Application of principle component analysis in differentiating the three types of *Amaranthus* based on their photoperiodic flowering response

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Abstract. Response of flowering has become an attracting field for plant breeders and is pre-requisite in plant introduction process from different ecogeographical origins. Time of flowering is mostly induced by the day-length or photoperiod and temperature. *Amaranthus* (*Amaranthus* spp.) is known as multipurpose plants and utilized as grains or as vegetables. Under tropical environment, likewise in Indonesia, the requirement of day-length to induce flowering in amaranths is unrevealed as the day-length remains always constant with approximately 12 hours per day. During the introduction of amaranth to Japan, we would like to confirm the type of amaranth, which in general has been theoretically classified as short day plants. Under the variation of day-length ranging from 9 up to 15 hours during the four seasons in Japan, thus, we confirmed that amaranth as short day plants. Although, the vegetable ones required day-length up to 12 hours. Such conclusion was obtained from the flowering response of 69 accessions representing amaranth’s *eco-geographical variations* within two consecutive years. Seeds were sown in two replicates. Plant nursery was conducted on the *Norin-Ijutsu Centre* belongs to the University of Tsukuba. Mean values from two replicates were obtained and the average was then further subjected to Principle Component Analysis (PCA) [JMP ver. 7.0 (SAS Institute, USA)]. Thus, PCA was able to differentiate the flowering tendency within the three types of amaranths in the form of three ellipsoids.

1. Introduction
Response of flowering has become an attracting field for plant breeders and is pre-requisite in plant introduction process from different ecogeographical origins. By manipulating flowering time in commercial crops, this would enable plant breeders to increase yield. Thus, time of flowering is recognized as an important adaptive trait [1]. In regard to flowering response, amaranth (*Amaranthus* spp.) generally has a wide variation of flowering types, ranging from short-day up to day length.
Insensitive types [2]. Such wide variation in terms of adaptive characteristics has enabled them to be distributed in various continents [3, 17]. Generally, it is classified as short day plants meaning that flowering will be promoted under the exposure of at least 8 hours’ day length or photoperiod and a maximum of 16 hours of day length per day or known as the critical limit. Although, some day neutral varieties are also exist [4].

Amaranth is also known as multipurpose plants and utilized as grains or vegetables depending on the regional preferences. The two types, moreover, show biologically a distinct character in regard to the biomass allocation in one plant [3]. The highly nutritious vegetables such as A. blitum, A. viridis, and A. tricolor L. are widely grown in the humid tropical low-lands and serve as high protein green leafy vegetables. The most representing and commercial one is A. tricolor L. [5]. Meanwhile, the grains of A. caudatus L., A. cruentus L., A. hybridus and A. hypochondriacus are more pronounced in most of the northern hemisphere regions. They are also known as pseudocereals plants, highly nutritious, and mostly consumed in the same way as cereals [6]. Beside the two cultivated types, some weedy relatives in amaranths including true-weeds such as the very early flowering represented by A. retroflexus are also exist with a wide variability of flowering time [7].

Photoperiodic flowering response is defined as the degree to which extent plant’s varieties can adjust their flowering under different environmental cues e.g. day length, sowing dates, temperature, that would affect growth characteristics in one plant [8], [9], [10], including in amaranth [6]. Although, previous studies concluded that variation of flowering was more relatively affected due to day-length in amaranths rather than temperature [2]. There are various differences between the grain and vegetable amaranths, such as: plant morphology and physiology, plant organ parts that are harvested, breeding targets, and different tendency of flowering response. These traits might be resulted from plant adaptation and man-made selection. In temperate growing regions, the varieties of grain amaranths with earlier flowering are preferred as this may facilitate the inflorescences to produce more seeds during the life cycle. Contrastingly, under the tropical climate, delayed flowering in vegetable amaranths is more favored as this may extend the vegetative growth period, which finally leads to increase the number of the produced biomass [11].

Under tropical condition at the equator such as in Indonesia, where day-length remains always constant with more or less 12 hours daily, the photoperiodic response of amaranth is unrevealed. When amaranth was dislocated and introduced into temperate regions such as in Japan, thus, we hypothesized that those amaranths originated from the tropics would show their photoperiodic flowering response. The collected data would be useful for preliminary data prior to their introduction as potential summer vegetable types in Japan. Therefore, in this study, we would like: 1) to investigate the photoperiodic flowering response in Amaranthus under the cool-temperate regions such as in Japan; 2) to differentiate the Amaranthus’ types based on the Principle Component Analysis (PCA).

2. Materials and Methods
2.1. Plant Materials
Table 1 listed the materials used during the experiment.
Table 1. List of the *Amaranthus*’ accessions

| Acc. No. | Accession Name | Type | Species | Average F.T. (d.a.s.) | Classification |
|----------|----------------|------|---------|----------------------|----------------|
|          | WORLD ACCESSIONS |      |         |                      |                |
| 1        | US’ 01/ Ames 5315/ IND | VG<sup>2</sup> | *A. blitum* | 44 | early |
| 2        | US 02/ PI 610262/ IND | VG | *A. blitum* | 44 | early |
| 3        | US 03/ PI 490298/ KEN | VG | *A. blitum* | 66 | middle |
| 4        | US 04/ PI 606281/ BGD | VG | *A. blitum* | 58 | early |
| 5        | US 05/ PI 606282/ BGD | VG | *A. blitum* | 58 | early |
| 6        | US 06/ PI 482049/ ZWW | GR | *A. cruentus* L. | 68 | early |
| 7        | US 07/ PI 482051/ ZWE | GR | *A. cruentus* L. | 72 | middle |
| 8        | US 08/ PI 490662/ BEN | GR | *A. cruentus* L. | 62 | middle |
| 9        | US 09/ PI 492777/ ZMB | GR | *A. cruentus* L. | 77 | middle |
| 10       | US 10/ PI 500267/ ZMB | GR | *A. cruentus* L. | 72 | middle |
| 11       | US 11/ PI 538319/ USA | GR | *A. cruentus* L. | 64 | middle |
| 12       | US 12/ PI 566897/ IND | GR | *A. cruentus* L. | 45 | early |
| 13       | US 13/ PI 604666/ USA | GR | *A. cruentus* L. | 66 | middle |
| 14       | US 15/ PI 605352/ JAM | VG | *A. dubius* | 49 | early |
| 15       | US 16/ PI 642737/ PRI | VG | *A. dubius* | 49 | early |
| 16       | US 17/ PI 608661/ IND | VG | *A. graecizans* | 20 | early |
| 17       | US 19/ PI 500249/ ZMB | GR | *A. hybridus* | 55 | early |
| 18       | US 20/ PI 605351/ GRC | GR | *A. hybridus* | 47 | early |
| 19       | US 21/ PI 604577/ MEX | GR | *A. hypochondriacus* | 56 | early |
| 20       | US 22/ 604796/ N.A | GR | *A. hypochondriacus* | 60 | early |
| 21       | US 23/ PI 607447/ JAM | WD | *A. retroflexus* | 35 | early |
| 22       | US 24/ Ames 5134/ USA | VG | *A. tricolor* L. | 78 | middle |
| 23       | US 25/ PI 349553/ PNG | VG | *A. tricolor* L. | 77 | middle |
| 24       | US 26/ PI 477918/ N.A | VG | *A. tricolor* L. | 66 | middle |
| 25       | US 27/ PI 566899/ IND | VG | *A. tricolor* L. | 46 | early |
| 26       | US 28/ PI 604669/ TWN | VG | *A. tricolor* L. | 45 | early |
| 27       | US 29/ PI 608761/ IND | VG | *A. tricolor* L. | 65 | middle |
|          | INDONESIAN ACCESSIONS |      |         |                      |                |
| 28       | PI 540445/ IDN/Java | WD | *A. viridis* | 58 | early |
| 29       | IDS 01/ Mongal | WD | *A. dubius* | 75 | middle |
| 30       | IDS02/ Daling | WD | *A. dubius* | 53 | early |
| 31       | IDS 04/ Bur biah | WD | *A. spinosus* | 91 | late |
| 32       | IDS 07/ Ulu Nuih | WD | *A. spinosus* | 67 | middle |
| 33       | IDS 08/ Asir-asir | WD | *A. dubius* | 85 | middle |
| 34       | IDS 09/ Asir-asir | WD | *A. dubius* | 64 | middle |
| 35       | IDS 10/ P-One-one | WD | *A. spinosus* | 73 | middle |
| 36       | IDS 11/ Tn.Depet | WD | *A. spinosus* | 66 | middle |
| 37       | IDS 14/ Mandua | WD | *A. dubius* | 61 | middle |
| 38       | IDS 15/ Mandua | WD | *A. dubius* | 39 | early |
| 39       | IDS 17/ Medan | WD | *A. blitum* | 80 | middle |
| 40       | IDS 18/ Medan | WD | *A. blitum* | 81 | Late |
| 41       | IDS 19/ Mandua | WD | *A. blitum* | 65 | middle |
| 42       | IDS 20/ Yogya | WD | *A. dubius* | 63 | middle |
| 43       | IDS22/ Yogya | WD | *A. dubius* | 58 | early |
| 44       | IDS 23/ Yogya | WD | *A. dubius* | 58 | early |
| 45       | IDS 24/ JKT | WD | *A. dubius* | 55 | early |
| Acc. No. | Accession Name | Type | Species | Average F.T. (d.a.s.)\(^d\) | Classification |
|----------|----------------|------|---------|-----------------------------|----------------|
| 46       | IDS 25/ JKT    | WD   | A. dubius | 54                          | early          |
| 47       | IDS 26/ JKT    | WD   | A. dubius | 63                          | middle         |
| 48       | IDS 27/ JKT    | WD   | A. dubius | 53                          | early          |
| 49       | IDS 30/ Kl.Urang | VG   | A. tricolor L. | 69                          | middle         |
| 50       | IDS 33/ Lembang | VG   | A. caudatus L. | 64                          | middle         |
| 51       | IDS 34/ Lembang | VG   | A. caudatus L. | 68                          | middle         |
| 52       | IDS 35/ BNA     | VG   | A. tricolor L. | 62                          | middle         |
| 53       | IDS 36/ BNA     | VG   | A. tricolor L. | 62                          | middle         |
| 54       | IDS 37/ BNA     | VG   | A. tricolor L. | 63                          | middle         |
| 55       | IDS 38/ BNA     | VG   | A. tricolor L. | 83                          | late           |
| 56       | IDS 43/ Marelan | VG   | A. tricolor L. | 83                          | late           |
| 57       | IDS 44/ Marelan | VG   | A. tricolor L. | 69                          | middle         |
| 58       | IDS 45/ Marelan | VG   | A. tricolor L. | 55                          | early          |
| 59       | IDS 46/ Kresek  | VG   | A. tricolor L. | 69                          | middle         |
| 60       | IDS 47/ Kresek  | VG   | A. tricolor L. | 66                          | middle         |
| 61       | IDS48/ Kresek   | VG   | A. tricolor L. | 53                          | early          |
| 62       | IDS 50/ P. Merah | VG   | A. tricolor L. | 55                          | early          |
| 63       | IDS51/ P. Merah | VG   | A. tricolor L. | 75                          | middle         |
| 64       | IDS 52/ SHS     | VG   | A. tricolor L. | 81                          | middle         |
| 65       | IDS 53/ SHS     | VG   | A. tricolor L. | 79                          | middle         |
| 66       | IDS 54/ Tanindo | VG   | A. tricolor L. | 60                          | early          |
| 67       | IDS 55/ P.kumbuh | WD   | A. hybridus | 65                          | middle         |
| 68       | IDS 56/ P.kumbuh | WD   | A. spinosus | 48                          | early          |
| 69       | IDS 57/P.kumbuh | WD   | A. hybridus | 47                          | early          |

\(^{a}\) US= USDA Collection. \\
\(^{b}\) Abbreviation of amaranth’s types: GR= grain; VG= vegetable; and WD= weedy type. \\
\(^{c}\) The worldwide accessions (N=27) were originated from 13 countries from the African, American and Asian continents. The 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. \\
\(^{d}\) The species identification was conducted by USDA personnel [USDA, ARS, National Genetic Resources Program Germplasm Resources Information Network (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland available at: http://www.ars-grin.gov/npgs/. \\
\(^{e}\) Average F.T. (Flowering Time) = The longest and the shortest period of flowering were divided into two.

### 2.2. Experimental Site

Previous experiment was stationed at the experimental field at the Agriculture and Forestry Research Centre belongs to the University of Tsukuba (Ibaraki, Japan). The city is located at 28 m above sea level (a.s.l.) at 36°07′01.71″ latitude and 140°05′40.24″ longitude. Ibaraki Prefecture been grouped in ‘Cfa’ according to the Köppen-Geiger climate classification. It has a significant amount of rainfall during the year with a humid sub-tropical climate. The Tsukuba city is located at the valley of the Tsukuba Mountain, known as the second highest mountain in Japan. Thus, the temperature is warm, mild, and temperate; ranging from -3°C in winter and 30°C in summer. Precipitation is the highest in...
September, while the lowest is taken place in January, with an average of 167 and 37 mm, respectively.

The capital city of Ibaraki Prefecture: Mito city was referred and its temperature and day length data were put in the graph. Mito is located about 38 km northwest from Tsukuba City, in which its climate tends to be warm and temperate, with a great deal of rainfall with an average of 1,392 mm, even in the driest month. The lowest and highest temperature are -2°C and 29°C, with an average of annual one is 13.7°C [13].

2.3. Germination
Within 2010 to 2012, seeds of amaranth were sown four times (table 2). The seeds were germinated in a quadratic plastic tray with 6*6 holes with 4 cm diameter and 4.7 cm depth. Each block was filled in with ready soil (‘Metromix’ 350, Sungro). Afterwards, 3 to 4 weeks old of seedlings (N= 3-4) were then selected for plant material. Thus, they were then transplanted into clay pots (18 cm diameter; 16.5 cm height) with added fertilizer soil containing 120:1,000:50 mg/l N:P2O5:K2O labelled as ‘Sumirin’ (pH= 6.7). Pots were then arranged in accordance to a completely randomized experimental design. At the end, only two plants were left in each pot and grown until their maturity. Each accession was prepared in duplicate.

Table 2. Four different sowing dates by the growing of Amaranthus.

| Season | Dates of Sowing | 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

2.4 Flowering Time
Amaranth were nursed and watered daily. Application of insecticide was done if necessary. For each accession number, two plants were reserved for the flowering trait data and the mean value from two plants for each accession was further applied for the statistical analysis. The flowering time was noted as the number of days starting from the sowing date until the time of flowering. This was distinguished by the first emergence of the terminal inflorescence; as some species in amaranths such as A. blitum L. and A. graecizans, they showed the emergence of the auxiliary inflorescences prior to the emergence of terminal one (personal observation). The time of flowering was noted as days after sowing (d.a.s.).

2.5. Temperature and Photoperiod Data
During the experiment, air temperature (maximum-, minimum-values) were recorded in grad Celsius (°C) by a Thermo Recorder RS 11 (Tabai Espec Corp., Osaka, Japan) daily. The Tsukuba City is located in the valley of the Tsukuba Mountain, known as the second highest mountain in Japan. Thus, the temperature is warm, mild, and temperate; ranging from -3°C in winter and 30°C in summer. Precipitation is the highest in September, while the lowest is taken place in January, with an average of 167 and 37 mm, respectively.

The capital city of Ibaraki Prefecture: Mito City was taken as a reference for taking the data of day length (photoperiod) in the graph because it was the nearest one from Tsukuba and the distance is 38 km northwest from Tsukuba City. Tsukuba City was not containing in the reference list [14; in Japanese]. Mito City’s temperature is more or less similar, only that it has higher precipitation even in the driest month; with an average of 1,392 mm. The lowest and highest temperature are -2°C and 29°C, with an average of annual one is 13.7°C [13].
2.6. Statistical Analysis

For further statistical data analysis, the average flowering times noted within four seasons (season I, II, III, and IV) were subjected in the data analysis. The distribution of flowering time data was plotted using Sigma Plot version 11. Various statistical analysis such as: one-way ANOVA, t-test, and F-test as well as the PCA were performed by JMP version 7.0 (SAS Institute, Cary, NC, USA). PCA is a data reduction technique or to simplify the data matrix of \( p \) variables measured on \( n \) objects or samples. Typically, some pairs of variables will be highly correlated and the samples will have a larger variability, measured by the variance or Standard Deviation (SD), on some variables than others. The principle components are each linear function of all the original variables and summarized as:

\[
Y_{i1} = a_{11}X_{i1} + a_{12}X_{i2} + a_{13}X_{i3} + \ldots + a_{1p}X_{ip}
\]

(1)

\[
Y_{i2} = a_{21}X_{i1} + a_{22}X_{i2} + a_{23}X_{i3} + \ldots + a_{2p}X_{ip}
\]

(2)

The scores of the Principal Components (PCs) were used in the analysis to detect major characteristics responsible for the majority of the measured variation. In addition to that, there are four major components necessary in order to explain the data: i) component, ii) Eigenvalue, iii) proportion, and iv) cumulative. Further explanation of PCA can be referred in [12].

3. Results and Discussion

3.1. Photoperiodic Flowering Response of Amaranthus

There were three major findings resulted based on our experiment. First, the time of flowering in amaranths can be classified into three categories: i) early flowering accessions (0-60 d.a.s.); ii) middle flowering (61-80 d.a.s.); and iii) late flowering (flowering time later than 80 days). The majority of accessions (51%) was classified in the middle flowering time, while about 44% had been classified in the early group. Very few (0.05%) had flowered more than 80 d.a.s.; mostly represented by the weedy or vegetable types (table 1). Under the cool temperate condition such as in Japan, where a mean daily temperature was ranging from 19 to 24°C, we found a pronounced difference in terms of day length, compared to Indonesia. From season I to IV, day length was ranging from 11 to 14 hours were observed. The longest day length or solstice in Japan occurred in June, namely with a day length period 14 hours and 36 minutes (figure 1).

Second, we confirmed that amaranths were short day plants [6]; as this tendency was performed among the true-weed types, such as \( A. \) graecizans (USDA 17/ PI 608661/ IND) and \( A. \) retroflexus (US 23/ PI 607447/ JAM). They required only up to 20 and 35 d.a.s. in order to flower in all seasons and this is in accordance with previous result [2]. Furthermore, we also confirmed that most of the grain species required at least a day length less than 12 hours in order enable them to flower. Similar phenomena was also observed in sorghum, which could perform their flowering more rapidly if day length was decreased. Such early flowering under less than 12 hours might be resulted due to human selection process [3].

Contrasting tendency was observed among the vegetable amaranths. Those required more than 12 hours in order to promote their flowering. In adjacent to that, they also performed a slightly tendency similar to the long day plant type. Such unique flowering characteristic was mostly observed among the tropical origin amaranths such as \( A. \) tricolor \( L. \) and \( A. \) blitum \( L. \). This evidence was noted by the data being presented in winter (season III), in terms of flowering response among the tropical amaranths. They significantly delayed their flowering time as a result of cool temperature and day length remained less than 12 hours. This result also confirmed that a day length of 12-13 hours is essential for vegetable ones in order to induce their flowering. Thus, vegetable amaranths are classified as warm-loving plants because they required a longer time to flower on average under
winter condition rather than in warmer seasons (summer, spring) [6]. Similar tendency was also observed by the grain ones as a result of plant adaptation process.

Third, the Indonesian amaranths were able to adapt and performed their growth until maturity despite a distinct environmental condition from their origins in the tropics. Although, short-day and cooler temperature within season III promoted the extreme delay of flowering up to 16 days than their regular flowering observed in the majority of amaranths (overall mean= 88 d.a.s.). Furthermore, season or different sowing dates had also affected very significantly the variation of flowering time in *Amaranthus*; besides the day length and temperature based on the Analysis of Variance (ANOVA) (table 3).

**Table 3.** Two-way ANOVA calculation in elucidating the factors affecting the flowering time variation

| Source           | DF | Sum of Square (SS) | Mean Square (MS) | F Value | P > F |
|------------------|----|--------------------|------------------|---------|-------|
| Accession        | 68 | 127,664            | 1,877            | 48      | ***   |
| Season           | 3  | 109,681            | 36,560           | 935     | ***   |
| Accession * Season | 204 | 198,795           | 975              | 25      | ***   |

*Accession’ collection (N= 69)*

**Figure 1.** Number of flowered *Amaranthus* accessions from season I, II, III, and IV. The total 69 accessions were grown under different day-length condition and seasonal temperature due to different sowing dates

3.1.1. Flowering Time vs. Quality

As a result of plant adaptation including the domestication of crop plants, these have tremendously changed some of the plant developmental or physiological traits such as seed morphology, plant-architecture determination or the patterns of branches such as in maize and rice, seed dormancy,
germination properties, and flowering time. As we know, that most of wild or weedy annual plant species present a wide array of natural intraspecific variation in those mentioned traits. Thus, domestication may often have led to a tremendous reduction in regard to seed dormancy trait in many crop plants, as well as shortening or extending the flowering time [15].

As general major goal, many plant breeders may have altered the seasonal timing of flowering in order to enable them to produce novel varieties with better adaptation traits with particular local environments or under seasonal changing conditions. Moreover, the introduction of new crops mostly requires well-adapted genotypes and an ability to flower in the new environment as this would guarantee their further existence. Thus, timing of flowering is highly regarded as an important adaptive key as well as yield determinant. However, the yield itself is majorly dependant on the current environment and optimum crop management practices [18].

In cereal plants, including in grain amaranths, flowering should be initiated as early as possible in order to prolong the phase of corn-filling, to avoid difficult environmental conditions, which could endanger seed production or harvest (e.g. extreme heat or frost) or to escape pest attack. In adjacent to that, the maturation of grain amaranth would face some difficulties such as slowing the plant dry down if it is grown under long photoperiods of the temperate climate. Contrastingly, extension of the flowering might be promoted in many crops grown for biomass (e.g. maize, sugar cane) or for leafy parts in vegetable crops. This tendency is also applicable for the vegetable amaranths, where much more leaves are expected to be produced during the vegetative growth period. Therefore, studying the flowering time variation is applicable to select appropriate parental lines for future breeding purposes in amaranths and finally, leading to yield improvement [16].

3.2. Principle Component Analysis (PCA)

Principle Component Analysis (PCA) was effective to differentiate visually the amaranths’ types (grain, vegetable, and weedy); based on their photoperiodic flowering response in three-dimensional axis (figure 2.A, B, and C). The ellipsoid from each type of amaranth had different size of area and forms; obviously by the grain ones (figure 2.C). The weedy types showed the biggest area of ellipsoid and a quite broad variation exist – compared to the cultivated ones (figure 2.A). This can be perceived that a wider variation in terms of flowering period is exist within those types and thus, they are mostly proposed as the putative progenitors from the grain and vegetable ones (figure 2.B and C). In general, many wild or weedy plants possess a high degree of variation not only in morphology but also related to their tendency in order to enable them to be widely distributed and to better adapt with the current environment. Such distinct characters; namely the high diversity in terms of photoperiodic flowering response along with an ability to produce great quantities of small seeds at once attributed in amaranths, is very advantageous adaptive character for their rapid widespread all over the world [6].

The second biggest ellipsoid was presented by the vegetables ones, in which the form is similar but the total area size was much smaller than the first one. Thus, this can be concluded that vegetable ones resembled their weedy progenitors in terms of the requirement of day length and growing period in order to initiate flowering, namely in the range of 12-13 hours and 58-80 days after sowing, respectively. In adjacent to that, this also showed that vegetable types do not depend greatly upon human intervention for survival. A significant much smaller size of area and different form of ellipsoid was presented by the grain ones. It seems that the ellipsoid is much more exerted at the longitudinal z-axis. Such remarkable differences in terms of form, can be interpreted that a very intense artificial selection had been applied by the grain ones; probably in the past during the domestication process of the grain types. Based on our results, we can conclude that PCA was able to be applied as practical tool to group the accessions based on the response of flowering and an effective method to further select potential line in any breeding efforts.
Figure 2. The ellipsoid forms of the three amaranths’ types based on the calculation of PCA. The multivariate analysis was using the mean values of the photoperiodic flowering response. 

4. Conclusion

The flowering variation was exist in the studied material of amaranths. Thus, the variation within the accessions applied in this experiment seemed to suffice to be inclusively applied by the investigation of amaranths’ photoperiod flowering response. In general, the variation of its photoperiodic flowering response was relatively great; ranging from 30 to 140 days under the cool-temperate condition such as in Japan. Amaranth is confirmed to be a short-day plant, although the vegetable and weedy require more than 8-hours to promote flowering. Based on their flowering tendency, they can be classified into three groups: early (0-60 d.a.s), middle (61-80 d.a.s) and late flowering (more than 80 days). Moreover, PCA application was also useful in order to distinguish the response of flowering observed in each accession. A greater variation distinguished with a wide three-dimensional ellipsoid was formed by the PCA results delivered by the weedy amaranths or true weed types; if compared with the other two domesticated ones. Further research should be conducted in the area of molecular works or in terms of Quantitative Trait Loci (QTL) in order to uncover the (responsible) major-genes involved in determination of flowering in amaranths.

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Supplement 1. PC Component based on JMP vers. 7 analysis

| WEEDY | Principle Component Based on Covariances |
|-------|------------------------------------------|
| No.   | Eigenvalue | Percent | Cum Percent |
| 1     | 685.82     | 61.24   | 61.24       |
| 2     | 235.93     | 21.07   | 82.31       |
| 3     | 160.94     | 14.37   | 96.68       |
| 4     | 37.23      | 3.33    | 100.00      |

| GRAIN | Principle Component Based on Covariances |
|-------|------------------------------------------|
| No.   | Eigenvalue | Percent | Cum Percent |
| 1     | 2837.63    | 89.46   | 89.46       |
| 2     | 231.39     | 7.29    | 96.75       |
| 3     | 90.49      | 2.85    | 99.60       |
| 4     | 12.58      | 0.39    | 100.00      |

| VEG.  | Principle Component Based on Covariances |
|-------|------------------------------------------|
| No.   | Eigenvalue | Percent | Cum Percent |
| 1     | 1372.79    | 53.78   | 53.78       |
| 2     | 689.44     | 27.01   | 80.79       |
| 3     | 334.76     | 13.12   | 93.91       |
| 4     | 155.47     | 6.09    | 100.00      |