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Morphological characterization of Italian weedy rice (*Oryza sativa*) populations

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Summary

Weedy rice (*Oryza sativa*) is one of the most widespread and problematic weeds in rice cultivation; it spans the globe worldwide and can cause high yield losses. In 2008, seeds of 149 weedy rice populations were collected from the major Italian rice cultivation area. In 2009, these populations were sown in a single field to determine their morphological characteristics, including plant height, flag leaf attitude and length, panicle attitude and length, auricle and node colour, seed weight and size, awn length, and germination rates at 0, 10 and 30 days of after-ripening (DAR). Of the collected populations, approximately 56% were awned, 17% mucronate, and 27% awnless. The morphological characteristics among the awned populations varied widely and displayed the highest average lengths of flag leaf (27.6 cm) and seed (8 mm). Mucronate populations were mainly characterised by small seeds and low germination rates. Awnless populations showed higher germination rates at 30 DAR (20%) and wider seeds (3.7 mm). Awn length and distribution, seed length, 1000 seed weight and germination rates were the most important traits influencing the variability among populations. Awned populations are expected to adapt better to differing environmental and cropping conditions, because of their larger variability.

Keywords: red rice, diversity, rice, plant traits, seed traits, awned, awnless.

Introduction

In Italy, the rice production area is mainly in Piemonte and Lombardia regions, between the Alps and the Po river (N-W Italy). Smaller rice areas are present in the regions of Veneto, Emilia-Romagna, Toscana, Calabria and Sardegna (Tinarelli, 2007). In 2010, the total area devoted to rice in Italy was about 250000 ha (Ente Nazionale Risi, 2010), representing about 50% of the total European rice area. Weedy or red rice (*Oryza sativa* L.) is one of the major weeds infesting rice fields worldwide (Fischer & Ramirez, 1993; Arrieta-Espinoza *et al.*, 2005). It is present in about 40-75% of the total rice area in Europe (Ferrero, 2003) and in at least 70% of the total rice area in Italy (Vidotto & Ferrero, 2005). The control of this weed is problematic because of the close affinity with cultivated rice, in terms of morphological and physiological characteristics. This similarity has even led weedy rice to be classified as the same species as cultivated rice (*Oryza sativa* L.) (Vaughan *et al.*, 2001).

Weedy rice in Italy was first reported at the beginning of the 19th century (Biroli, 1807). After that period, weedy rice continued to spread, despite infestation control through transplanting the cultivated rice; this practice favoured better crop development so as to out-compete the weedy rice. Transplanting was abandoned in the 1960s, as a consequence of increased labour costs and the availability of the first herbicides. The return to direct seeding re-established weedy rice infestations. The introduction of less-competitive *indica* varieties and the use of commercial rice seed containing weedy rice seeds (Vidotto *et al.*, 2001) worsened the problem. These days, weedy rice is controlled through an integration of mechanical, chemical and agronomical means, that aim to reduce plant and seed densities and lessen their contributions to the weedy rice soil seed bank (Ferrero, 2003). Control is necessary because the weed can cause severe yield losses in rice production and can also affect rice milling and seed trade, reducing the value of marketed rice (Diarra *et al.*, 1985).

While the difficulty of discrimination between the crop and weed forms, especially at early growth stages, complicates control (Diarra *et al.*, 1985), there are some morphological traits, belonging both to the plant and to the seed, which allow for their distinction. The weedy rice plant is usually taller (from 40% to 57%) than cultivated rice and has a faster increase in height (Sánchez-Olguín *et al.*, 2007). It also has long, hispid, pale, droopy leaves and more culms, conferring it a more open canopy structure compared with cultivated rice (Kwon *et al.*, 1992; Delouche *et al.*, 2007).

Weedy rice seeds have a red-pigmented pericarp, due to the presence of anthocyanins, catechins and cathecolic tannins (Baldi, 1971). For this reason, it is often called red rice. The weedy rice panicle is usually longer, wider and more slender (20-30 cm), but generally produces fewer and lighter seeds compared with cultivated rice (Arrieta-Espinoza *et al.*, 2005; Delouche *et al.*, 2007; Shivrain *et al.*, 2010b). Likewise, some weedy rice populations often have awned seeds and more variable hull coloration compared with cultivated rice.

The biological cycle of weedy rice is generally longer than the crop, although some populations are able to flower earlier then the crop (Agostinetto *et al.*, 2001). Weedy rice seeds also have a peculiar trait, in that they shatter easily at maturity and have a variable degree of seed dormancy. These characteristics together contribute to seed dispersion in the environment and to persistence in the soil seed bank (Valverde, 2005).

Morphological traits are variably expressed in weedy rice and some of them have been used to identify different populations that can be distinguished in the field. Spikelet traits and hull colour are two such traits. Awnless strawhull populations are the most dominant group of weedy rice across the world and are genetically different from the awned blackhulls (Gealy *et al.*, 2002; Shivrain *et al.*, 2010a), which are less common (Delouche *et al.*, 2007). Wide phenotypic variations in many other plant and seed traits are found in weedy rice populations all over the world, such as plant height, tillering, panicle morphology, seed size, seed weight and phenology. In general, Italian weedy rice populations are mainly strawhull and characterised by large variability of different morphological traits. Differences among weedy rice populations were also found for some biological traits, such as seed germination (Fogliatto *et al.*, 2010). In spite of the strong infestation pressure and the relatively long history of weedy rice in Italian rice fields, limited information is available on morphological diversity among different populations.

The present study had several objectives: a) to assess the morphological variability of weedy rice and its distribution in the Italian rice area, b) to find underlying structures of the collected populations by identifying groups related to distinctive morphological traits, and c) to evaluate the impact of morphological variability on weedy rice management strategies and on the overall weediness of the species.

Materials and Methods

Seed collection

The study was carried out in Northwest Italy in a territory comprised of about 90% of the total Italian rice area. The territory was divided in three zones (Fig. 1): the north-west, characterized by heavy textured soils, rice water seeding and mono-cropping; the south-west, comprised of loamy soils, rice water seeding and mono-cropping; and east, covered in light-textured soils, often dry seeded with delayed flooding, and frequently rotated with maize, soybean, or wheat. Across the three zones, we chose a total of 40 areas (about 10x10 km quadrats). Within each area, environment, cultivation practices and weedy rice populations can be considered homogeneous.

Fig 1 near here

During weedy rice flowering in 2008 (mid-August), the fields displaying the most vigorous infestation and the highest morphological diversity were selected from each area for subsequent seed collection. During the latter half of August 2008, weedy rice plants from the selected fields were attributed to homogeneous groups, differentiated on the basis of a visual evaluation of the following morphological traits: plant height, node coloration, panicle attitude, hull coloration, awn presence and length. Each of these homogeneous groups was then designated as a "population." We then hand harvested all the seeds from ten different plants randomly selected within each population. From this process, a total of 149 different populations were ultimately identified and collected.

Field experiment

In spring 2009, all the weedy rice populations collected in 2008 were sown in a field usually cultivated with rice, but without a history of weedy rice. Before sowing, awns of weedy rice seeds were manually removed when present to facilitate mechanical sowing. Weedy and cultivated rice seeds were drilled into dry soil with a plot seeder (Wintersteiger - HEGE 90, series H090. 2005. 0310) on 2 May 2009. The 1x2 m plots were arranged according to a randomised complete block design with three replicates. Within each block, the plots were aligned in three adjacent rows. The field was flooded, starting after plant rooting and lasting up to maturation. Penoxsulam (Viper®, 20.4 g a.s. L^{-1}, OD, Dow Agrosciences B.V.) was applied at 40.8 g a.i. ha^{-1} when most of weedy rice populations were at growth stage BBCH 12-13 (2 to 3 leaves). Further flushes of emergence were controlled by hand-weeding. At the first visible signs of seed shattering, the seeds from each population were collected to assess germinability. Simultaneously, three panicles from each population were carefully cut, transferred into paper bags and then examined to evaluate the number of grains per panicle.

Morphological evaluations

All weedy rice populations and cultivated varieties were evaluated at the growth stages of stem elongation-booting, anthesis, milk development, dough development, ripening and after harvest. A total of 19 morphological traits (Table 1) were chosen, using a combination of the IRRI Standard Evaluation System for Rice (IRRI, 2002) and the UPOV Protocol for distinctness, uniformity and stability test for *O. sativa* (UPOV, 2004). Depending on the trait selected, the evaluation was carried out at specific growth stages and the results were expressed according to a continuous (e.g. plant height, flag leaf length), ordinal (e.g. attitude of flag leaf blade) or nominal scale (e.g. auricle colour, anthocyanin colouration of nodes). Awn length, whole and dehulled seed length and width were measured on 50 seeds per population via digital images acquired with a flatbed scanner. Seeds having an awn shorter than two millimetres were deemed mucronates. The images were analysed with the open source program ImageJ, version 1.41 (Abramoff *et al.*, 2004). The total number of seeds per panicle was estimated by counting pedicels per panicle and assuming that a seed was formed at each pedicel (Baker *et al.*, 1986).

Table 1 near here

Germination test

Seed germinability was assessed immediately after harvest (0 DAR), at ten days of after-ripening (10 DAR) and at 30 days of after-ripening (30 DAR). About 60 seeds per population were sterilised by soaking them in a 0.23% sodium hypochlorite solution for 3 minutes and were then rinsed with deionised water. For each population, three Petri dishes lined with a filter paper (Whatman no.1) were sowed with 20 seeds imbibed with 7 ml of deionised water. The Petri dishes were then incubated in a growth chamber at a constant 25 °C with alternating light/dark (16/8 h). Total seed germination was assessed after 14 days, as prescribed by the International Rules for Seed Testing (ISTA, 2009) for *O. sativa*.

Statistical Analyses

The collected populations were grouped on the basis of awn shape into awned, mucronate, and awnless. In previous studies on weedy rice morphological characterisation, results have often been presented after grouping the populations according to hull colouration (Arrieta-Espinoza *et al.*, 2005; Shivrain *et al.*, 2010b). In this study, we preferred to group the populations by awn character, as it showed more variability than hull coloration (more than 90% of populations were, in fact, strawhull). Moreover, classification of populations on the basis of awn shape permitted us to obtain groups of discrete size.

Continuous data (flag leaf length, plant height, panicle emission, awn length, whole and dehulled seed length and width, weight of 1000 seeds, number of seeds per panicle, panicle length, germination at 0, 10, and 30 DAR) were analysed using the GLM Analysis of Variance procedure of the statistical package SPSS (version 17), after assigning the weedy rice populations to one of three groups based on awn character. Mean comparison within each group was conducted by Tukey test at $P = 0.05$.

Continuous variables measured on all of the 149 populations formed the basis for calculation of the Principal Components, according to the Principal Component Analysis (PCA) method, using the Factor Analysis procedure in SPSS. Both quantitative and categorical data (auricle colour, flag leaf attitude, node colour, panicle emission, distribution of awns, panicle attitude of branches, panicle attitude in relation to stem, awn colouration and hull colouration) were grouped using the Two-step cluster analysis procedure. This analysis was applied to the dataset because of its ability to handle large datasets constituted of both continuous and categorical data, and to find the optimal number of clusters according to a specific algorithm. Two-step cluster procedures automatically standardise all the variables, a requirement when using variables expressed on different scales.

Results

Among the collected populations, about 56% were awned, 17% mucronate, and the remaining 27% were awnless. No remarkable proportional differences among the awned, mucronate, or awnless populations were found in the north-western, south-western and eastern zones. Among all the weedy rice plant and seed traits considered, germination at 30 DAR, the number of seeds per panicle, the flag leaf length and the percentage of panicle emission recorded during the late observation (Table 2) were the most variable among the three groups representing awn character.

Table 2 near here

Plant traits

Among the continuous variables, plant height averaged about 75 cm in mucronate populations, while awned and awnless did not differ relative to each other and were significantly taller. The flag leaf length differed significantly among the populations and averaged 28.5, 29.7 and 31.6 cm, in the awnless, mucronate and awned groups (Table 2), respectively. In terms of categorical variables, nodes and auricles were mainly green for all three groups. Purple nodes were observed in six awned populations. Purple auricles were found in 34 awned, but only two were found in the awnless populations. The attitude of the flag leaf blade was semi-erect in the majority of the populations of all three groups, at both early and late observations. Only 30% and 14% of the awned group populations showed horizontal or recurved flag leaves, respectively.

Seed traits

The mean 1000 seed weight of mucronate populations was 25.7 g and significantly lower than both awned and awnless populations, which averaged 27.5 g. The mean number of seeds per panicle was about 115 in the awnless populations and was significantly less in the awned and mucronate populations, which had about 130 seeds. Awned populations produced significantly longer whole seeds (8 mm) compared with the other groups; no differences were found in dehulled seeds among the weedy rice typologies. This could indicate thicker hulls in awned populations (at least in the apical palea-lemma apiculi and pedicellar ends), or a larger free space between hull and kernel in the same regions. Mucronate and awnless populations produced the narrowest and widest seeds, respectively. Awned seeds had an intermediate width, not significantly different from the other two populations.

Panicle emission initially proceeded slightly faster in awned and mucronate populations. In fact, at the first assessment in mid-July, panicles that emerged completely were observed in up to about 5% of the plants in these two populations and in only 1.3% of the awnless. At the second assessment, the highest percentages were recorded in mucronate populations (90%). Panicle length showed no significant differences among weedy rice groups, with average values of about 20 cm. Panicle branch attitude was semi-erect in awned populations; the erect attitude dominated in both the mucronate and awnless ones. Panicle attitude in relation to stem was more frequently semi-upright for mucronate populations, but upright for awned and awnless populations. Awned populations mainly showed awns distributed all along the panicle and the predominant colour was black, with some differences according to hull colouration. Black awns were present in all the brownhull and blackhull populations and in about 60% of the strawhull ones. The remaining 40% of the strawhull populations had straw awns. The mucronate seeds always displayed very short and black awns.

All weedy rice populations were highly dormant at harvest, reaching less than 2% of germinability after 14 days in Petri dishes. After ten days of after-ripening at room temperature, the germination was still low, ranging from 1.6% (mucronate) to 3.8% (awnless). At 30 DAR, awnless populations had germinability rates of about 20%, while awned and mucronate populations at 15% and 17% had lower rates).

Principal component analysis

The first two components calculated by PCA explained about 38% of the variation of the original variables, while the inclusion of the third component allowed for explanation of an additional 12% (Table 3). The first component explained 22.9% of the variation and was positively correlated mainly with 1000 seed weight, plant height, flag leaf length and whole seed length. It was also correlated, albeit to a lower degree, with whole seed width, dehulled seed length and awn length. A negative correlation was found with panicle emission. The second component accounted for 15.3% of the variation and had the following characteristics: high positive correlation with germination at 0, 10, and 30 DAR, lower positive correlation with whole seed width and negative correlation with awn length.

Table 3 near here

Representing all the populations in a bi-dimensional space of the first two components, it was not possible to identify well-separated groups of populations, even though the awnless and mucronate populations were less dispersed than the awned ones and were concentrated around slightly negative values of the first component (Fig. 2). Awned populations were largely spread from negative to positive values of both components. Awned populations were generally characterised by values of 1000 seed weight, plant height, flag leaf length and whole seed length, all at levels higher than mucronate and awnless ones. This was true of late panicle emission, as well. All ordination behaviours were in agreement with those observed in the ANOVA analysis.

Figs 2 near here

The higher dispersion of the awned populations along the second component indicated a higher variability of traits associated with germinability behaviour during the first 30 days of after-ripening. When the populations were labelled on the basis of hull colouration, brownhull ones were mostly located towards positive values of the first component (data not shown). However, it should be noted that the number of brownhull populations was considerably lower than strawhull ones, making comparisons and generalisations difficult.

Two-step cluster analysis

Two-step cluster analysis was performed on both continuous and categorical variables, from which two clusters were identified based on Schwarz's Bayesian Criterion (BIC). Comparing the mean of each variable in the cluster to the overall mean, the analysis showed the quantitative variables contributed most to cluster 1 formation in descending order (Fig. 3). Among all these variables, only awn length and germination at 0 and 10 DAR play a significant role in defining this cluster. Overall, cluster 1 was composed of awned populations having long awns (about 28 mm), delayed panicle emission, long seeds (8.0 mm) and low germination (less than 1.6% at 10 DAR) (Table 4). The morphological traits showing the largest variation in both clusters were panicle emission, germination at 30 DAR and the number of seeds per panicle.

Fig 3 near here

Table 4 near here

Cluster 2 was associated with awn length, whole seed-length and 1000 seed weight. Panicle emission (late observation) was barely significant (Fig. 4). Cluster 2 was mainly characterised by awnless populations with short awns (4.3 mm), short seeds (7.7 mm) and low 1000 seed weight (26.7 g) (Table 4). Cluster analysis indicated that some variables, such as 1000 seeds, awn length and whole seed length, were important both for cluster formation and for definition of the principal components.

Fig 4 near here

Cluster 1 included blackhull and brownhull populations and about 45% of strawhull ones. Moreover, the same cluster included all the populations with purple nodes and straw coloration of the awns (Data not shown). The categorical variables that contributed most to the formation of both clusters were awn distribution, auricle and awn colouration and panicle attitude (Fig. 5).

Fig 5 near here

Discussion

The populations collected in the Italian rice area were differentiated by awn character. For this reason, the comparisons among morphological traits were performed after grouping the populations into awned, mucronate and awnless. As seen in the literature (Sánchez-Olguín *et al.*, 2007), weedy rice plants in this study were taller than the main rice varieties cultivated in Italy. Plant height is one of the traits conferring competitive advantage to cultivated rice (Kwon *et al.*, 1992) and is usually correlated with other growth parameters, such as flag leaf length. Taller weedy rice plants are able to capture more light and shade their neighbouring rice plants, particularly when infestation levels are high (Shivrain *et al.*, 2010b).

Concomitantly, taller weedy rice plants could be more easily identified and better controlled during the post-emergence period. Some control means, such as cutting bars, sponge or rope bars wetted with herbicides, can be effectively applied because of the difference in crop and weed plant height. Even though such interventions are often performed too late to avoid yield losses from competition, they can help to reduce seed shattering and prevent an increase in the soil seed bank (Ferrero & Vidotto, 1999). At the same time, plants that mimic the crop, especially in terms of height, are difficult to locate and consequently have a better chance to reach maturity and then shatter on the soil surface. Mucronate populations were significantly shorter than awned and awnless ones, with values not dissimilar to those of some cultivated rice varieties.

Mucronate population seed sizes were smaller in length and width as compared with the others; as a consequence, seed weight was also lower compared with awnless and awned populations. However, mucronates showed a high seed density per panicle and earlier panicle emission, which may compensate for small seed size. Smaller seeds, in general, have fewer stored energy reserves and hence a lower germination and seedling production that reduces population survival chances (Baskin & Baskin, 1998). Germination percentages of mucronate populations at 30 days of after-ripening were, in fact, lower than for the awnless populations. Awned populations showed levels of germination similar to those of mucronate ones, despite a greater seed size, probably due to stronger dormancy. Previous studies have reported that seed dormancy is associated with the presence of awns (Gu *et al.*, 2005). In the present study, awned populations showed possibly thicker hulls or larger free space between hull and kernel, which might have contributed to the higher dormancy of awned seeds through slowed water absorption and inhibitor release during germination.

Principal component analysis suggested that several characteristics played an important role in explaining part of the variability among weedy rice populations. In particular, 1000 seed weight, plant height, flag leaf length, whole seed-length, awn length and germination were the traits most correlated with the two extracted components. Awned populations were largely scattered in the two-component space; this implies a greater diversity in the morphological characteristics compared with awnless and mucronate populations. Other weedy rice morphological studies agree partially with our results, indicating that diversity among populations is affected by plant height, 1000 seed weight and seed size (Arrieta-Espinoza *et al.*, 2005; Zainudin *et al.*, 2010). Cluster analysis supported the results of the PCA, as awn length and distribution, seed length, 1000 seed weight and germination were the factors associated with the division into two clusters.

In general, the most relevant differentiation among populations that was highlighted by the morphological study was the trait variability among awned, mucronate, and awnless plants . In particular, the awned populations showed the greater diversity in traits that can impact species' weediness. We found large variability for two major areas: traits related to crop competitiveness (overall plant size including plant height, flag leaf length, etc.), and germination pattern characteristics known to influence seed bank dynamics and infestation evolution. From an evolutionary point of view, awned populations would be favoured under different environmental and cropping systems, being able to adapt more easily to conditions in which cultivation practices change from year to year.

Despite the fact that the entire Italian rice area is under mono-cropping cultivation with a standard irrigation system, different environmental conditions (in particular, pedological and climatic conditions) and different management operations are represented. Many practices can impact rice weed dynamics in a major way: water management, soil tillage, seeding system (broadcast in flooded field or drilled in dry sol), varietal choice, mineral and organic fertilisation and weed management (adoption of stale seed bed, use of pre-emergence or post-emergence herbicides, herbicide choice, and so forth). Some of these practices vary greatly among farms, but some can even vary within a single farm. This variability might be one of the reasons that awned populations were more prevalent across the rice growing area.

The high biological diversity found in awned populations implies a greater adaptability to variable environments and management practices and, as a consequence, the control of these populations could be more challenging. According to this study, awned populations are usually dormant, with tall plants able to produce many seeds. Since these traits influence weedy rice population dynamics and eventually the competitiveness of this weed, management practices have necessarily to take them into account and be adapted accordingly. In this context, practices such as tillage delayed to spring, associated with stale seed bed cultivations, can favour dormancy release and seed bank depletion and may therefore be successfully adopted for controlling awned populations.

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Figure legends

Fig. 1 Rice territory included in the study, divided in east, north-west and south-west zones. Each zone is divided into 10x10 km areas.

Fig. 2 Distribution of awned (+), mucronate (●), and awnless (Δ) populations on the first and second Principal Component.

Fig. 3 Quantitative variables that mainly contributed to the formation of cluster 1. Histograms represent the t Student values of each variable, while dashed lines refer to the significant critical values.

Fig. 4 Quantitative variables that mainly contributed to the formation of cluster 2. Histograms represent the t Student values of each variable, while dashed lines refer to the significant critical values.

Fig. 5 Categorical variables that mainly contributed to the formation of cluster 1 and 2. Histograms represent the Chi Square values of each variable, while dashed lines refer to the significant critical values.

Table 1 Morphological traits used to evaluate weedy rice populations

Morphological traits	Growth stage[1]	Description
Auricle colour	30-40	1. light green; 2. purple
Flag leaf: attitude of blade (early observation)	60	1. erect; 3. semi-erect; 5. horizontal; 7. recurved
Flag leaf: attitude of blade (late observation)	90	1. erect; 3. semi-erect; 5. horizontal; 7. recurved
Flag leaf: length	60	measured (cm)
Stem: anthocyanin colouration of nodes	70	1. absent; 9. present
Plant height (stem length)	70	measured from the base of the stem to the last node, excluding the panicle (cm)
Panicle emission (early observation)	70	% of plants with panicle completely emerged on the total plants present in the plot
Panicle emission (late observation)	80	% of plants with panicle completely emerged on the total plants present in the plot
Panicle: distribution of awns	70-80	1. tip only; 2. ¼ upper only; 3. upper half only; 4. ¾ of the total length; 5. whole length
Panicle: attitude of branches	90	1. erect; 3. semi-erect; 5. spreading
Panicle: attitude in relation to stem	90	1. upright; 2. semi-upright; 3.slightly drooping; 4. strongly drooping
Seed: awn length	90	measured on about 50 seeds (mm)
Seed: awn colouration	90	1. straw; 2. black; 3. brown
Seed: hull colouration	90	1. straw; 2. black; 3. brown
Whole seed: length	90	measured on about 50 seeds (mm)
Whole seed: width	90	measured on about 50 seeds (mm)
Dehulled seeds: length	90	measured on about 50 seeds (mm)
Dehulled seeds: width	90	measured on about 50 seeds (mm)
Seeds: weight of 1000 seeds	90	measured (g)
Number of seeds per panicle	90	counted on three panicles per population
Panicle length	90	measured on three panicles per population (cm)

[1]Growth stage according to BBCH scale (Bleiholder *et al.*, 1997).

Table 2 Plant and seed characteristics of weedy rice populations grouped according to awn character (awned, mucronate and awnless)

Continuous variable	**Awned**	**SE[2]**	**Mucronate**	**SE**	**Awnless**	**SE**
Germination 0 DAR[1] (%)	0.8 a	0.35	0.6 a	0.27	2.2 a	1.08
Germination 10 DAR (%)	2.1 a	0.47	1.6 a	0.56	3.8 a	1.40
Germination 30 DAR (%)	15.0 a	1.67	17.3 a	3.46	20.1 b	2.92
1000 seed weight (g)	27.5 b	0.10	25.7 a	0.06	27.5 b	0.07
Seeds per panicle (number)	129.8 b	2.43	129.7 b	2.29	115.6 a	3.26
Whole seed-length (mm)	8.0 b	0.06	7.6 a	0.06	7.6 a	0.05
Whole seed-width (mm)	3.6 ab	0.02	3.5 a	0.02	3.7 b	0.02
Dehulled seed-length (mm)	6.1 a	0.06	6.0 a	0.08	6.0 a	0.06
Dehulled seed-width (mm)	3.0 ab	0.02	2.9 a	0.04	3.1 b	0.03
Panicle length (cm)	20.2 a	0.17	20.5 a	0.25	20.5 a	0.22
Panicle emission (%)-early observation	5.2 b	0.83	3.0 ab	0.86	1.3 a	0.68
Panicle emission (%)-late observation	72.0 a	3.28	90.7 b	2.59	77.5 ab	2.96
Awn length (mm)	27.6 b	1.28	4.9 a	1.55	0.0 a	-
Plant height (cm)	82.2 b	0.25	75.7 a	0.35	82.3 b	0.33
Flag leaf length (cm)	31.6 c	0.23	29.7 b	0.34	28.5 a	0.28

[1]DAR: Days of after-ripening; [2]SE: Standard Error. Values sharing the same letter are not significantly different according to Tukey's test ($P \leq 0.05$). Comparisons were made among awn character groups within each variable.

Table 3 Rotated component matrix of the first two principal components and the respective loadings

Variables	1st component (22.9%)[1]	2nd component (15.3%)
1000 seed weight	0.745	0.111
Panicle emission-early observation	-0.081	0.084
Panicle emission-late observation	-0.358	0.221
Plant height	0.657	0.068
Flag leaf length	0.641	0.045
Whole seed-length	0.873	-0.011
Whole seed-width	0.313	0.290
Dehulled seed-length (mm)	0.441	-0.180
Dehulled seed-width (mm)	-0.006	0.057
Awn length	0.580	-0.258
Germination at 0 DAR	-0.037	0.820
Germination at10 DAR	0.036	0.913
Germination at 30 DAR	0.080	0.713
Seeds per panicle	0.003	-0.043
Panicle length	0.031	0.013

[1]Values in brackets represent the percentage of the variance explained by each component.

Table 4 Mean of each variable associated with Clusters 1 and 2 identified in Cluster Analysis

Variables	**Cluster 1** mean	**Cluster 2** mean
1000 seed weight (g)	27.7	26.7
Panicle emission (%) early	3.9	3.2
Panicle emission (%) late	69.3	82.6
Plant height (cm)	82.4	79.9
Flag leaf length (cm)	31.6	29.5
Whole seed length (mm)	8.0	7.7
Whole seed width (mm)	3.6	3.6
Dehulled seed length (mm)	6.1	6.0
Dehulled seed width (mm)	3.0	3.1
Awn length (mm)	28.4	4.3
Germination at 0 DAR (%)	0.6	1.6
Germination at 10 DAR (%)	1.6	4.1
Germination at 30 DAR (%)	13.9	20.6
Seeds per panicle	128.0	122.7
Panicle length (cm)	20.2	20.4

Fig. 1

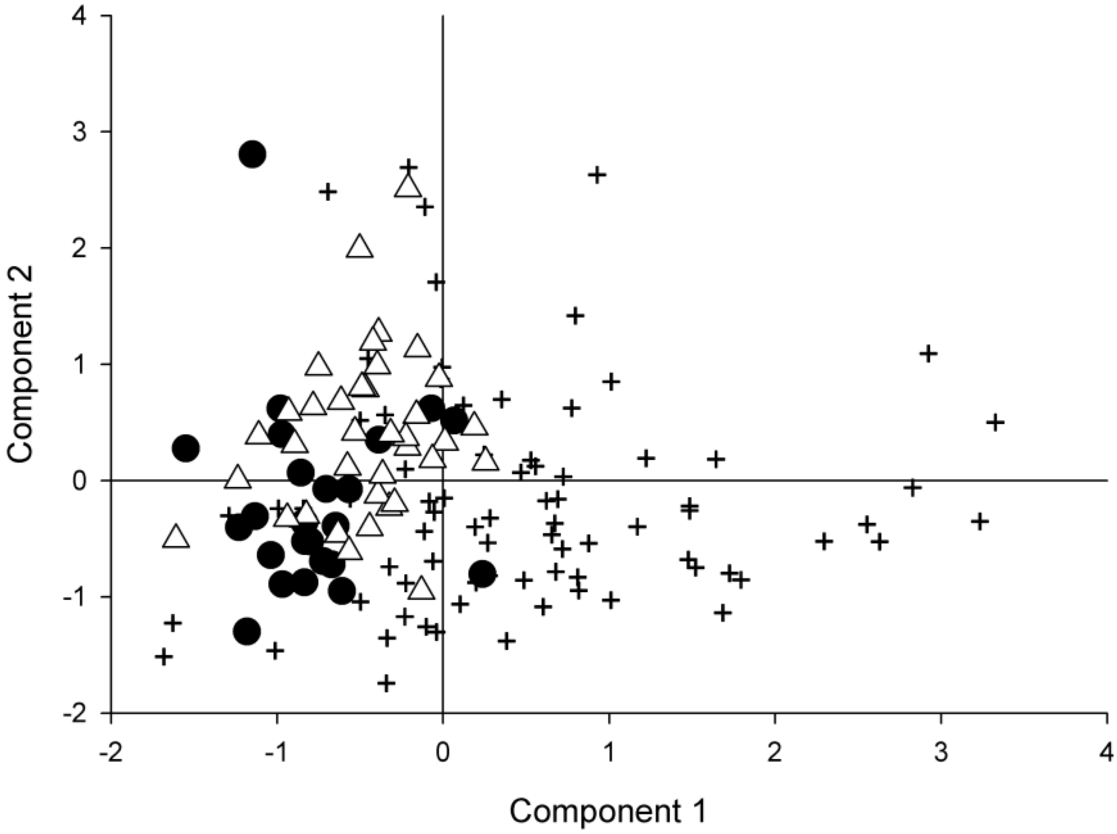
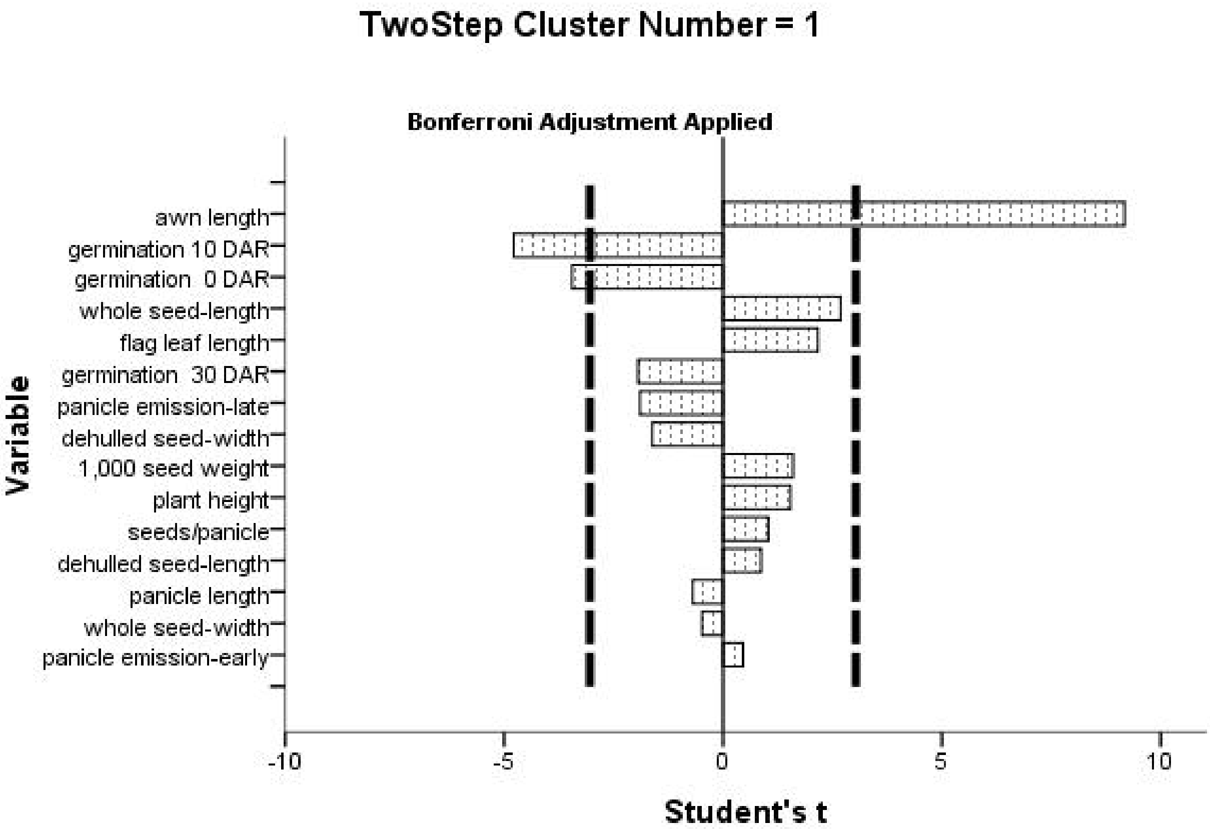


Fig. 2



TwoStep Cluster Number = 1
Bonferroni Adjustment Applied
Variable
awn length
germination 10 DAR
germination 0 DAR
whole seed-length
flag leaf length
germination 30 DAR
panicle emission-late
dehulled seed-width
1,000 seed weight
plant height
seeds/panicle
dehulled seed-length
panicle length
whole seed-width
panicle emission-early
-10
-5
0
5
10
Student's t

Fig. 3

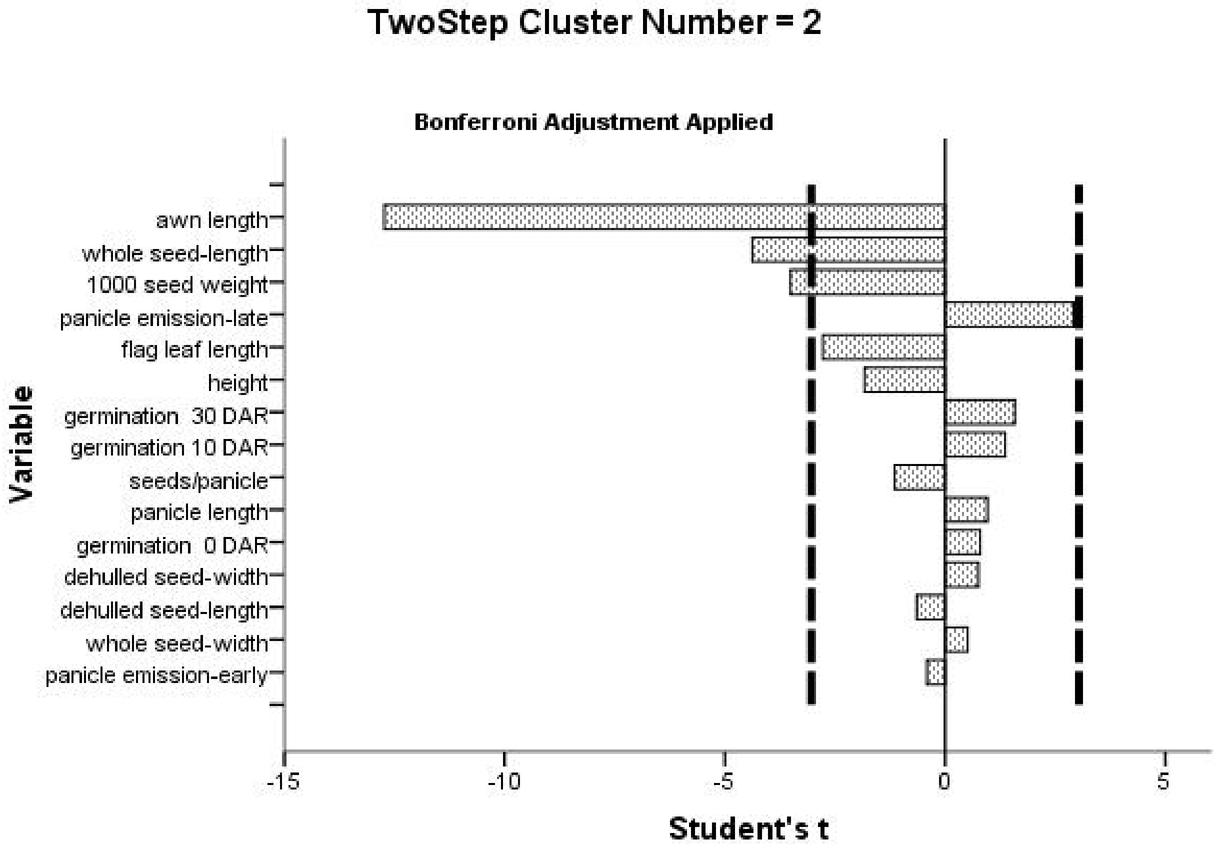

TwoStep Cluster Number = 2
Bonferroni Adjustment Applied
awn length
whole seed-length
1000 seed weight
panicle emission-late
flag leaf length
height
germination 30 DAR
germination 10 DAR
seeds/panicle
panicle length
germination 0 DAR
dehulled seed-width
dehulled seed-length
whole seed-width
panicle emission-early
Variable
-15
-10
-5
0
5
Student's t

Fig. 4

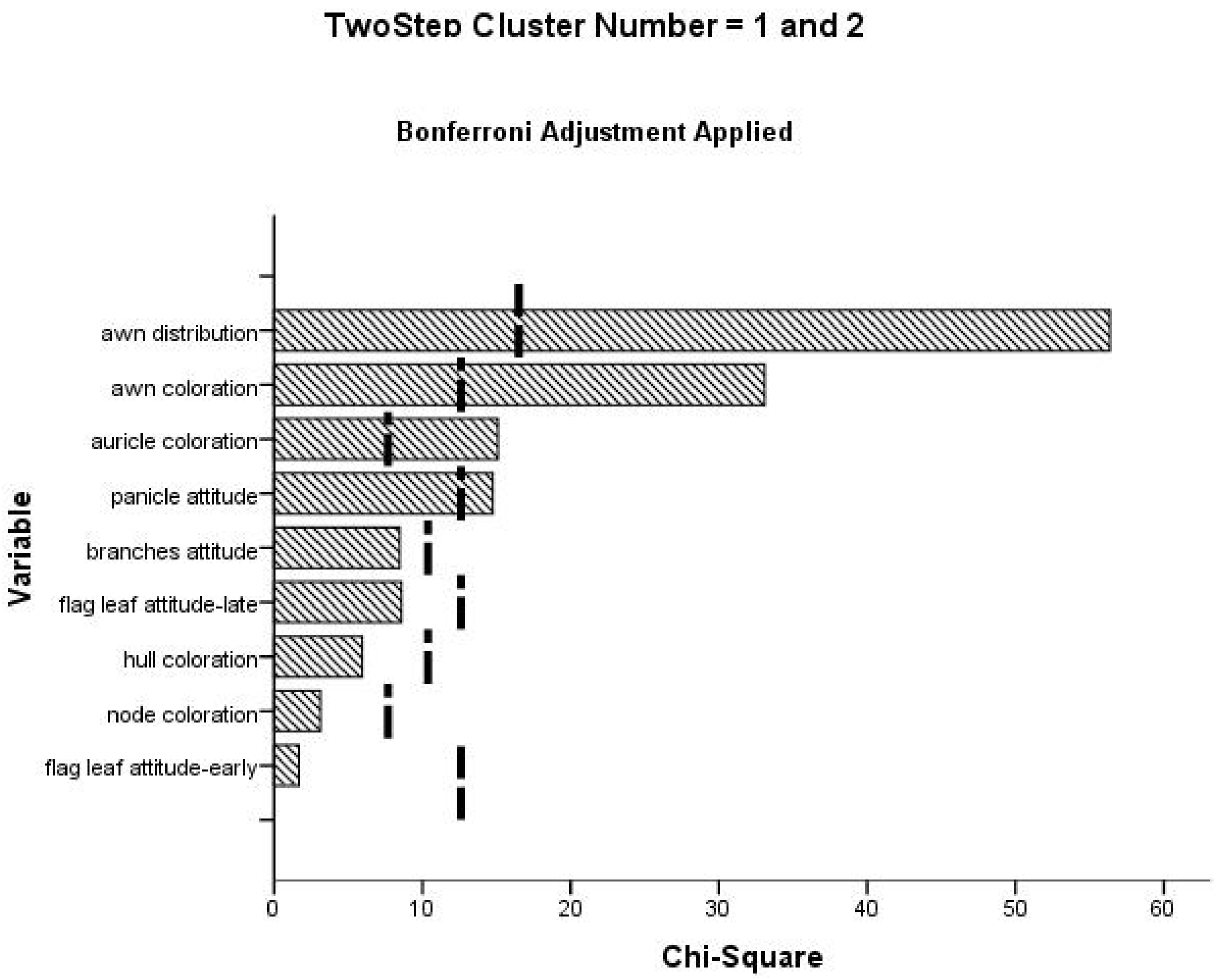

TwoStep Cluster Number = 1 and 2
Bonferroni Adjustment Applied
Variable
awn distribution
awn coloration
auricle coloration
panicle attitude
branches attitude
flag leaf attitude-late
hull coloration
node coloration
flag leaf attitude-early
0
10
20
30
40
50
60
Chi-Square

Fig. 5