

n.s., not significant; * and **, *** represent significance at P <0.05 and P < 0.01, P < 0.001, respectively

Figure 4.1. The leaves of three representative types of amaranths. A= grain (*A. cruentus* L.); B= vegetable (*A. tricolor* L.); C= edible weedy (*A. dubius*).

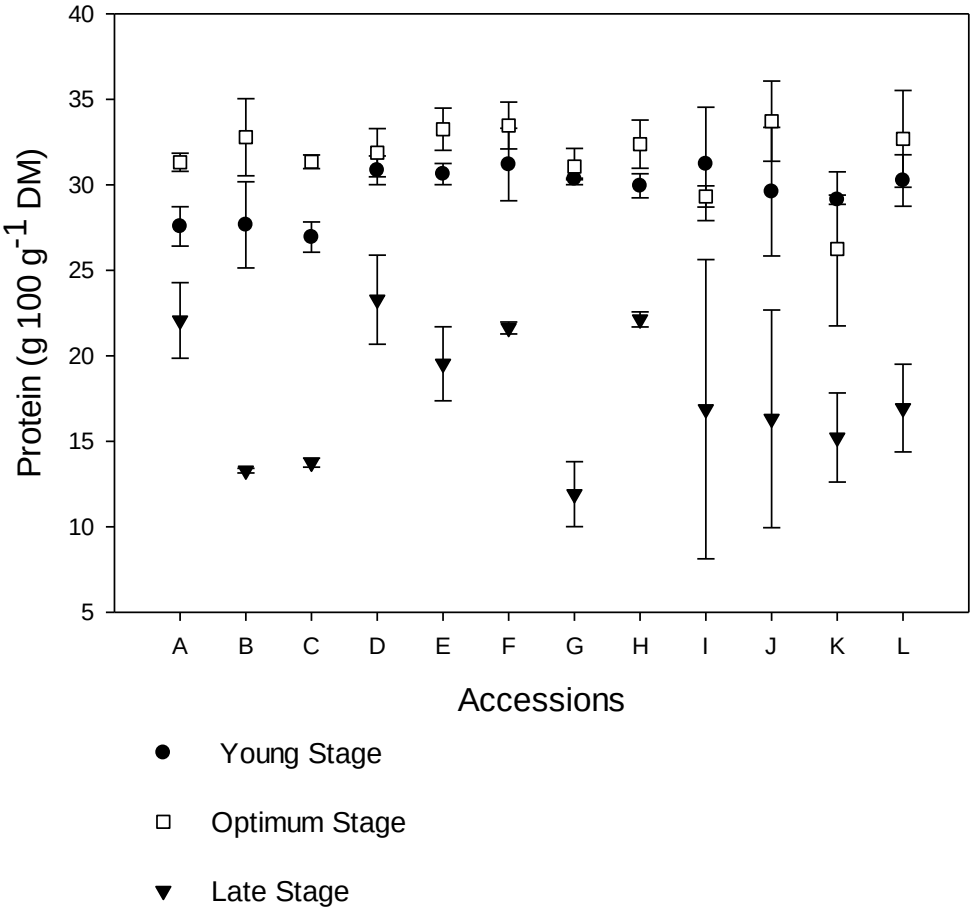


Figure 4.2. Effect of growth stage on protein content. In the y-axis: Protein content.

CHAPTER 5

Polyploidy assessment in *Amaranthus* spp.

5.1. Introduction

Polyploidy means the heritable condition of having more than two complete sets of chromosome. Thus, the organism is called as polyploids (Comai 2005). Polyploidy is counted as an important evolutionary and speciation process in many plants and some animals (Liu and Wendel 2003). Many advantages to be polyploids in the nature since many of the agriculturally important species that provide our needs for food, fodder and fibre; such as maize, wheat, barley, *Beta vulgaris* and *Brassica* are closely associated with polyploidy (Becker 1993, Comai 2005, Kaushal *et al.* 2009). Therefore, polyploidy is an area of interest to many plant biologists due to their inextricable link with many important crops. There are two types of polyploids. First, the naturally exist due to changes in the environment (Liu and Wendel 2003). The second is the artificial ones which can be generated *via* seed treatment with colchicine (Becker 1993).

Amaranthus is a genus with up to 75 species; of which 50 are native to the Americas and another 15 can be found in Europe, Asia, Africa and Australia (Kigel 1994). Amaranths show a wide range of morphological diversity of which for some researcher still remain as a puzzle (Das 2011). Based on our morphological studies in Indonesian amaranths in Chapter 1, we found a high variability of morphological variation and high nutritional qualities in the Indonesian weedy of *A.dubius* (further referred as *A.dubius*) may exist as diploid or as tetraploid in the nature (Grubben 2004). Ploidy variations cause usually alterations in morphology (Kaushal *et al.* 2009). Furthermore, most of polyploids in *Amaranthus* exhibit distinct morphological characteristics than the diploid ones, such as shorter and thicker stem, higher seed production and significant changes in the nutritional values (Brenner *et al.* 2000). Therefore, we hypothesized that the differences observed in the morphology of

Indonesian amaranths, particularly those from *A. dubius* were resulted due to polyploidy level. Therefore, the objective of this study is to: investigate the polyploidy level in *Amaranthus*.

5.2. Materials & Methods

5.2.1. Plant materials

Fresh young leaves from 3-4 weeks old plants were harvested in May 2012. The polyploidy level of 87 accessions consists of grain, vegetable, ornamental and weedy amaranths were assessed.

The preparation followed the method suggested by the filter producer company (PARTEC GmbH, Münster, Germany). About 0.5 cm^2 leaf tissues was put in a glass petri dish and was added with 400 µl extraction buffers (CyStain UV precies P -05-5002). Extraction and stain buffer (PARTEC GmbH, Münster, Germany) were available in handle as one set ready solution. Plant materials were chopped using a sharp razor blade for 30 -60 seconds and then, incubated for about 30-60 seconds. After the incubation, the suspension was filtered through a Partec 50 µm cell trics disposable filter (Nr. Serie 04-0042-2317) and placed into a glass container. Afterwards, 150 µl staining solution was added into the container and incubate for 30-60 seconds. The suspension was ready to be analyzed with flow cytometer. Fresh suspension was applied for each analysis.

5.2.2. Polyploidy assessment

Rapid screening for identification of the polyploidy within accessions was done with Attune Acoustic focusing flow cytometer (Applied Biosystem, California, USA). The company provides detail information regarding the principle theory of this machine on the company's website: http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/flow-cytometry.html. Three sources of laser was applied, FSC, VL1-H and VL1-A. Analysis was conducted automatically in few seconds. Number of peak produced on the graph represents the polyploidy level of each sample.

5.3. Results

This is the first result which reported the polyploidy level in *Amaranthus* based on flow cytometer. Previously, conventional method was applied such as chromosome study to detect the number of chromosome in amaranths (Pal 1972, Greizerstein and Poggio 1995). Table 5.1 presents the polyploidy results based on flow cytometer. The number of chromosome presented (Table 5.1) was developed based on several references from previous reports (Grubben 1976, Greizerstein and Poggio 1995). *Amaranthus* showed a variation of chromosome number but no polyploids were detected in our materials albeit the remarkable morphological variation observed in the three types of *Amaranthus* (Fig. 5.1). Out of our expectation, all ornamental types of *Celosia* spp. (USDA 31, USDA 32, Nepalese and Indonesian accessions) as well as *A. dubius* were diploid. The grain species of *A. cruentus* and *A. hypochondriacus* showed *2n*= 34. The majority of *Amaranthus* had a chromosome number of *2n*= 32. This was detected in the dual purpose *A. caudatus*, *A. hybridus*, in most of the vegetable species (*A. tricolor*, *A. blitum*, *A. graecizans* and *A. viridis*) and in the real weed species such as *A. retroflexus* and *A. spinosus*. In contrast, only *A. dubius* was found to be *2n*= 32. A variation of *2n*= 18 or 36 by the chromosome number of the ornamental *Celosia* spp. was noted.

5.4. Discussion

A great number of morphological diversity due to high genetic diversity occurs in amaranths (Das 2011). Such great deal of diversity in amaranths was proposed to be affected by the genotypic and environmental condition (Shukla *et al.* 2006). The high genetic diversity in amaranths might be resulted from three possibilities. First, noted the variability of chromosome number might lead to higher genetic diversity in amaranths (Grubben 1976). The variation of chromosome number *2n*= 32 or 34 in most of amaranths are in accordance with Greizerstein and Poggio (1995). *Amaranthus* are actually classified as allotetraploids as their basic number (*x*) are *x*= 8 for most of the grain and vegetable species, meanwhile, *x*=9 for *Celosia*. However, in the nature they exhibit in secondary basic numbers forms; *n*=16 instead of *x*= 8 or *n*=17 (8 + 9 = 17) (Grubben 1976, Greizerstein and Poggio

1995). For *Celosia*, chromosome number of *2n*= 18, *2n*= 36 or (*2n*= 72, 108) were also common (Denton 2004). Second, hybridization was accounted as the main contributor affecting the high variability in amaranths (Kulakow and Hauptli 1994). This ability to hybridize was supported by the outcrossing possibility among *Amaranthus* (Jain *et al.* 1982, Hauptli and Jain 1985). Outcrossing rates between 10- 20% were reported by Jain *et al.* (1982), Hauptli and Jain (1985).

The environmental variation that contributes to high phenotypic plasticity by the Nepalese grain amaranths was also noted (Brenner *et al.* 2000). The variation of phenotypes exists albeit similar genotype is referred as "phenotypic plasticity" (Brenner *et al.* 2000). A high level of morphological variation (e.g. branching index, stem and inflorescence color variation) was observed in the highland adapted amaranths growing on the elevation more than 1,000 m a.s.l. or in some grain amaranth such as in cultivar "Plainsman" (Brenner *et al.* 2000). Such high variation was proposed as a result of unique adaptation process or due to genotype x environment interaction (Espitia-Rangel 1994, Brenner *et al.* 2000). Similar tendencies were also reported in the genus *Brassica* which has been adapted in the mountainous regions. Those highland adapted *Brassica* usually show differences in their phenotypes such as larger stem diameter, greater leaf numbers and biomass (Murren and Pigliucci 2005). Geographical isolation during domestication process as another component of environment might contribute to the genetic variability in several important crop species (Dempewolf *et al.* 2012). Similar condition might be applied for those amaranths (*A. dubius*) obtained from the highland Takengon which is located in the highland areas of central Aceh, Sumatra. Such geographical barrier might contribute to the alteration of gene flow and finally, genetic differences between populations were formed.

The analysis of polyploidy and chromosome number in this work indicates the possibility of interspecific gene exchange that could be useful for plant breeding. No polyploidy found in *A. dubius* opens new possibilities to transfer desired traits via interspecific crossing. In this meaning, breeding of new varieties in amaranths would be possible. So far, chromosome differences have been pointed out as the main reproductive barriers for producing viable hybrids in amaranths (Greizerstein and Poggio 1995). Another challenge that might be encountered after reproductive barrier is hybrid

sterility and inviability such as retarded growth in F_1 plants (Pal 1972, Pal and Khoshoo 1972, Gudu and Gupta 1988). Conventional breeding such backcrossing (Pal 1972) and advance technique (recombinant DNA or cytoplasmic male sterility) (Sodhi *et al.*, 2006) were being suggested as effective attempts to overcome such constraints.

5.5. Conclusions

This study reported the polyploidy level in *Amaranthus* accession (N= 87) from three types of amaranths via flow cytometer. No tetraploid of *A. dubius* was found in this study albeit higher morphology observed in our amaranths' collection. The chromosome number was varied between *2n*=32 or *2n*=34 in most of grain, vegetable and weedy amaranths. Meanwhile, a variation of chromosome number *2n*= 18 or 36 was found by the ornamental *Celosia*. No polyploidy level may open new possibility to breed new variety of amaranths with desired characteristics. Further interspecific crossing should be conducted in the near future in order to investigate the stability of chromosome number in the progenies of amaranths.

Table 5.1. Polyploidy level and chromosome number (*2n*) in *Amaranthus* (N=87).

Nr.	Origin	Accession	Place of Origin	Type	Species	Polyploidy Level	Nr. of Chromosome (*2n*)
1	WW	USDA 01/ Ames 5315/ IND	IND- Maharastra	VG	*A. blitum*	Diploid	34
2	WW	USDA 02/ PI 610262/ IND	IND-Gujarat	VG	*A. blitum*	Diploid	34
3	WW	USDA 03/ PI 490298/ KEN	KENYA	VG	*A. blitum*	Diploid	34
4	WW	USDA 04/ PI 606281/ BGD	BANGLADESH	VG	*A. blitum*	Diploid	34
5	WW	USDA 05/ PI 606282/ BGD	BANGLADESH	VG	*A. blitum*	Diploid	34
6	WW	USDA 06/ PI 482049/ ZWE	ZIMBABWE	GR	*A. cruentus*	Diploid	34
7	WW	USDA 07/ PI 482051/ ZWE	ZIMBABWE	GR	*A. cruentus*	Diploid	34
8	WW	USDA 08/ PI 490662/ BEN	BENIN	GR	*A. cruentus*	Diploid	34
9	WW	USDA 09/ PI 494777/ ZMB	ZAMBIA	GR	*A. cruentus*	Diploid	34
10	WW	USDA 10/ PI 500267/ ZMB	ZAMBIA	GR	*A. cruentus*	Diploid	34
11	WW	USDA 11/ PI 538319/ USDA	USA	GR	*A. cruentus*	Diploid	34
12	WW	USDA 12/ PI 566897/ IND	INDIA	GR	*A. cruentus*	Diploid	34
13	WW	USDA 13/ PI 604666/ USDA	USA	GR	*A. cruentus*	Diploid	34
14	WW	USDA 15/ PI 605352/ JAM	JAMAICA	VG	*A. dubius*	Diploid	32
15	WW	USDA 16/ PI 642737/ PRI	PUERTO RICO	VG	*A. dubius*	Diploid	32
16	WW	USDA 17/ PI 608661/ IND	INDIA	VG	*A.graecizans*	Diploid	34
17	WW	USDA 19/ PI 500249/ ZMB	ZAMBIA	GR	*A. hybridus*	Diploid	32
18	WW	USDA 20/ PI 605351/ GRC	GREECE	GR	*A. hybridus*	Diploid	32
19	WW	USDA 21/ PI 604577/ MEX	MEX-Puebla	GR	*A. hypochondriacus*	Diploid	34
20	WW	USDA 22/ PI 604796/ N.A	UNKNOWN	GR	*A. hypochondriacus*	Diploid	34
21	WW	USDA 23/ PI 607447/ JAM	JAMAICA	WD	*A. retroflexus*	Diploid	34
22	WW	USDA 24/ Ames 5134/ USDA	USA	VG	*A. tricolor*	Diploid	34
23	WW	USDA 25/ PI 349553/ PNG	PAPUA NEW G	VG	*A. tricolor*	Diploid	34
24	WW	USDA 26/ PI 477918/ N.A	UNKNOWN	VG	*A. tricolor*	Diploid	34
25	WW	USDA 27/ PI 566899/ IND	INDIA	VG	*A. tricolor*	Diploid	34
26	WW	USDA 28/ PI 604669/ TWN	TAIWAN	VG	*A. tricolor*	Diploid	34
27	WW	USDA 29/ PI 608761/ IND	INDIA	VG	*A. tricolor*	Diploid	34
28	IDN	USDA 30/ PI 540445/ IDN/ Java	IDN-Java	WD	*A. viridis*	Diploid	34
29	WW	USDA 31/ PI 586680/ USA	USA	OR	*C. argentea*	Diploid	36
30	WW	USDA 32/ PI 482244/ ZWE	ZIMBABWE	OR	C.trigyna	Diploid	18
31	WW	NPL 01/ KTM	Kathmandu-Nepal	OR	*C. argentea*	Diploid	36
32	WW	NPL 02/ KTM	Kathmandu-Nepal	OR	*C. argentea*	n.a	n.a
33	WW	NPL 03/ KTM	Kathmandu-Nepal	OR	*C. argentea*	n.a	n.a
34	WW	NPL 04/ KTM	Kathmandu-Nepal	OR	*C. argentea*	n.a	n.a
35	WW	NPL 05/ KTM	Kathmandu-Nepal	OR	*C. argentea*	Diploid	36
36	IDN	IDN 01/ Mongal	Mongal-TKG	WD	*A. dubius*	Diploid	32

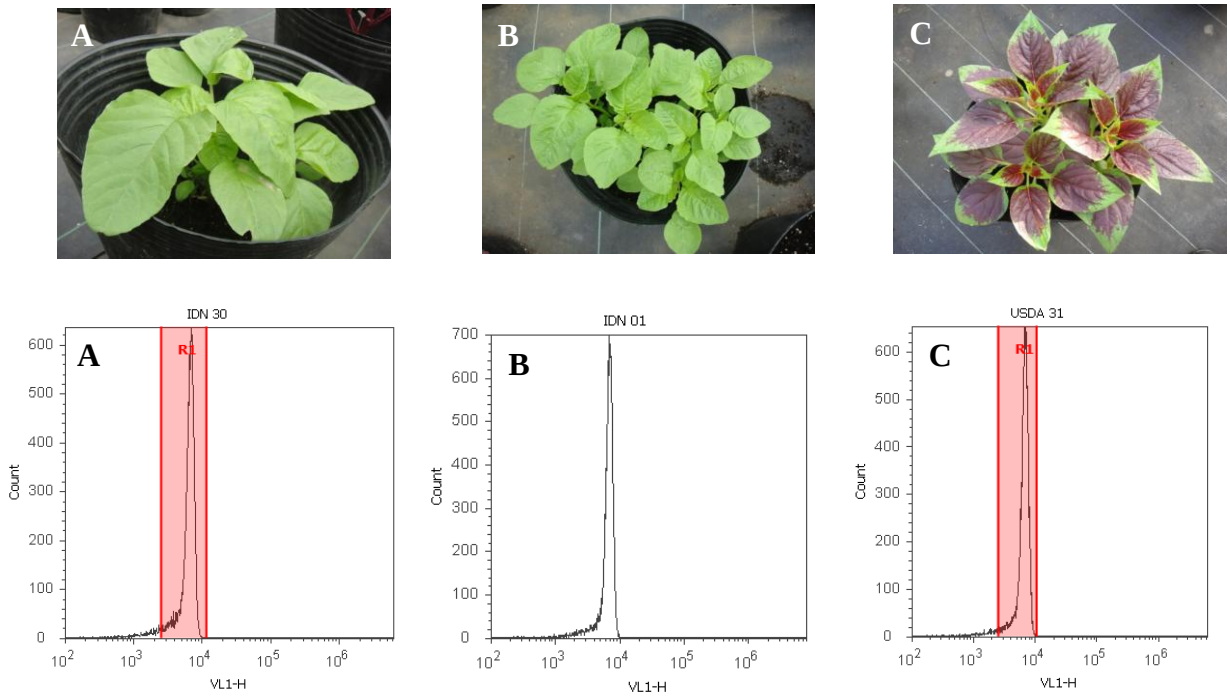
Table5.1 (continued)

37	IDN	IDN 02/ Daling	Kp.Glg-TKG	WD	*A. dubius*	Diploid	32
38	IDN	IDN 03/ Daling	Kp.Glg-TKG	WD	*A. dubius*	Diploid	32
39	IDN	IDN 04/ Bur biah	Kp.Glg-TKG	WD	*A. spinosus*	Diploid	34
40	IDN	IDN 07/ Ulu Nuih	Kp.Bbh-TKG	WD	*A. spinosus*	Diploid	34
41	IDN	IDN 08/ Asir-asir	Hilir-TKG	WD	*A. dubius*	Diploid	32
42	IDN	IDN 09/ Asir-asir	Kp.asir-TKG	WD	*A. dubius*	Diploid	32
43	IDN	IDN 10/ P. One-one	One-one-TKG	WD	*A. dubius*	Diploid	32
44	IDN	IDN 11/ Tn.Depet	Tn.Debet-TKG	WD	*A. dubius*	Diploid	32
45	IDN	IDN 13/ Mandua	Mandua-TKG	WD	*A. dubius*	n.a	n.a
46	IDN	IDN 14/ Mandua	Mandua-TKG	WD	*A. dubius*	Diploid	32
47	IDN	IDN 15/ Mandua	Mandua-TKG	WD	*A. dubius*	Diploid	32
48	IDN	IDN 17/ Medan	Medan	WD	*A. blitum*	Diploid	34
49	IDN	IDN 18/ Medan	Medan	WD	*A. blitum*	Diploid	34
50	IDN	IDN 19/ Mandua	Mandua-TKG	WD	*A. blitum*	Diploid	34
51	IDN	IDN 20/ Yogya	Yogyakarta	WD	*A. dubius*	Diploid	32
52	IDN	IDN 22/ Yogya	Yogyakarta	WD	*A. dubius*	Diploid	32
53	IDN	IDN 23/ Yogya	Yogyakarta	WD	*A. dubius*	Diploid	32
54	IDN	IDN 24/ JKT	Jakarta	WD	*A. dubius*	Diploid	32
55	IDN	IDN 25/ JKT	Jakarta	WD	*A. dubius*	Diploid	32
56	IDN	IDN 26/ JKT	Jakarta	WD	*A. dubius*	Diploid	32
57	IDN	IDN 27/ JKT	Jakarta	WD	*A. dubius*	Diploid	32
58	IDN	IDN 28/ Kl.Urang	Kl.Urg-Ygy	VG	*A. tricolor*	Diploid	34
59	IDN	IDN 29/ Kl.urang	Kl.Urg-Ygy	VG	*A. tricolor*	Diploid	34
60	IDN	IDN 30/ Kl.Urang	Kl.Urg-Ygy	VG	*A. tricolor*	Diploid	34
61	IDN	IDN 32/ Kl.Urang	Kl.Urg-Ygy	VG	*A. tricolor*	Diploid	34
62	IDN	IDN 33/ Lembang	West Java	VG	*A. caudatus*	Diploid	32
63	IDN	IDN 34/ Lembang	West Java	VG	*A. caudatus*	Diploid	32
64	IDN	IDN 35/ BNA	B.Ach-Sum	VG	*A. tricolor*	Diploid	34
65	IDN	IDN 36/ BNA	B.Ach-Sum	VG	*A. tricolor*	Diploid	34
66	IDN	IDN 37/ BNA	B.Ach-Sum	VG	*A. tricolor*	Diploid	34
67	IDN	IDN 38/ BNA	B.Ach-Sum	VG	*A. tricolor*	Diploid	34
68	IDN	IDN 39/ BNA	B.Ach-Sum	OR	*C. argentea*	Diploid	36
69	IDN	IDN 40/ BNA	B.Ach-Sum	VG	*A. tricolor*	Diploid	34
70	IDN	IDN 41/ Marelan	Mrl-Medan	VG	*A. tricolor*	Diploid	34
71	IDN	IDN 42/ Marelan	Mrl-Medan	VG	*A. tricolor*	Diploid	34
72	IDN	IDN 43/ Marelan	Mrl-Medan	VG	*A. tricolor*	Diploid	34
73	IDN	IDN 44/ Marelan	Mrl-Medan	VG	*A. tricolor*	Diploid	34
74	IDN	IDN 45/ Marelan	Mrl-Medan	VG	*A. tricolor*	Diploid	34
75	IDN	IDN 46/ Kresek	Kresek-JKT	VG	*A. tricolor*	Diploid	34
76	IDN	IDN 47/ Kresek	Kresek-JKT	VG	*A. tricolor*	Diploid	34

Table5.1 (continued)

77	IDN	IDN 48/ Kresek	Kresek-JKT	VG	*A. tricolor*	Diploid	34
78	IDN	IDN 49/ Yates	JKT	OR	*C. argentea*	Diploid	36
79	IDN	IDN 50/ P.Merah	Pn.Merah-JKT	VG	*A. tricolor*	Diploid	34
80	IDN	IDN 51/ P.Merah	Pn.Merah-JKT	VG	*A. tricolor*	Diploid	34
81	IDN	IDN 52/ SHS	Sang Hy-JKT	VG	*A. tricolor*	Diploid	34
82	IDN	IDN 53/ SHS	Sang Hy-JKT	VG	*A. tricolor*	Diploid	34
83	IDN	IDN 54/ Tanindo	Tanindo-JKT	VG	*A. tricolor*	Diploid	34
84	IDN	IDN 55/ P.Kb	West Sumatra	WD	*A. hybridus*	Diploid	32
85	IDN	IDN 56/ P.Kb	West Sumatra	WD	*A. spinosus*	Diploid	34
86	IDN	IDN 57G/P.Kb	West Sumatra	WD	*A. hybridus*	Diploid	32
87	IDN	IDN 57R/ P.Kb	West Sumatra	WD	*A. hybridus*	Diploid	32

Figure 5.1. Three representatives of *Amaranthus*: A= vegetable type (*A. tricolor*); B= weedy type (*A. dubius*); C= ornamental (*Celosia* spp.) based on their morphology (in white font) and the results of flow cytometer (in black font) showing their diploid level.



CHAPTER 6

General Discussion

Being as one of the rich biodiversity countries such as Indonesia does not always contribute to better diets in its inhabitants (Termote *et al.* 2012). Many technical and non technical including many social and economical problems (e.g. the tremendous increase food prices) are interlinked which finally lead to a malnutrition problem in Indonesia. Almost 30% of the number of population and most of them are young age children do not have an access for a good quality food with high protein. Having dealt with such problem for more than one decade, consumption of amaranth can be promoted as one of the potential solution. Many scientists agree that amaranths show great potential to be prepared as promising food for the 21st century. Despite their still lower inference as underutilized crop, their superior characteristics in term of nutritional benefits and wide variability were confirmed in this study. However, their utilization and management mainly depend on adequate knowledge of existing genetic diversity (Van *et al.* 2011).

The assessment of plant genetic diversity can be derived in three possible ways: (i) morphological characterization; (ii) biochemical analysis such as nutritional values; (iii) molecular analysis by using DNA markers or chromosome number (Mondini *et al.* 2009). Here, we tried to assess amaranths based on their phenotypes such as morphological-, nutritional variation, their adaptive characteristics such as photoperiodic flowering response and polyploidy assessment by their chromosome number detection. All these gathered information would serve as a basic platform by their future utilization and further determination for breeding high quality vegetable with good features (Siemonsma and Piluek 1994).

Plant morphology is the simplest and easiest way to assess the diversity in a species. Morphology data give breeder a general concept of the total range of variability exist (Hawkes 1981). Measurement at the extent of diversity in our amaranths' accession may help us to determine the

limits of selection for improvement. The extent of the diversity from this study was presented as standard deviations. Thus, the Indonesian resources emphasizing the weedy types performed a range of diversity similar to that one by the worldwide amaranths. Previously, a high morphological diversity in vegetable amaranths was being reported by Jain *et al.* (1980). Ray and Roy (2009) proposed the long cultivation history of *Amaranthus* in the Indo-Gangetic plains have produced local *Amaranthus* species adapted to the local environmental condition. In similar view with Ray and Roy (2009), we hypothesized that the high genetic diversity found in Indonesian amaranths was resulted as a result of long environmental adaptation process, introgression and genetic drift. This result may also be applied to encounter the theory proposed by Jain *et al.* (1980) that Indo Gangetic Plain as the centre of diversity of vegetable amaranths. To verify this hypothesis, molecular studies such as SSR or AFLP should be conducted in order to reveal the evolutionary and phylogenetic in Indonesian amaranths (Mondini *et al.* 2009).

Moreover, the Indonesian resources showed distinct advantageous characteristics in most of yield contributing traits such as stem diameter, leaf thickness and number. Beyond our expectation, some of weedy Indonesian species had extraordinarily high leaf number which affirms the possibility to increase the yield performance of *A. tricolor* as the essential vegetable amaranth in Indonesia. The possibility to increase the leaf number in one plant may increase the competitiveness and attractiveness of amaranths with "kangkong" which so far acknowledged as the most productive leafy vegetable in the tropics (Edie and Ho 1968). Moreover, leaf number was very strongly correlated with biomass shown as total leaf area (r =0.607**). However, an increase in leaf number for increasing the yield biomass might affect leaf area as well. This result implies that an increase in one factor would allow a compensating decrease in the others. These have been encountered by Hauptli and Jain (1984) who revealed the tendency of relationship between leaf length with the yield of grain amaranths. They proposed that one of the yield indicators should be emphasized as breeding target (either leaf length or leaf number) but an increase in both parameters at once would not be possible.

Moreover, the high protein content observed in most of (edible) weedy types may open opportunity for breeding new varieties of amaranths with high quality protein or to enhance the

protein content in *A. tricolor*. This study showed that most of Indonesian weedy resources may contain up to 29 % protein. Thus, an increase up to 18 or 20% by the level of protein content can be set up as breeding targets. Nevertheless, these efforts to enhance the protein in amaranths cannot be achieved instantly as it requires a long time (more than 10 years) period, full determination and eagerness from many stakeholders including from the government side to achieve such target. Similar works by the enhancement of protein content and other nutrient traits in other countries such as in Africa and Brazil have been reported by Awoyinka *et al.* (1995) and Chavez *et al.* (2000).

Timing of flowering should be considered as one of many efforts to increase biomass yield in most of vegetable crops, including in amaranths (Jung and Müller 2009). Late flowering tendency in vegetable amaranths was one of the major selected targets during the domestication process. This was supported with the results of photoperiodic flowering response. In contrast, a shorter flowering period was shown by the weedy types including the weeds. This unique early flowering characteristics and wide range of adaptability to various climates in weedy types has enabled them to be widely dispersed around the globe (Grubben 1976). Interestingly, we were able to distinguish the dual purpose types with the major grain amaranths, and the other vegetable and weedy types based on their day length requirement to promote their flowering. We hypothesized that both performed different requirement of day length and temperature as a result of selection and adaptation process (Espitia-Rangel 1994). This is a novel finding and complements the previous results by the characterization of amaranths as grain or vegetable types (Mapes *et al.* 1996).

Photoperiodic flowering response can be applied as a complementary tool to characterize the types of amaranths. These results suggest that the two types are distinct not only bio-physiologically but also in their flowering tendency. We proposed further work at the molecular level (e.g. QTL) should clarify whether such biological response is mainly affected by specific regions in the chromosome or particular gene such as reported in maize (Ducrocq *et al.* 2008). Study of the photoperiodic flowering response may bring two advantageous. First, selection of potential parental lines can be achieved. The appropriate candidate should be not strongly affected by day length changing (based on PCA results), have late flowering tendency and high leaf number such as USDA

03/ PI 490298/ KEN, USDA 04/ PI 606281/ BGD, USDA 05/ PI 606282/ BGD, USDA 24/ Ames 5134/ USA, USDA 25/ PI 349553/ PNG. These accessions had 16-100 leaf number and middle to late flowering (60-100 days after sowing). By the Indonesian accessions, IDN 08/Asir-asir, IDN 38/ BNA and IDN 52/ SHS are also noted with good characteristics as potential parental lines with late flowering tendency (70-90 days after sowing) and 20-185 leaves per plant were performed. Second advantageous is that the important factors affecting the flowering in amaranths were revealed in this study. Deutsch (1977) who initiated the first work was unable to detect the important factors. Meanwhile, previous researcher (Kohli and Sawhney 1979, Kulakow and Jain 1985, Ahmed 2005) had only dealt with day length as the major determinants. In our results, we were able to confirm both temperature and day length are interlinked as crucial factors in determining flowering.

This is the first report dealt with the nutritional assessment of the grain, vegetable and weedy amaranths compiled in one extensive study. Up to now, the nutritional values of amaranths' seeds are the most extensively studied and reported (Barba de la Rosa *et al.* 1992, Caselato-Sousa and Amaya-Farfán 2012), followed with the nutritional benefits of vegetable amaranths (Steffensen *et al.* 2011, Shukla et al 2009, Prakash and Pal 1991). These unique characteristics by their nutrition have broaden the utilization of amaranths other than as protein rich source but also as source of high protein gene by the protein improvement of tuber crops via genetic engineering or as functional foods with nutraceutical properties (Chakraborty *et al.* 2010, Barba de la Rosa *et al.* 2007).

Very limited reports have dealt with the nutritional assessment of the weedy types especially the edible ones. In this report, we delivered the nutritional characteristics of weedy amaranths such as *A. blitum*, *A. dubius*, *A viridis* that have not been much explored. Their superiority noted with high protein scores had been noted. This study also affirmed the remarkably high content of lysine not only in their seeds but also in the leaves of amaranths which are in similar view with Barba de La Rosa *et al.* (2007). The leaves of amaranths present high content of most of essential amino acids, except in cysteine and methionine. Cysteine and methionine concentrations are usually combined in the FAO – WHO list of amino acids requirement for human (WHO/FAO/UNU 2007). Both are found to be limited in most of non-animal source protein (Sarwar 1997). A diverse food intake was suggested as

an alternative to compensate these lack points in amaranths (National Academy of Sciences 2006, Schaafsma 2000).

The assessment of nutritional values of the leaves of grain, vegetable and weedy amaranths presented support their potential to feed the high densely populated nations and to address the malnutrition problem in the developing countries. The RDA of protein for human is estimated 0.66 g/ kg body weight/day and 3.8-12 mg/ kg body weight/ day of lysine (WHO/FAO/UNU 2007, Gropper *et al.* 2009). Based on this standard, if the (lowest-) average protein content in amaranths of 12 g 100 g^{-1} DW is considered in the calculation, then a consumption of 100 grams or 275-550 grams of vegetable amaranths would cover the protein requirement for children or (adults) people with 50-100 kg of body weight. In adjacent to that, a consumption of 100-220 grams of vegetable amaranths would fulfill the RDA values of lysine in children and adults. From these results, we can conclude that a minimum consumption of 50 to 100 g fresh leaves of amaranths daily would suffice the nutritional requirement of healthy body (Grubben 1976).

Many of advantageous traits in our study (morphology, nutritional and relative stabile flowering response) were shown within *A. dubius*. The over competence in *A. dubius* has been also reported previously (Grubben 1976, Grubben 2004, Hadisoeganda 1996, Ugas *et al.* 2008). However, some limitations of *A. dubis* which sometimes limit its further exploitation as breeding materials have been reported, such as: the difficulties to transfer the desirable traits in *A. dubius* due to the incompatibility of chromosome number (Greizerstein and Poggio 1995). Hybrid obtained from *A. dubius* as one the parent was shown to be fertile or even no seed production. Backcrossing with one of the parents should restore the fertility; though partially fertile was observed within the progenies (Grubben 1976). Moreover, he also noted that in term of number of its production, seeds are moderately produced in *A. dubius* with very small seeds. The very small size of seeds make the utilization of *A. dubius* is less practicable if compared with the seeds of cultivated types of *A. tricolor* L. (van der Meer and Linders 1990). And last, many diversity of leaf form is exist within *A. dubius*. Nevertheless, some of them have very small size of leaves. An increase of leaf shape (in term of length and width ratio) might be also proposed for further improvement in *A. dubius*.

We had tried to assess the phenotypical variation in *Amaranthus* from various angles (morphology, nutrition and flowering response). Brenner *et al.* (2000) suggested that genetic variability in *Amaranthus* are strongly influenced both by genetic and environmental variation. Therefore, the application of molecular marker is required to complement the data of phenotype and to extent the accurate degree in genetic variation. Moreover, the application of molecular marker will be more substantial to better understand the genetic variation in a quantitative manner (Dekkers and Hospital 2002). By having the information at their molecular level, several breeding strategies such as marker-assisted selection (MAS) can be applied. Thus, more efficient and quick verification and identification for further genetic utilization or conservation can be achieved. Therefore, further molecular work of this study should be conducted in the future.

Summary

Malnutrition is one of the major public health problems in developing countries including in Indonesia. About 32% of children under the age of 5 years are malnourished. Biodiversity such as assessment and utilization of underutilized crop with high nutritional values are promoted as a promising potential to alleviate malnutrition.

Amaranthus or commonly known as amaranths include 75 species which are distinguished as 1) the grain; 2) vegetable; 3) ornamental 4) weedy types including weeds. The grain amaranths are represented by *A. caudatus*, *A. cruentus*, *A. hybridus*, *A. hypochondriacus*; with the first two function as the "dual purpose" types due to their edible leaves in their young stage. The most prominent vegetable species in South East Asia including in Indonesia is *A. tricolor* which serves as cheap protein source and as livelihoods for many small farmers. *Celosia* spp. is the renowned ornamental amaranths. The weedy types referred as "weeds" are *A. retroflexus* and *A. spinosus* which are usually not edible. Some of weedy types which closely resemble the cultivated vegetable types can be used for human consumption such as *A. dubius*, *A. blitum*, *A. viridis.*

Despite of the popularity of amaranths, no so far breeding efforts have been initiated in Indonesia. A diversity of amaranths exists in Indonesia which bears great potential for genetic improvement. Nevertheless, such huge variability remains untapped or not yet been directed to alleviate the malnutrition problem. Amaranths are available throughout the year and are classified as easy to grow crop within a short period (up to 4 weeks). These good characteristics in amaranths bear great potential to solve the malnutrition problem in Indonesia. Prior to that, their performances by the yield and nutritional should be enhanced in order to increase their attractiveness and competitiveness with other tropical vegetables types such as *Ipomoea aquatica* and *Manihot esculenta* (cassava leaves). Based on the current situation on the field and concerning the opportunities exist in Indonesia, therefore, the objective of this study is to build a basic foundation for breeding of vegetable amaranths via: 1) assessing the phenotypical & genetical variation of Indonesian amaranth resources from their

morphology, nutritional values, their photoperiodic flowering response and polyploidy level; 2) valuing the potential of Indonesian amaranth resources by comparing them with the worldwide variation of amaranths; 3) selecting the potential parental lines for future breeding efforts.

The high variability of Indonesian amaranths was affirmed from this study. Their variation was almost similar to that one exhibited the worldwide amaranths in most of morphological traits and protein content. Interesting, Indonesian amaranths were shown to be more superior the essential parameters determining in vegetable yield such as leaf number, stem diameter, and leaf thickness. Some accessions belong to *A. blitum* (USDA 04/ PI 606281/ BGD), *A. dubius* (IDN 01/ Mongal, IDN 02/ Daling, IDN 24/ JKT) and *A viridis* (USDA 30/ PI 540445/ IDN/ Java) can be selected as promising parental lines for increasing leaf number and leaf protein. No selection pressures, high out crossing rates which may facilitate introgression and polyploidy were proposed as the major factors contributing to such higher diversity of Indonesian amaranths.

Amaranths showed exhibit photoperiodic flowering response and they can be classified based on their flowering time as: early flowering (0-60 d.a.s), middle (61-80 d.a.s) and late flowering type (more than 80 days). Flowering in amaranths was majorly affected by accumulative temperature and day length. Photoperiod flowering response can be applied as an alternative tools for the characterization of the three types in amaranths. Amaranths are generally classified as short day plant, nevertheless, the vegetable and the dual purpose types seemly behave long day plant which means that they require longer than 8 hours per day to induce their flowering.

The three types of amaranths (grain, vegetable and weedy types) showed a wide array of highly nutritional characteristics. The weedy species such as *A. dubius*, *A. blitum* and *A. viridis* can be promoted as alternative plants for solving malnutrition problem due to their relatively high content of protein (up to 29 g 100 g^{-1} DW) and the values of lysine was close to an ideal protein score. A consumption of at least 100 grams of vegetable amaranths is sufficed to fulfill the Recommended Dietary Allowance. The dual type species such as *A. caudatus* and *A. cruentus* contain an acceptable high content of protein (about 18 g 100 g^{-1} DW) and they can be prospected as alternative protein rich

vegetable or as fodder. The quality of protein was significantly affected by the growth stage in amaranths. Therefore, good timing of harvesting amaranth (31-39 d.a.s) should be considered as quality control for consumer.

The polyploidy level in *Amaranthus* accession (N= 87) were investigated via flow cytometer and they showed variation of chromosome number. The ornamental *Celosia* showed diploid choromosome number with *2n*= 18 or 36. Meanwhile, the grain, vegetable and weedy amaranths had a chromosome number variation between *2n*=32 or *2n*=34. No tetraploid form in *A. dubius* was found. The "no polyploidy" level in the studied materials may open new possibility to breed new variety of amaranths with desired characteristics.

I was succeed to assess the genetic diversity of amaranths, particularly those Indonesian resources based on their phenotypic, nutritional values, flowering characteristics and the level of polyploidy which brings new opportunity to improve their genetics. Thus, based on this information, the basic platform for amaranth breeding efforts has just been set up. Moreover, some accessions having high nutritional qualities (high protein content) were also selected. These materials can be promoted to enrich the quality of nutrition for Indonesian people including to combat the malnutrition problem in the rural areas of Indonesia.

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Appendix (A)

List of Tables

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Table A.1. Morphology data of *Amaranthus* accession (N= 84 accessions).

Acc.No.	Accession Name	Type	Species	H (cm)	SDM (mm)	NBR (unit)	NIN (unit)	NLV (unit)	LAR (cm2)	TLA (cm2)	RLW	LTK (mm)	PRT (%)	PP	LSH	LCL
	WORLDWIDE															
1	Ames 5315/ IND	VG	*A.blitum*	56.13	4.40	10.00	12.25	60.40	2.80	168.95	1.83	0.29	12.19	1	1	2
2	PI 610262/ IND	VG	*A.blitum*	65.67	5.27	14.40	19.00	45.80	7.95	364.15	1.89	0.21	14.75	1	2	2
3	PI 490298/ KEN	VG	*A.blitum* subsp. oleraceus	43.30	10.43	18.20	11.80	98.80	5.02	495.49	1.75	0.24	17.44	1	2	2
4	PI 606281/ BGD	VG	*A.blitum*	28.60	9.14	13.00	9.40	42.80	51.60	2208.57	1.54	0.22	28.03	1	2	5
5	PI 606282/ BGD	VG	*A.blitum*	44.50	10.82	16.80	12.80	24.60	48.39	1190.51	1.60	0.30	24.59	1	2	5
6	PI 482049/ ZWE	GR	*A.cruentus*	26.00	7.08	18.20	14.80	22.00	63.94	1406.59	2.02	0.29	17.56	3	3	2
7	PI 482051/ ZWE	GR	*A.cruentus*	26.80	8.32	15.40	12.75	15.40	62.27	958.97	1.96	0.24	17.25	5	2	2
8	PI 490662/ BEN	GR	*A.cruentus*	31.60	8.50	18.80	14.80	18.40	65.20	1199.63	2.54	0.32	15.84	1	3	2
9	PI 494777/ ZMB	GR	*A.cruentus*	14.10	5.06	11.20	8.80	9.20	42.67	392.57	2.04	0.25	16.25	1	2	2
10	PI 500267/ ZMB	GR	*A.cruentus*	29.60	7.08	17.00	13.40	19.20	50.08	961.49	5.45	0.23	18.78	3	8	2
11	PI 538319/ USA	GR	*A.cruentus*	34.40	9.42	12.80	10.20	16.00	78.78	1260.54	1.71	0.41	17.28	1	2	2
12	PI 566897/ IND	GR	*A.cruentus*	27.00	9.80	18.00	15.00	17.00	86.27	1466.51	2.08	0.40	15.41	1	2	2
13	PI 604666/ USA	GR	*A.cruentus*	43.40	9.96	14.60	10.60	18.80	94.35	1773.69	1.78	0.38	14.16	1	1	2
14	PI 605352/ JAM	VG	*A.dubius*	32.00	6.58	13.60	12.80	35.80	54.09	1936.59	1.59	0.26	16.09	1	2	2
15	PI 642737/ PRI	VG	*A.dubius*	49.00	5.40	14.00	14.20	57.80	28.44	1643.64	1.59	0.13	18.13	1	2	2
16	PI 608661/ IND	VG	*A.graecizans*	25.60	5.20	13.00	13.60	101.80	25.52	2598.08	2.01	0.25	19.47	9	1	5
17	PI 500249/ ZMB	GR	*A.hybridus*	9.40	5.30	9.60	6.80	13.60	47.83	650.51	2.32	0.25	20.31	1	2	3
18	PI 605351/ GRC	GR	*A.hybridus*	30.80	7.92	20.00	17.40	53.40	63.47	3389.21	1.83	0.20	12.28	1	2	3
19	PI 604577/ MEX	GR	*A.hypochondriacus*	12.50	5.25	12.50	8.25	15.50	30.77	476.89	1.98	0.20	12.59	1	2	3
20	Ames 5134/ USA	VG	*A.tricolo.*	12.30	5.04	15.00	12.20	49.40	23.65	1168.49	3.46	0.14	11.59	1	3	2
21	PI 349553/ PNG	VG	*A.tricolor*	9.60	8.00	10.75	5.60	15.60	9.82	153.13	1.78	0.21	12.19	1	4	3

Acc.No.	Accession Name	Type	Species	H (cm)	SDM (mm)	NBR (unit)	NIN (unit)	NLV (unit)	LAR (cm2)	TLA (cm2)	RLW	LTK (mm)	PRT (%)	PP	LSH	LCL
22	PI 566899/ IND	VG	*A.tricolor*	30.25	11.34	18.20	16.00	54.20	32.55	1764.08	1.62	0.13	12.03	1	2	2
23	PI 604669/ TWN	VG	*A.tricolor*	4.00	7.25	9.50	5.50	23.50	44.41	1043.65	1.22	0.12	21.28	1	6	2
24	PI 586680/ USA	OR	*C.argentea*	17.20	7.08	34.80	33.80	44.20	33.90	1498.58	2.84	0.20	8.91	1	2	4
25	PI 482244/ ZWE	OR	*C.trigyna*	1.75	7.10	10.00	9.50	28.50	43.02	1226.21	1.94	0.25	23.50	1	2	2
26	PI 608761/ IND	VG	*A.tricolor*	17.40	6.89	16.20	15.00	52.80	23.17	1223.38	1.77	0.29	17.06	1	2	5
27	NPL 01/ KTM	OR	*Celosia* spp.	2.00	3.70	7.00	7.00	4.00	22.25	88.99	2.16	0.27	24.22	1	2	2
28	NPL 02/ KTM	OR	*Celosia* spp.	13.02	6.08	7.20	5.80	29.40	7.88	231.77	2.74	0.23	19.94	1	2	2
29	NPL 03 /KTM	OR	*Celosia* spp.	10.30	6.08	9.20	8.40	35.00	24.34	852.05	2.29	0.25	19.16	1	2	2
30	NPL 04 /KTM	OR	*Celosia* spp.	18.20	4.80	11.00	11.00	32.00	33.59	1074.94	2.53	0.19	19.25	1	7	2
31	NPL 05 /KTM	OR	*Celosia* spp.	33.20	6.75	12.00	11.25	34.00	35.42	1204.13	2.55	0.27	13.66	1	7	2
	INDONESIA															
32	PI 540445/IDN/Java	WD	*A.viridis*	2.40	4.76	9.20	7.60	52.50	31.26	1641.37	1.37	0.17	29.06	2	2	3
33	IDN 01/ Mongal	WD	*A.dubius*	13.40	9.68	16.40	12.60	50.80	68.72	3490.96	1.54	0.21	28.34	1	2	2
34	IDN02/ Daling	WD	*A.dubius*	32.70	8.80	21.40	20.00	334.40	15.16	5069.53	1.54	0.12	24.19	1	1	2
35	IDN 03/ Daling	WD	*A.dubius*	35.10	6.80	17.00	15.20	147.80	18.50	2734.43	1.56	0.15	24.28	1	1	2
36	IDN 04/ Bur biah	WD	*A.spinosus*	15.40	7.38	14.60	14.40	32.40	35.66	1155.43	1.50	0.35	19.56	1	2	2
37	IDN 05/ Bur biah	OR	*Celosia* spp.	14.20	6.80	18.60	16.00	26.00	18.34	476.78	2.16	0.18	19.34	8	2	2
38	IDN 06/ Bur biah	OR	*Celosia* spp.	9.20	6.72	16.40	12.60	22.60	30.49	689.08	2.12	0.20	11.63	1	7	2
39	IDN 07/ Ulu Nuih	WD	*A.spinosus*	12.30	7.08	15.40	13.20	22.60	32.31	730.21	2.10	0.28	11.84	1	7	3
40	IDN 08/ Asir-asir	WD	*A.dubius*	19.80	10.03	21.50	20.50	183.75	18.93	3479.03	2.13	0.13	21.25	9	2	3
41	IDN 09/ Asir-asir	WD	*A.dubius*	23.60	7.38	18.60	17.80	191.80	5.78	1107.80	1.88	0.11	19.59	9	1	2
42	IDN 10/P.One-one	WD	*A.dubius*	25.75	6.18	13.25	13.25	155.50	4.03	626.79	1.72	0.15	15.38	9	2	2
43	IDN 11/ Tn.Depet	WD	*A.dubius*	33.00	5.64	10.00	10.00	125.60	10.33	1298.03	1.71	0.17	24.09	9	1	2
44	IDN 13/ Mandua	WD	*A.dubius*	26.20	10.28	16.60	16.40	179.80	19.76	3552.65	1.72	0.17	17.16	9	2	1

Acc.No.	Accession Name	Type	Species	H (cm)	SDM (mm)	NBR (unit)	NIN (unit)	NLV (unit)	LAR (cm2)	TLA (cm2)	RLW	LTK (mm)	PRT (%)	PP	LSH	LCL
45	IDN 14/ Mandua	WD	*A.dubius*	18.20	12.28	17.60	17.40	55.80	48.07	2682.36	1.15	0.28	18.19	1	2	2
46	IDN 15/ Mandua	WD	*A.dubius*	23.70	6.26	16.20	13.80	24.00	10.23	245.56	1.38	0.18	13.69	1	2	2
47	IDN 17/ Medan	WD	*A.blitum*	16.60	21.70	13.00	13.00	31.80	49.01	1558.48	1.42	0.25	18.28	1	2	2
48	IDN 18/ Medan	WD	*A.blitum*	1.67	4.07	10.00	8.00	20.33	9.85	200.21	1.95	0.24	25.59	2	2	2
49	IDN 19/ Mandua	WD	*A.blitum*	3.80	5.08	10.40	8.40	49.00	14.00	685.79	1.54	0.19	24.91	2	2	2
50	IDN 20/ Ygy	WD	*A.dubius*	17.90	7.76	14.60	14.40	64.00	24.97	1598.20	1.53	0.31	14.53	1	2	2
51	IDN 22/ Ygy	WD	*A.dubius*	16.90	7.96	15.40	13.60	76.60	43.63	3341.68	1.47	0.32	14.34	1	2	2
52	IDN 23/ Ygy	WD	*A.dubius*	20.80	2.28	13.00	13.40	43.40	17.96	779.28	1.52	0.17	16.44	1	2	1
53	IDN 24/ JKT	WD	*A.dubius*	20.80	8.62	14.80	14.20	137.20	47.29	6488.40	1.65	0.42	17.53	1	2	2
54	IDN 25/ JKT	WD	*A.dubius*	18.90	7.88	15.80	14.80	116.60	30.57	3564.17	1.68	0.33	22.75	1	2	2
55	IDN 26/ JKT	WD	*A.dubius*	20.40	8.50	13.60	13.80	71.40	31.17	2225.63	1.41	0.31	19.53	1	2	2
56	IDN 27/ JKT	WD	*A.dubius*	18.70	8.18	16.20	14.60	88.00	36.07	3174.50	1.64	0.32	13.88	1	2	2
57	IDN 29/ Kl.Urang	VG	*A.tricolor*	11.90	8.41	10.00	8.60	31.20	36.86	1149.90	1.70	0.28	13.41	1	2	5
58	IDN 30/ Kl.Urang	VG	*A.tricolor*	22.90	7.64	13.40	12.60	28.60	35.89	1026.41	1.51	0.34	15.22	1	1	1
59	IDN 33/ Lembang	VG	*A.caudatus*	28.10	9.08	16.00	16.20	45.00	46.67	2100.20	1.70	0.35	12.56	1	2	2
60	IDN 34/ Lembang	VG	*A.caudatu .*	15.50	9.46	11.20	9.80	33.20	84.29	2798.29	1.30	0.50	15.78	1	2	3
61	IDN 35/ BNA	VG	*A.tricolor*	28.30	10.26	15.40	13.80	96.60	61.02	5894.53	1.70	0.38	12.59	1	2	2
62	IDN 36/ BNA	VG	*A.tricolor*	23.00	9.17	11.20	10.20	30.60	24.30	743.50	1.41	0.25	14.97	1	2	2
63	IDN 37/ BNA	VG	*A.tricolor*	23.10	9.98	11.60	10.40	47.40	34.21	1621.76	1.69	0.30	16.44	1	2	2
64	IDN 38/ BNA	VG	*A.tricolor*	20.38	8.75	11.00	10.25	23.50	29.17	685.41	1.50	0.33	15.19	1	2	5
65	IDN 39/ BNA	OR	*Celosia* spp.	13.60	8.81	11.40	8.20	18.40	24.76	455.66	2.69	0.41	16.03	1	7	2
66	IDN 40/ BNA	VG	*A.tricolor*	17.40	9.76	5.40	4.80	15.80	9.96	157.39	1.37	0.23	13.91	1	2	2
67	IDN 41/ Marelan	VG	*A.tricolo.*	34.00	10.82	6.00	5.75	7.50	24.79	185.91	1.37	0.37	14.19	1	2	5
68	IDN 42/ Marelan	VG	*A.tricolor.*	15.10	8.25	7.00	5.75	15.25	31.16	475.27	1.61	0.42	14.56	1	2	5

Acc.No.	Accession Name	Type	Species	H (cm)	SDM (mm)	NBR (unit)	NIN (unit)	NLV (unit)	LAR (cm2)	TLA (cm2)	RLW	LTK (mm)	PRT (%)	PP	LSH	LCL
69	IDN 43/ Marelan	VG	*A.tricolor*	37.40	11.76	10.60	9.60	52.60	48.11	2530.82	1.42	0.33	16.53	1	2	5
70	IDN 44/ Marelan	VG	*A.tricolor*	24.44	10.86	8.60	7.20	30.60	30.23	925.09	1.53	0.36	15.81	1	2	2
71	IDN 45/ Marelan	VG	*A.tricolor*	30.10	9.88	6.40	5.80	7.80	39.42	307.46	1.27	0.35	18.19	1	2	5
72	IDN 46/ Kresek	VG	*A.tricolor*	28.10	9.11	10.20	8.60	51.20	26.88	1376.17	1.80	0.31	21.28	1	2	2
73	IDN 47/ Kresek	VG	*A.tricolor*	29.20	10.15	9.00	8.40	36.20	31.94	1156.12	1.59	0.40	16.94	1	2	5
74	IDN 48/ Kresek	VG	*A.tricolor*	32.00	10.02	13.80	12.60	45.40	25.59	1161.68	1.93	0.43	16.25	1	2	2
75	IDN 49/ Yates	OR	*Celosia* spp.	20.52	7.81	8.20	7.40	13.60	10.74	146.04	3.87	0.41	18.63	1	3	2
76	IDN 50/ Pn. Merah	VG	*A.tricolor*	25.40	9.23	15.20	14.20	88.40	37.50	3315.04	1.60	0.40	18.88	1	2	2
77	IDN 51/ Pn.Merah	VG	*A.tricolor*	23.30	7.96	10.60	9.60	30.40	36.76	1117.57	1.58	0.36	13.94	1	2	3
78	IDN 52/ SHS	VG	*A.tricolor*	21.90	9.85	7.40	6.20	18.60	54.79	1019.11	1.22	0.37	15.97	1	2	5
79	IDN 53/ SHS	VG	*A.tricolor*	17.30	9.81	11.20	8.40	27.40	47.42	1299.41	1.39	0.31	13.53	1	2	2
80	IDN 54/ Tanindo	VG	*A.tricolor*	29.40	7.69	12.20	10.60	21.60	14.42	311.49	1.73	0.25	13.81	1	2	2
81	IDN 55/ P.Kumbuh	WD	*A.hybridus*	26.08	9.01	25.60	21.20	20.40	57.39	1170.80	2.38	0.43	14.13	1	3	2
82	IDN 56/ P.Kumbuh	WD	*A.spinosus*	22.00	6.39	13.60	12.80	116.00	3.55	412.04	2.61	0.36	21.53	1	2	1
83	IDN 57G/P.Kumbuh	WD	*A.hybridus*	5.20	6.04	16.50	14.50	11.00	5.11	56.22	1.93	0.15	17.25	1	2	2
84	IDN 57R/P.Kumbuh	WD	*A.hybridus*	24.83	8.76	24.00	21.67	42.50	42.60	1810.36	2.31	0.35	16.56	1	2	2

GR, grain; OR, ornamental; VG, vegetable; WD, weedy; 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; PP, plant posture; LSH, leaf shape; LCL, leaf color.

Table A.2. Accumulative temperature (°C), accumulative day length (hours), mean flowering time of the 13 species of amaranths represented the grain, vegetable and weedy amaranths.

Nr.	TYPE	Species	N	Season I (LD)			Season II (LD to SD)			Season III (SD)			Season IV (LD)		
				Mean FT	*Acc. T (oC) until FT*	**Acc. DL (hours) until FT**	Mean FT	*Acc. T (oC) until FT*	**Acc. DL (hours) until FT**	Mean FT	*Acc. T (oC) until FT*	**Acc. DL (hours) until FT**	Mean FT	*Acc. T (oC) until FT*	**Acc. DL (hours) until FT**
1	GR	*A. cruentus*	8	75	*1205*	**861**	65	*1663*	**843**	79	*1187*	**796**	67	*1535*	**881**
2	GR	*A. hybridus*	4	57	*991*	**638**	64	*1630*	**826**	74	*1087*	**742**	78	*1811*	**1042**
3	GR	*A. hypochondriacus*	2	69	*1104*	**787**	49	*1337*	**638**	79	*1180*	**791**	76	*1733*	**1001**
4	VG	*A. blitum*	5	61	*1026*	**683**	47	*1269*	**616**	87	*1323*	**870**	63	*1444*	**827**
5	VG	*A. dubius*	2	59	*999*	**650**	39	*1118*	**515**	75	*1093*	**747**	64	*1471*	**844**
6	VG	*A. graecizans subsp. silvestris*	1	n.f	*n.a.*	**n.a.**	40	*1143*	**527**	124	*2089*	**1269**	78	*1811*	**1044**
7	VG	*A. viridis*	1	67	*1082*	**762**	65	*1656*	**840**	87	*1304*	**861**	57	*1287*	**730**
8	VG	*A. caudatus*	2	80	*1258*	**929**	60	*1555*	**770**	94	*1457*	**941**	80	*1850*	**1066**
9	VG	*A. tricolor*	21	80	*1262*	**930**	59	*1530*	**757**	114	*1877*	**1160**	82	*1915*	**1106**
10	WD	*A. retroflexus*	1	38	*714*	**412**	32	*904*	**413**	42	*487*	**420**	35	*838*	**443**
11	WD	*A. dubius*	14	78	*1247*	**909**	45	*1245*	**589**	81	*1225*	**813**	64	*1470*	**843**
12	WD	*A. spinosus*	5	73	*1180*	**838**	48	*1297*	**619**	100	*1582*	**1005**	66	*1499*	**861**
13	WD	*A. blitum*	2	76	*1203*	**877**	68	*1719*	**877**	115	*1877*	**1163**	67	*1540*	**886**
			69	68	*1106*	**773**	52	*1390*	**679**	88	*1367*	**891**	67	*1554*	**890**

LD, Long Day; SD, Short Day; GR, Grain; VG, Vegetable; WD, Weedy; Acc.T, Accumulative Temperature; DL, Day Length; FT, Flowering Time

Table A.3. List of materials, biomass, leaves' dry matter, protein and amino acids of *Amaranthus* (N= 76)

Nr.	Accession Name	Type	Species	BMS	Leaf DM	Protein	Glx	Asx	Leu	Ala	Lys	Gly	Ser	Arg	Thr	Phe	Pro	Val
				(% DM)	(% DM)	(g 100 g^{-1} DW)	(g 100 g^{-1} DW Protein)											
	WORLDWIDE																	
1	USDA 01/ Ames 5315/ IND	VG	*A.blitum*	25.75	24.42	12.19	11.11	9.09	6.82	5.21	5.18	5.50	6.08	4.50	5.12	4.04	4.78	4.03
2	USDA 02/ PI 610262/ IND	VG	*A.blitum*	21.21	13.13	14.75	11.30	9.95	8.04	6.13	5.74	5.64	5.47	5.22	4.97	4.70	4.49	4.97
3	USDA 03/ PI 490298/ KEN	VG	*A.blitum*	13.75	21.92	17.44	12.21	9.58	7.75	6.27	5.95	6.29	5.93	5.14	5.22	4.74	4.60	4.94
4	USDA 04/ PI 606281/ BGD	VG	*A.blitum*	13.56	19.78	28.03	12.49	9.78	7.85	6.33	5.71	5.42	5.18	5.22	4.79	4.72	4.37	4.57
5	USDA 05/ PI 606282/ BGD	VG	*A.blitum*	13.46	18.75	24.59	12.36	10.10	7.84	6.72	5.87	5.75	5.31	5.11	4.67	4.91	4.12	4.20
6	USDA 06/ PI 482049/ ZWE	GR	*A.cruentus*	15.47	21.48	17.56	11.01	9.95	7.56	6.14	5.91	6.22	5.69	4.76	4.93	4.52	4.69	4.39
7	USDA 07/ PI 482051/ ZWE	GR	*A.cruentus*	16.07	21.41	17.25	11.29	10.27	7.61	5.85	6.14	6.16	5.59	4.91	5.03	4.67	4.52	4.48
8	USDA 08/ PI 490662/ BEN	GR	*A.cruentus*	17.78	24.37	15.84	10.46	9.65	6.85	5.67	5.55	5.95	5.30	4.31	4.63	4.13	4.40	4.09
9	USDA 09/ PI 494777/ ZMB	GR	*A.cruentus*	17.17	19.36	16.25	11.05	9.90	6.71	5.74	5.68	6.03	4.55	4.41	4.44	4.12	4.59	3.67
10	USDA 10/ PI 500267/ ZMB	GR	*A.cruentus*	13.21	1.13	18.78	10.91	10.05	6.92	6.18	5.50	6.96	5.53	4.27	4.41	4.30	4.25	3.84
11	USDA 11/ PI 538319/ USA	GR	*A.cruentus*	14.16	19.55	17.28	12.03	10.42	7.13	6.12	5.82	6.82	5.66	4.55	5.02	4.23	4.34	4.20
12	USDA 12/ PI 566897/ IND	GR	*A.cruentus*	15.40	17.60	15.41	10.59	9.75	6.92	6.18	5.50	6.96	5.53	4.27	4.41	4.30	4.25	3.84
13	USDA 13/ PI 604666/ USA	GR	*A.cruentus*	13.20	17.20	14.16	11.91	9.96	8.19	6.55	6.42	5.59	5.27	5.07	5.03	4.91	4.56	5.18
14	USDA 15/ PI 605352/ JAM	VG	*A.dubius*	19.35	23.50	16.09	12.29	9.90	8.25	5.97	6.10	5.70	5.07	5.08	5.08	4.93	4.87	4.92
15	USDA 16/ PI 642737/ PRI	VG	*A.dubius*	16.92	25.40	18.13	12.55	10.01	8.36	6.16	6.12	5.77	5.13	5.23	4.99	5.12	4.82	5.11
16	USDA 17/ PI 608661/ IND	VG	*A.graecizans*	18.45	16.34	19.47	12.83	10.55	8.48	6.05	6.31	6.06	2.63	5.56	5.13	5.06	5.04	5.25
17	USDA 19/ PI 500249/ ZMB	GR	*A.hybridus*	17.10	22.16	20.31	12.82	9.96	8.15	6.53	6.27	5.96	5.57	5.21	5.28	4.92	4.85	4.69
18	USDA 20/ PI 605351/ GRC	GR	*A.hybridus*	15.29	22.79	12.28	11.10	10.20	7.81	5.61	6.16	6.10	5.56	4.86	5.01	4.59	5.09	4.68

Nr.	Accession Name	Type	Species	BMS	Leaf DM	Protein	Glx	Asx	Leu	Ala	Lys	Gly	Ser	Arg	Thr	Phe	Pro	Val
				(% DM)	(% DM)	(g 100 g^{-1} DW)	(g 100 g^{-1} DW Protein)											
19	USDA 21/ PI 604577/ MEX	GR	*A.hypochondriacus*	13.96	12.64	12.59	10.87	9.95	7.95	6.00	5.98	5.88	5.41	5.27	5.04	4.74	4.34	4.76
20	USDA 22/ PI 604796/ N.A	GR	*A.hypochondriacus*	17.81	18.61	10.78	10.67	9.88	7.72	5.65	6.03	5.95	5.35	4.75	4.96	4.43	4.68	4.92
21	USDA 24/ Ames 5134/ US	VG	*A. tricolor*	7.57	24.14	11.59	11.63	9.60	7.55	6.06	5.99	5.90	5.19	5.19	4.92	4.69	5.67	4.70
22	USDA 25/ PI 349553/ PNG	VG	*A.tricolor*	12.91	16.09	12.19	13.74	10.45	7.77	5.76	6.16	6.09	5.52	5.11	5.05	4.69	5.08	4.29
23	USDA 26/ PI 477918/ N.A	VG	*A.tricolor*	20.81	28.65	20.81	12.62	10.00	7.35	6.36	5.57	6.15	5.11	4.92	4.84	4.52	4.62	4.21
24	USDA 27/ PI 566899/ IND	VG	*A.tricolor*	13.54	26.77	12.03	12.15	10.38	7.23	6.16	5.43	5.85	5.04	4.67	4.70	4.08	4.28	4.61
25	USDA 28/ PI 604669/ TWN	VG	*A.tricolor*	17.22	20.88	21.28	13.42	10.12	7.83	6.42	5.69	5..54	4.89	5.07	4.82	4.47	4.42	4.84
26	USDA 29/ PI 608761/ IND	VG	*A. tricolor*	13.17	25.74	17.06	12.24	9.89	7.95	6.60	6.18	5.95	5.38	5.38	5.13	4.65	4.65	4.90
27	USDA 30/ PI 540445/IDN	VG	*A. viridis*	10.01	17.09	29.06	12.11	10.28	8.09	6.96	6.24	5.83	5.14	5.80	4.90	4.92	4.67	4.54
	INDONESIA																	
28	IDN 01/ Mongal	WD	*A.dubius*	16.01	20.02	28.34	14.87	10.72	8.20	6.54	6.23	5.69	5.38	5.37	5.05	4.90	5.01	4.51
29	IDN 02/ Daling	WD	*A.dubius*	5.85	22.97	24.19	14.30	9.85	8.06	6.10	6.18	6.21	5.65	5.31	5.12	4.87	4.42	4.56
30	IDN 03/ Daling	WD	*A.dubius*	n.a.	n.a.	24.28	16.09	10.38	7.47	6.79	5.67	6.25	5.77	5.30	4.77	4.79	4.72	3.30
31	IDN 04/ Bur biah	WD	*A.spinosus*	16.59	22.86	19.56	13.76	11.37	7.65	6.57	6.00	6.32	6.11	5.05	5.11	4.91	4.26	3.48
32	IDN 07/ Ulu Nuih	WD	*A.spinosus*	21.45	11.55	11.84	12.00	9.87	7.38	5.89	5.64	6.49	5.47	4.66	4.70	4.38	4.48	3.41
33	IDN 08/ Asir-asir	WD	*A.dubius*	22.48	30.69	21.25	15.58	10.66	7.76	7.69	5.63	6.01	5.96	4.87	4.68	4.87	4.46	3.25
34	IDN 09/ Asir-asir	WD	*A.dubius*	13.58	27.38	19.59	13.84	10.07	7.80	7.01	6.14	5.91	5.79	5.00	5.19	4.62	4.99	3.95
35	IDN 10/P.One-one	WD	*A.dubius*	15.00	24.44	15.38	13.13	9.97	7.84	6.64	6.22	5.92	5.79	5.12	5.31	4.72	4.91	4.21
36	IDN 11/ Tn.Depet	WD	*A.dubius*	15.22	27.43	24.09	15.53	10.31	7.92	7.06	5.99	5.57	6.01	5.43	4.91	4.77	4.62	4.20
37	IDN 13/ Mandua	WD	*A.dubius*	n.a.	n.a.	17.16	13.77	10.45	8.05	7.07	5.75	5.58	5.57	5.02	5.23	4.79	5.00	4.09
38	IDN 14/ Mandua	WD	*A.dubius*	20.03	22.24	18.19	13.15	10.22	8.11	7.28	6.38	5.57	5.77	5.25	5.39	4.91	4.78	4.38

Nr.	Accession Name	Type	Species	BMS	Leaf DM	Protein	Glx	Asx	Leu	Ala	Lys	Gly	Ser	Arg	Thr	Phe	Pro	Val
				(% DM)	(% DM)	(g 100 g^{-1} DW)	(g 100 g^{-1} DW Protein)											
39	IDN 15/ Mandua	WD	*A.dubius*	14.81	37.70	13.69	13.51	10.93	7.46	6.13	5.95	6.11	5.96	4.82	5.05	4.43	5.14	3.55
40	IDN 17/ Medan	WD	*A.dubius*	21.93	12.39	18.28	13.54	10.67	7.96	7.68	6.16	5.58	5.70	5.00	5.19	4.79	4.75	3.93
41	IDN 18/ Medan	WD	*A.blitum*	22.41	21.40	25.59	13.05	10.83	7.93	5.77	6.00	5.92	5.40	5.59	5.02	4.90	4.89	4.68
42	IDN 19/ Mandua	WD	*A.blitum*	21.22	21.99	24.91	13.55	11.01	7.98	5.83	6.10	5.91	5.38	5.77	4.86	5.03	4.92	4.48
43	IDN 20/ Ygy	WD	*A.dubius*	17.75	24.92	14.53	13.13	9.59	8.19	6.90	6.42	5.29	5.07	4.98	5.01	4.77	5.52	5.31
44	IDN 22/ Ygy	WD	*A.dubius*	19.43	23.59	14.34	13.12	9.83	8.37	6.80	6.47	5.31	5.13	5.20	5.21	4.92	4.67	5.25
45	IDN 23/ Ygy	WD	*A.dubius*	21.01	29.99	16.44	13.37	9.92	8.40	6.84	6.53	5.26	5.02	5.14	5.14	4.94	4.68	5.32
46	IDN 24/ JKT	WD	*A.dubius*	19.73	26.90	17.53	12.40	10.33	8.19	6.40	5.94	5.64	5.45	5.17	5.17	4.95	4.25	5.09
47	IDN 25/ JKT	WD	*A.dubius*	21.28	24.57	22.75	15.48	10.22	7.99	7.24	6.10	5.53	5.22	5.25	4.79	4.81	4.28	4.91
48	IDN 26/ JKT	WD	*A.dubius*	21.52	27.07	19.53	13.22	11.02	8.38	6.15	6.36	5.64	5.23	5.48	5.10	5.06	4.34	5.05
49	IDN 27/ JKT	WD	*A.dubius*	25.42	34.25	13.88	13.01	11.16	8.15	6.13	6.21	5.77	5.21	5.28	5.07	4.95	4.16	4.88
50	IDN 28/ JKT	WD	*A.dubius*	22.28	20.19	13.41	12.72	10.72	7.71	5.67	6.59	5.85	5.15	5.16	5.06	4.60	4.23	4.89
51	IDN 29/ Kl.Urang	VG	*A.tricolor*	n.a.	n.a.	14.59	12.81	10.50	8.00	6.00	6.47	5.88	5.09	5.35	5.02	4.89	4.21	5.19
52	IDN 30/ Kl.Urang	VG	*A.tricolor*	27.57	20.99	15.22	13.44	10.57	8.24	6.08	6.60	5.90	5.28	5.39	5.15	4.80	4.62	5.14
53	IDN 33/ Lembang	VG	*A.caudatus*	13.33	22.40	12.56	13.31	10.44	7.60	5.89	6.47	5.82	5.47	5.02	4.91	4.24	4.59	4.49
54	IDN 34/ Lembang	VG	*A.caudatus*	10.55	21.18	15.78	14.34	11.22	7.68	6.03	6.63	5.86	5.41	5.18	5.07	4.50	4.71	4.74
55	IDN 35/ BNA	VG	*A.tricolor*	11.32	12.35	12.59	13.76	11.04	7.71	5.83	6.51	6.16	5.59	5.27	4.90	4.27	4.69	4.84
56	IDN 36/ BNA	VG	*A.tricolor*	11.10	18.30	14.97	13.29	10.87	7.99	5.80	6.55	5.80	5.33	5.28	5.12	4.69	4.37	5.21
57	IDN 37/ BNA	VG	*A.tricolor*	7.79	18.20	16.44	13.49	10.50	7.45	6.05	6.05	6.11	5.51	4.90	5.10	4.41	4.86	4.56
58	IDN 38/ BNA	VG	*A.tricolor*	10.60	13.51	15.19	13.54	10.63	7.36	5.98	6.07	5.91	5.64	4.81	5.10	4.29	4.62	4.23
59	IDN 40/ BNA	VG	*A.tricolor*	15.77	13.55	13.91	13.31	10.09	7.82	5.76	6.53	5.86	5.45	4.97	5.10	4.44	5.29	4.83

Nr.	Accession Name	Type	Species	BMS	Leaf DM	Protein	Glx	Asx	Leu	Ala	Lys	Gly	Ser	Arg	Thr	Phe	Pro	Val
				(% DM)	(% DM)	(g 100 g^{-1} DW)	(g 100 g^{-1} DW Protein)											
60	IDN 41/ Marelan	VG	*A.tricolor*	14.43	16.22	14.19	14.02	10.56	7.51	6.04	6.06	5.89	5.64	4.88	4.87	4.40	4.79	4.29
61	IDN 42/ Marelan	VG	*A.tricolor*	12.71	17.84	14.56	14.29	10.61	7.51	5.91	6.16	5.79	5.75	4.94	4.98	4.61	4.59	4.53
62	IDN 43/ Marelan	VG	*A.tricolor*	11.55	15.71	16.53	14.30	10.53	7.78	5.93	5.86	5.50	5.48	4.94	4.97	4.53	5.02	4.83
63	IDN 44/ Marelan	VG	*A.tricolor*	11.16	13.34	15.81	13.45	10.19	7.97	5.92	6.36	5.80	5.53	5.23	5.07	4.63	4.73	4.86
64	IDN 45/ Marelan	VG	*A.tricolor*	16.68	26.60	18.19	13.96	10.75	8.09	6.09	6.00	5.86	5.41	5.06	5.04	4.71	4.75	4.85
65	IDN 46/ Kresek	VG	*A.tricolor*	10.79	10.97	21.28	12.83	10.22	7.82	6.27	5.87	5.90	5.26	5.43	5.10	4.72	4.56	5.08
66	IDN 47/ Kresek	VG	*A.tricolor*	13.16	13.66	16.94	14.12	9.88	6.99	6.63	5.76	6.09	5.61	6.01	4.81	4.40	3.89	4.59
67	IDN 48/ Kresek	VG	*A.tricolor*	11.12	15.31	16.25	11.04	10.50	7.90	6.26	6.05	6.06	5.60	5.20	5.06	4.58	5.24	4.55
68	IDN 50/ Pn. Merah	VG	*A.tricolor*	10.09	10.97	18.88	10.97	10.51	8.02	6.03	5.68	5.68	5.35	5.24	5.05	4.71	5.24	4.86
69	IDN 51/ Pn.Merah	VG	*A.tricolor*	19.44	29.22	13.94	10.64	10.09	8.22	6.04	5.77	6.33	5.53	5.06	5.15	4.96	4.89	4.86
70	IDN 52/ SHS	VG	*A.tricolor*	14.17	13.50	15.97	11.15	10.11	8.27	5.88	5.61	6.23	5.36	5.27	5.06	5.00	4.87	5.17
71	IDN 53/ SHS	VG	*A.tricolor*	17.15	25.90	13.53	12.14	10.76	8.13	6.11	5.89	5.79	5.40	5.15	4.87	4.72	5.17	4.72
72	IDN 54/ Tanindo	VG	*A.tricolor*	13.36	14.44	13.81	11.36	9.92	8.24	6.12	6.30	5.79	5.37	5.56	5.13	4.72	4.75	5.22
73	IDN 55/ P.Kumbuh	WD	*A.hybridus*	17.35	17.47	14.13	11.37	10.32	7.74	5.91	6.54	5.98	6.05	5.24	4.89	4.51	3.99	4.80
74	IDN 56/ P.Kumbuh	WD	*A.spinosus*	18.56	16.74	21.53	12.39	10.00	7.89	5.37	6.16	6.70	5.43	5.15	4.79	5.36	4.81	4.76
75	IDN 57G/ P.Kumbuh	WD	*A.hybridus*	14.71	14.67	17.25	12.16	9.81	5.44	6.99	6.18	5.72	5.26	5.18	5.01	4.70	4.51	5.14
76	IDN 57R/ P.Kumbuh	WD	*A.hybridus*	n.a.	n.a.	16.56	11.71	9.76	8.00	6.19	6.28	6.20	5.60	5.22	5.16	4.80	5.07	5.22

GR, grain; VG, vegetable; WD, weedy; WW, worldwide; IDN, Indonesia; BMS, biomass; DM, dry matter; Glx, glutamic acid or glutamine; Asx, aspartic acid or asparagine; Leu, leucine; Ala, alanine; Lys, lysine; Gly, glysine; Ser, serine; Arg, arginine; Thr, threonine; Phe, phenylalanine; Pro, proline; Val, valine; Ile, isoleucine; Tyr, tyrosine; His, histidine; Cys, cysteine; Met, methionine; P-Ser, P-serine; Orn, ornithine; Cit, citrulline, Total AA, total Amino Acids; Total NPAA, total Non Protein Amino Acids

Table A.3. (Continued)

Nr.	Accession Name	Type	Species	Ile	Tyr	His	Cys	Met	P-Ser	Orn	Cit	Total AA	Total NPAA
				(g 100 g^{-1} DW Protein)									
	WORLDWIDE												
1	USDA 01/ Ames 5315/ IND	VG	*A.blitum*	3.53	2.87	1.56	0.36	0.00	0.62	0.09	0.90	79.77	2.44
2	USDA 02/ PI 610262/ IND	VG	*A.blitum*	4.13	3.48	1.77	0.39	0.66	0.97	0.07	0.53	87.03	2.84
3	USDA 03/ PI 490298/ KEN	VG	*A.blitum*	4.13	3.20	2.10	0.48	0.17	0.59	0.50	0.47	88.71	2.13
4	USDA 04/ PI 606281/ BGD	VG	*A.blitum*	4.15	3.77	1.96	0.47	0.51	0.54	0.13	0.29	87.28	1.54
5	USDA 05/ PI 606282/ BGD	VG	*A.blitum*	3.77	3.83	1.89	0.55	0.80	0.34	0.05	0.40	87.82	1.18
6	USDA 06/ PI 482049/ ZWE	GR	*A.cruentus*	3.81	3.86	2.00	0.59	0.50	0.00	0.06	0.65	86.51	1.47
7	USDA 07/ PI 482051/ ZWE	GR	*A.cruentus*	3.87	3.89	2.06	0.53	0.49	0.33	0.04	0.46	87.36	1.62
8	USDA 08/ PI 490662/ BEN	GR	*A.cruentus*	3.57	3.44	1.87	0.55	0.19	0.49	0.18	0.52	80.62	2.06
9	USDA 09/ PI 494777/ ZMB	GR	*A.cruentus*	3.17	3.38	1.96	0.57	0.38	0.66	0.00	1.24	80.35	2.77
10	USDA 10/ PI 500267/ ZMB	GR	*A.cruentus*	3.47	3.87	2.02	0.62	0.75	0.60	0.12	0.57	83.83	2.17
11	USDA 11/ PI 538319/ USA	GR	*A.cruentus*	3.34	3.72	2.10	0.55	0.00	0.58	0.00	0.86	86.05	2.08
12	USDA 12/ PI 566897/ IND	GR	*A.cruentus*	3.51	2.74	1.97	0.47	0.42	1.38	0.11	0.55	80.65	3.10
13	USDA 13/ PI 604666/ USA	GR	*A.cruentus*	4.33	3.75	2.06	0.52	0.18	0.00	0.05	0.56	89.47	1.24
14	USDA 15/ PI 605352/ JAM	VG	*A.dubius*	4.18	3.68	2.00	0.37	0.12	1.10	0.00	0.56	88.50	2.13
15	USDA 16/ PI 642737/ PRI	VG	*A.dubius*	4.25	3.98	1.90	0.47	0.56	0.29	0.00	0.38	90.52	1.28
16	USDA 17/ PI 608661/ IND	VG	*A.graecizans*	4.60	3.82	2.06	0.41	0.39	0.32	0.00	0.41	90.25	1.10
17	USDA 19/ PI 500249/ ZMB	GR	*A.hybridus*	3.94	4.19	2.02	0.56	0.32	0.35	0.00	0.35	91.24	1.05
18	USDA 20/ PI 605351/ GRC	GR	*A.hybridus*	4.03	3.54	1.87	0.47	0.00	0.60	0.00	0.66	86.66	2.17

Nr.	Accession Name	Type	Species	Ile	Tyr	His	Cys	Met	P-Ser	Orn	Cit	Total AA	Total NPAA
				(g 100 g-^{1} DW Protein)									
19	USDA 21/ PI 604577/ MEX	GR	*A.hypochondriacus*	4.01	4.12	1.98	0.51	0.00	0.44	0.00	0.71	86.80	1.98
20	USDA 22/ PI 604796/ N.A	GR	*A.hypochondriacus*	4.26	3.26	1.94	0.45	0.00	0.51	0.21	0.83	84.91	2.79
21	USDA 24/ Ames 5134/ US	VG	*A. tricolor*	4.04	3.91	1.91	0.67	0.19	0.31	0.00	0.66	87.81	1.60
22	USDA 25/ PI 349553/ PNG	VG	*A.tricolor*	3.62	3.54	1.93	0.83	0.00	0.34	0.00	0.58	89.62	1.23
23	USDA 26/ PI 477918/ N.A	VG	*A.tricolor*	3.60	2.88	1.85	0.25	0.00	1.14	0.00	0.72	84.85	2.75
24	USDA 27/ PI 566899/ IND	VG	*A.tricolo.*	3.86	2.87	1.75	0.48	0.00	0.85	0.12	0.83	83.54	3.16
25	USDA 28/ PI 604669/ TWN	VG	*A.tricolor*	4.13	2.88	1.81	0.10	0.14	1.58	0.07	0.37	86.59	2.99
26	USDA 29/ PI 608761/ IND	VG	*A. tricolor*	4.19	3.59	1.62	0.44	0.17	0.46	0.00	0.58	88.92	2.32
27	USDA 30/ PI 540445/IDN	VG	*A. viridis*	3.97	3.77	1.81	0.55	0.00	0.40	0.00	0.31	89.57	3.30
	INDONESIA												
28	IDN 01/ Mongal	WD	*A.dubius*	3.77	3.82	1.93	0.42	0.54	0.47	0.03	0.26	92.96	1.06
29	IDN02/ Daling	WD	*A.dubius*	4.14	3.73	1.97	0.60	0.45	0.41	0.17	0.43	91.51	1.33
30	IDN 03/ Daling	WD	*A.dubius*	2.84	3.78	1.79	0.81	0.81	0.27	0.00	0.38	91.33	1.11
31	IDN 04/ Bur biah	WD	*A.spinosus*	2.93	3.90	1.73	0.75	0.52	0.64	0.10	0.52	90.43	1.62
32	IDN 07/ Ulu Nuih	WD	*A.spinosus*	2.67	3.91	1.75	0.61	1.09	2.07	0.00	0.90	84.40	3.63
33	IDN 08/ Asir-asir	WD	*A.dubius*	2.83	3.94	1.71	0.50	0.71	0.64	0.06	0.48	91.11	1.55
34	IDN 09/ Asir-asir	WD	*A.dubius*	3.26	3.70	1.79	0.58	0.44	0.94	0.00	0.53	90.08	1.82
35	IDN 10/P.One-one	WD	*A.dubius*	3.47	3.69	1.96	0.58	0.00	0.52	0.00	0.59	89.48	1.72
36	IDN 11/ Tn.Depet	WD	*A.dubius*	3.56	3.76	1.81	0.62	0.75	0.36	0.00	0.33	92.81	0.89
37	IDN 13/ Mandua	WD	*A.dubius*	3.37	3.62	1.68	0.32	0.39	0.52	0.00	0.49	89.75	2.56

Nr.	Accession Name	Type	Species	Ile	Tyr	His	Cys	Met	P-Ser	Orn	Cit	Total AA	Total NPAA
				(g 100 g-^{1} DW Protein)									
38	IDN 14/ Mandua	WD	*A.dubius*	3.61	3.82	1.86	0.56	0.46	0.18	0.00	0.48	91.49	1.20
39	IDN 15/ Mandua	WD	*A.dubius*	3.08	3.63	1.72	0.56	0.78	0.61	0.00	0.91	88.81	2.02
40	IDN 17/ Medan	WD	*A.blitum*	3.23	3.62	1.79	0.48	0.58	0.37	0.00	0.53	90.64	1.57
41	IDN 18/ Medan	WD	*A.blitum*	4.19	3.81	1.85	0.58	0.57	0.52	0.05	0.37	90.99	1.48
42	IDN 19/ Mandua	WD	*A.blitum*	3.93	3.96	1.79	0.89	0.94	0.25	0.00	0.39	92.32	1.22
43	IDN 20/ Ygy	WD	*A.dubius*	4.39	3.44	1.87	0.48	0.26	0.00	0.00	0.60	90.64	0.91
44	IDN 22/ Ygy	WD	*A.dubius*	4.46	3.31	1.87	0.30	0.22	0.28	0.00	0.59	90.43	1.21
45	IDN 23/ Ygy	WD	*A.dubius*	4.50	3.43	1.92	0.35	0.20	0.32	0.00	0.59	90.96	1.23
46	IDN 24/ JKT	WD	*A.dubius*	4.34	3.50	1.89	0.33	0.10	0.19	0.12	0.64	89.14	1.98
47	IDN 25/ JKT	WD	*A.dubius*	4.12	3.30	1.86	0.24	0.57	1.22	0.00	0.35	91.90	1.57
48	IDN 26/ JKT	WD	*A.dubius*	4.18	3.78	1.91	0.24	0.50	0.48	0.00	0.39	91.64	1.58
49	IDN 27/ JKT	WD	*A.dubius*	4.12	3.59	1.80	0.00	0.00	0.75	0.00	0.52	89.49	2.28
50	IDN 28/ JKT	WD	*A.dubius*	4.16	3.49	1.85	0.00	0.00	0.97	0.00	0.65	87.84	2.85
51	IDN 29/ Kl.Urang	VG	*A.tricolor*	4.42	3.42	1.94	0.00	0.00	0.31	0.00	0.43	89.21	1.65
52	IDN 30/ Kl.Urang	VG	*A.tricolor*	4.32	3.64	1.87	0.23	0.00	0.57	0.00	0.43	91.24	1.68
53	IDN 33/ Lembang	VG	*A.caudatus*	3.67	3.54	1.82	0.00	0.00	0.96	0.00	0.57	87.30	2.60
54	IDN 34/ Lembang	VG	*A.caudatus*	3.87	3.62	1.86	0.26	0.00	0.45	0.00	0.33	90.99	1.52
55	IDN 35/ BNA	VG	*A.tricolor*	3.90	3.61	1.75	0.00	0.00	0.93	0.00	0.54	89.82	2.18
56	IDN 36/ BNA	VG	*A.tricolor*	4.32	3.55	1.95	0.00	0.00	0.36	0.00	0.57	90.12	0.93
57	IDN 37/ BNA	VG	*A.tricolor*	3.72	3.75	1.70	0.63	0.35	0.37	0.06	0.58	89.15	2.21
58	IDN 38/ BNA	VG	*A.tricolor*	3.48	3.62	1.77	0.61	0.49	0.50	0.00	0.69	88.15	2.02

Nr.	Accession Name	Type	Species	Ile	Tyr	His	Cys	Met	P-Ser	Orn	Cit	Total AA	Total NPAA
				(g 100 g-^{1} DW Protein)									
59	IDN 40/ BNA	VG	*A.tricolor*	4.11	3.57	1.63	0.53	0.16	0.20	0.06	0.67	89.43	1.53
60	IDN 41/ Marelan	VG	*A.tricolor*	3.51	3.89	1.41	0.60	1.21	0.81	0.00	0.26	89.59	1.51
61	IDN 42/ Marelan	VG	*A.tricolor*	3.73	3.71	1.59	0.60	0.38	0.53	0.00	0.59	89.68	1.63
62	IDN 43/ Marelan	VG	*A.tricolor*	3.90	3.62	1.71	0.47	0.66	0.21	0.00	0.56	90.03	1.33
63	IDN 44/ Marelan	VG	*A.tricolor*	4.14	3.75	1.72	0.65	0.71	0.44	0.04	0.59	90.70	1.77
64	IDN 45/ Marelan	VG	*A.tricolor*	4.18	3.76	1.72	0.51	0.65	0.49	0.00	0.44	91.39	1.54
65	IDN 46/ Kresek	VG	*A.tricolor*	4.35	4.11	1.94	0.69	0.29	0.79	0.00	0.47	90.43	1.65
66	IDN 47/ Kresek	VG	*A.tricolor*	3.70	4.15	2.05	0.70	0.52	0.56	0.00	0.51	89.90	1.41
67	IDN 48/ Kresek	VG	*A.tricolor*	3.89	3.73	1.41	0.49	0.68	0.78	0.00	0.56	88.23	2.78
68	IDN 50/ Pn. Merah	VG	*A.tricolor*	4.25	3.76	1.63	0.43	0.33	0.49	0.00	0.62	87.73	2.50
69	IDN 51/ Pn.Merah	VG	*A.tricolor*	4.40	3.88	1.66	0.51	0.00	0.86	0.21	0.66	87.99	3.31
70	IDN 52/ SHS	VG	*A.tricolor*	4.76	3.71	1.62	0.57	0.24	0.78	0.00	0.56	88.90	2.62
71	IDN 53/ SHS	VG	*A.tricolor*	4.02	4.00	1.67	0.48	0.85	0.91	0.00	0.48	89.89	2.51
72	IDN 54/ Tanindo	VG	*A.tricolor*	4.53	3.58	1.62	0.40	0.56	0.87	0.00	0.56	89.16	2.82
73	IDN 55/ P.Kumbuh	WD	*A.hybridus*	3.95	3.93	1.73	0.43	0.67	1.16	0.22	0.68	88.06	3.43
74	IDN 56/ P.Kumbuh	WD	*A.spinosus*	4.58	4.46	2.22	1.06	0.00	0.52	0.00	0.38	91.13	1.16
75	IDN 57G/ P.Kumbuh	WD	*A.hybridus*	6.25	3.54	1.73	0.32	2.39	0.63	0.00	0.40	90.34	2.69
76	IDN 57R/ P.Kumbuh	WD	*A.hybridus*	4.44	3.96	1.90	0.40	0.11	0.47	0.00	0.56	90.04	1.98

GR, grain; VG, vegetable; WD, weedy; WW, worldwide; IDN, Indonesia; BMS, biomass; DM, dry matter; Glx, glutamic acid or glutamine; Asx, aspartic acid or asparagine; Leu, leucine; Ala, alanine; Lys, lysine; Gly, glysine; Ser, serine; Arg, arginine; Thr, threonine; Phe, phenylalanine; Pro, proline; Val, valine; Ile, isoleucine; Tyr, tyrosine; His, histidine; Cys, cysteine; Met, methionine; P-Ser, P-serine; Orn, ornithine; Cit, citrulline, Total AA, total Amino Acids; Total NPAA, total Non Protein Amino Acids.

A. *A. caudatus* **B**. *A. cruentus* **C**. *A. hybridus* **D**. *A. hypochondriacus*

E. *A. blitum* **F**. *A. dubius* **G**. *A. spinosus* **H**. *A. viridis*

Figure A.1. Genetic diversity of *Amaranthus* : Grain amaranths (A-D) and weedy amaranths (E-H).

I. *A. blitum*

J. *A. tricolor (green)*

K. *A. tricolor (red)*

L. *A. tricolor* (variegated)

M. *Celosia* spp

J. *Celosia* spp.

K. *Celosia* spp.

L. *Celosia* spp.

M. *Celosia* spp.

N. *Celosia* spp.

Figure A.1. Genetic diversity of *Amaranthus*. Vegetable amaranths (I-L) and ornamental amaranths (M-N).