Morphological and biochemical diversity among ecotypes of redroot pigweed (Amaranthus retroflexus L.) and lamb's quarters (Chenopodium album L.)

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Author contribution statement

Shiva Hamidzadeh Moghadam performed the experiments and data collection, data analysis, and figure preparation, writing of the manuscript. Mohammad Taghi Alebrahim conceived the original idea and formulated the research plan, oversaw the research and writing of the manuscript. Ahmad Tobeh, Mehdi Mohebodini, Danièle Werck-Reichhart and Dana MacGregor contributed to data analysis and writing of the manuscript.

Keywords

Cluster analysis, Morphological and biochemical traits, obnoxious weeds, principle component analysis, Climate classification

Abstract

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Amaranthus retroflexus L. and Chenopodium album L. are obnoxious weeds that have a cosmopolitan distribution. These species successfully invade and are adapted to a wide array of habitats. In this paper we evaluated various morphological and biochemical properties of 16 ecotypes of A. retroflexus L. and 17 ecotypes of C. album L. collected in 2016-2017 from Spain, France and Iran. These seeds were grown together at the experimental field of the agriculture research of University of Mohaghegh Ardabili in 2018 and morphological traits and biochemical traits were assessed. Significant differences were observed for all of the morphological and biochemical characteristics measured among the ecotypes of A. retroflexus L. and of C. album L. The maximum coefficient of variation value was recorded for number of branches for A. retroflexus L. (12.22) and inflorescence length (14.34) for C. album L. Principle component analysis of these data identified four principal components for each species that explained 88.22 (A. retroflexus L.) and 89.01 (C. album L.) of the total variation. The dendrogram generated, based on unweighted neighbor-joining method, clustered all the A. retroflexus L. and C. album L. into two main clusters and four sub-clusters. This analysis revealed no separate groups among ecotypes along climate classification suggesting high levels of morphological and biochemical diversity among them. Canonical correlation analysis was used to evaluate relationships between climate classification and traits. Measured characteristics among ecotypes also did not group along Köppen climate classification. The high diversity of biochemical compounds measured in them indicates ecotypes of A. retroflexus L. and C. album L. can use different metabolic programmes in response to environmental conditions and these changes can be manifested in subsequent generation of plants. Several of the biochemical constituents identified in our study could serve as effective indices for indirect selection of stresses resistance/tolerance of A. retroflexus L. and C. album L. The diversity of the morphological and biochemical traits observed among these ecotypes illustrates the role that the environment and genetics play in shaping the biology of these plants and demonstrate how plastic and adaptable these species can be.

Contribution to the field

Amaranthus retroflexus L. and Chenopodium album L. are the most costly category of agricultural pests. Worldwide, these weeds cause more yield loss and add more to farmers production costs. They are examples of nature struggling to bring about ecological succession, plants that are especially successful at colonizing disturbed, but potentially productive sites, and at maintaining their abundance under conditions of repeated disturbance. In addition, create unexpectedly severe problems when these plants grow amok in new habitats in the absence of their natural checks and balances. Although they appear to degrade many natural ecosystems, quantitative measures of their impact on those systems are relatively rare. Information needed to establish priorities for the control of weeds in natural ecosystems include determination of the morphological and biochemical diversity for examination of weed invasion mechanisms, the ecological impact on the weeds and provide information to guide crop improvement through new mechanisms.

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Morphological and biochemical diversity among ecotypes of redroot pigweed (*Amaranthus retroflexus* L.) and lamb's quarters (*Chenopodium album* L.)

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Keywords: cluster analysis, climate classification, morphological and biochemical traits, obnoxious weeds, principle component analysis

Abstract

Amaranthus retroflexus L. and *Chenopodium album* L. are obnoxious weeds that have a cosmopolitan distribution. These species successfully invade and are adapted to a wide array of habitats. In this paper we evaluated various morphological and biochemical properties of 16 ecotypes of *A. retroflexus* L. and 17 ecotypes of *C. album* L. collected in 2016-2017 from Spain, France and Iran. These seeds were grown together at the experimental field of the agriculture research of University of Mohaghegh Ardabili in 2018 and morphological traits and biochemical traits were assessed. Significant differences were observed for all of the morphological and biochemical characteristics measured among the ecotypes of *A. retroflexus* L. and of *C. album* L. The maximum coefficient of variation value was recorded for number of branches for *A. retroflexus* L. (12.22) and inflorescence length (14.34) for *C. album* L. Principle component analysis of these data identified four principal components for each species that explained 88.22 (*A. retroflexus* L.) and 89.01 (*C. album* L.) of the total variation. The dendrogram generated, based on unweighted neighbor-joining method, clustered all the *A. retroflexus* L. and *C. album* L. into two main clusters and four sub-clusters. This analysis revealed no separate groups among ecotypes along climate classification suggesting high levels of morphological and biochemical diversity among them. Canonical correlation analysis was used to evaluate relationships between climate classification and traits. Measured characteristics among ecotypes also did not group along Köppen climate classification. The high diversity of biochemical compounds measured in them indicates ecotypes of *A. retroflexus* L. and *C. album* L. can use different metabolic programmes in response to environmental conditions and these changes can be manifested in subsequent generation of plants. Several of the biochemical constituents identified in our study could serve as effective indices for indirect selection of stresses resistance/tolerance of *A. retroflexus* L. and *C. album* L. The diversity of the morphological and biochemical traits observed among these ecotypes illustrates the role that the environment and genetics play in shaping the biology of these plants and demonstrate how plastic and adaptable these species can be.

1 Introduction

Amaranthus retroflexus L. (redroot pigweed) and *Chenopodium album* L. (lamb's quarters) are fast-growing weedy annual plants that belong to the *Amaranthaceae* family. They are both listed among the most common dicotyledonous weeds in the world and are widely distributed in many agricultural areas (Horak and Loughin, 2000; Alebrahim et al., 2012) where they cause significant problems. They severely reduce the yield of several crops, moreover, their powerful destructive growth and allelopathic activity make them very competitive and result in significantly decrease crop yield and quality (Ma et al., 2015; Bajwa et al., 2019).

A. retroflexus is is a C_4 plant (Baskin and Baskin, 1978) considered to be native to North America, but it now is distributed worldwide (Frankton and Mulligan, 1987). Where it has been introduced, this annual weed can be found as a casual weed on cultivated land and in waste places such as rubbish tips (Clapham et al., 1987; Stace, 1997; Bond et al., 2007). It grows best at higher temperatures, light intensities and nitrogen levels (Costea et al., 2003). *A. retroflexus* is reported to have a negative influence on row crops, such as sugar beet (Brimhall et al., 1967), soybean (Dieleman et al., 1995), potato (Weaver, 1991), cotton (Buchanan et al., 1980), and corn (Kenzevic et al., 1995).

C. album is native to Western Asia (Poonia and Upadhayay, 2015) but even in the early 1950s was considered to be one of the five most widely distributed plants in the world (Williams, 1963). *C. album* have been reported to grow as weed in fields of wheat, barley, mustard, gram and other crops (Sarabi et al., 2013; Jabran et al., 2017). This weed is low growing while the cultivated plants in which it grows are frequently tall and leafy (Bhattacharjee, 2001).

Both species interfere with human land use as they are successful colonizers and have considerable impact on plant growth (Garbari and Pedulla, 2001). They are adapted to highly unstable and unpredictable environments, as they can compete with other plants for nutrients, water, light, and space through different survival tactics, and can be harbor crop for pests or diseases (Rodenburg et al., 2010).The number of herbicides that can be used to control them ,.is limited and the herbicides not very efficient (Alebrahim et al2011). Learning more about . their diversity in different geographical locations is necessary to design and employ effective management practices.

The ability of plants to vary their morphological traits has long been recognized as a beneficial survival strategy that enables plants to acclimatize to changing habitats (Gambino and Vilela, 2011). Postembryonic development is unique to plants and enables plants to incorporate information from the environment into decisions about their morphology. Root (MacGregor et al., 2008) and shoot (Teichmann and Muhr, 2015) architecture can vary dramatically in response to different environmental conditions and changesin morphology are often connected to the conditions under which the plant is growing (Mandák et al., 2011) . Hence, the same species of plant can occupy and be maintained in diverse habitats by appropriately adjusting plant morphology (Urbas and Zobel, 2000).

Morphological parameters are one of the most critical factors for taxonomic classification (Jannatabadi et al., 2014). Plant breeders mostly use morphological markers for evaluation of preferable genetic material as they are simple and cost-effective (Geleta et al., 2006). Also, morphological parameters are sensitive to phenotypic plasticity and allow the evaluation of diversity in the environmental instabilities (Mondini et al., 2009). Analysis of morphological and biochemical traits of various ecotypes has been used to explain the level of genetic diversity and population genetic structure for management and evolution of conservation strategies (Thompson et al., 1999).

Although the traditional approach to study weeds is to examine their control or management (Rodenburg et al., 2010), the main goal of weed management is to understand their capacity to be invasive. Therefore, we investigate here several populations of *A. retroflexus* and *C. album*

collected from contrasting habitats. This collection was examined for morphological and biochemical variations in order to understand the strategies that have enabled their successful invasion into a wide range of habitats by providing a selective advantage for competitiveness these varied environments.

2 Method and materials

2.1 Plant materials

In order to investigate the morphological and biochemical characteristics of these weeds, seeds of 16 *A. retroflexus* and 17 *C. album* populations were collected in 2016 and 2017 from different provinces of Iran, Spain and France **(Table 1)**. The seeds provided by Research Institute of Forests and Rangelands (RIFR) were cultivated at the experimental field of the agriculture research of University of Mohaghegh Ardabili **(Figure 1)**.
To assess the morphological and biochemical traits, three replicates of 5 seedlings per replicate were planted outdoors at the experimental field of the agriculture research of University of Mohaghegh Ardabili during the summer of 2018. Seeds were planted at distance of 20 cm in row and 30 cm between rows. At the end of the growing season, eleven morphological traits were evaluated on ten randomly selected plants: plant height (PH), inflorescence length (FL), leaf length (LL), leaf width (LW), leaf area (LA), number of leaves (LN) number of branches (BN), diameter of stem (SD), fresh weight (FW), dry weight (DW) and seed weight (SW). For the analyses of some of the biochemical parameters: chlorophyll a (Ca), chlorophyll b (Cb), total chlorophyll (TC), carotenoid (Car) and total protein content (TP), catalase (CAT), peroxides (POD) and polyphenol oxidase (PPO), the fresh leaf samples were collected and stored at -70°C until analyses.

2.2 Determination of leaf photosynthetic pigments

To determine leaf photosynthetic pigment content, approximately 0.25 g of fresh plant leaf sample was homogenized in 5 ml 80% acetone. Homogenates were centrifuge at 10,000 rpm for 15 min at 4°C and 0.25 ml of the clarified supernatant was mixed with 2.5 ml of 80% acetone. The absorbance of acetone extracts was measured at 662 nm, 645 nm and 470 nm for determination of chlorophyll a, chlorophyll b and carotenoids content using a spectrophotometer. The leaf photosynthetic pigments were expressed as mg g^{-1} on fresh weight basis using the formula listed below (Lichtenthaler and Wellburn, 1983).

$$Ca = 12.25\,A - 2.798\,A646.8$$
$$Cb = 21.50\,A646.8 - 5.10\,A663.2$$
$$TC = Ca + Cb$$
$$Car = (1000\,A470 - 1.82\,Ca - 85.02Cb)/198$$

2.3 Determination of Protein Content

Total protein content was measured using the method of Bradford (Bradford, 1976) using bovine serum albumin standard (BSA) as a standard. Protein concentrations were measured using a NanoDrop spectrophotometer (Thermo One C., Termo scientific, Inc., USA) at 595 nm.

2.4 Extraction of antioxidant enzymes

To extract proteins for antioxidant enzyme analysis, 200 mg of leaf samples were flash-frozen in liquid nitrogen and homogenized in 10 ml of Tris-HCl buffer (pH 7.5, 0.1 M). The

homogenate was centrifuged at 13000 rpm for 15 min at 4℃ and supernatants collected to determine catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) activities using established protocols described in Sudhakar et al. (2001).

2.5 Determination of enzymatic activities

To determine CAT activity (EC 1.11.1.6), the method described by Chance and Maehly (1995) was used with the following modifications. Degradation of H_2O_2 in a reaction medium containing 300 µM tris buffer (pH 7.5), 100 µM H_2O_2 and 1 ml of plant extract mixed in an ice bath was monitored at 240 nm for 2 min. The same reaction medium free of plant extract was used as a blank.

The activity of PPO (EC 1.10.3.1) was determined according to Kar and Mishra (1976) with minor modifications. The reaction medium consisted of the same assay mixture as that of peroxidase without H_20_2 and was incubated at 25℃. Readings were taken at 560. Enzymatic activities were expressed in absorbancy units (Unit mg^{-1} protein min^{-1}).

The activity of POD (EC 1.11.1.7) was determined by reading absorbance at 420 nm according to Kar and Mishra (1976) with minor modifications. The reaction consisted of 125 µM tris buffer (pH 7.5), 50 µM pyrogallol, 50 µM H_2O_2 and 1 ml of the total plant extract incubated for 5 min at 25℃. As a control, the same reaction medium was incubated in the absence of plant extract under the same conditions.

2.6 Statistical Analysis

ANOVA tests were performed for each morphological and biochemical parameter using SAS package (9.3 SAS Institute, Inc., USA). The simple correlation coefficient among the studied variables using the Pearson's correlation coefficient method and principal component analysis were made using the SPSS software (22, SPSS, Inc, Chicago, IL, USA). Unweighted pair-group method of arithmetic averages method (UPGMA) were performed using SPSS 16 to determine the individual relationship among ecotype by adopting the Ward method based on Squared Euclidean distance and to determine the best cut-off point of the dendrogram, a canonical discriminant function analysis (Manly 2005). CCA (canonical correlation analysis) was used to evaluate relationships between Köppen climate classification and morphological and biochemical traits by PROC CANCORR procedure of SAS program version 9.3.

3 Results

3.1 Morphological traits

To determine if the ecotypes of *A. retroflexus* and *C. album* exhibited different morphological traits, plant height (PH), inflorescence length (FL), leaf length (LL), leaf width (LW), leaf area (LA), number of leaves (LN) number of branches (BN), diameter of stem (SD), fresh weight (FW), dry weight (DW) and seed weight (SW) were assessed. The data demonstrate that for all traits these morphological characteristics differed significantly among the ecotypes in *A. retroflexus* and *C. album* **(Table 2A, 2B)**.

***A. retroflexus*:** Mean comparison of ecotypes indicated shortest plant height (22.6 cm) in Spain 1, and longest (93.6 cm) in Spain 2. Ecotype Zarand showed the maximum inflorescence length (28 cm), followed by Bojnurd (26.63 cm), while minimum (1.96 cm) was noted in Sari. The leaf length, leaf width and leaf area were highest (12.77 cm, 5.1 cm and 65.08 cm^2 respectively) in Spain 2 and lowest (2.5 cm, 1 cm and 2.5 cm^2 respectively) in Yazd. The least numbers of leaves and branches (34.66 and 2.67, respectively) were obtained in Zarand and Bojnurd, and the highest number of leaves and branches (107 and 9.67 respectively) in Ilam and Rudsar. The

thickest shoot (11.32 cm) was measured in Spain 2 and thinnest (1.99 cm) in Yazd. Ecotype Spain 2 showed the highest fresh and dry weights (95.36 g and 17.17 g, respectively) while the lowest (24.15 g and 4.29 g, respectively) was found for fresh and dry weight in Gorgan. Seed weight was the highest (1.83 g) in Spain 2 and the lowest in Spain 2 (0.43 g), followed by ecotype Gorgan (0.42 g) **(Figure 2A)**.

C. album: Mean comparison of ecotypes showed minimum plant height in ecotype Dehloran (22 cm) and maximum in Rudsar (97.5 cm). Maximum inflorescence length was observed in Boyer- Ahmad (20.4 cm) and minimum (3.1 cm) was noted for Moghan, followed by Rudsar (3.2 cm) and Rasht (3.3 cm). The shortest leaf length (1.6 cm) was observed for Spain 2 (1.6 cm) followed by Dehloran (2 cm), and the longest for Rudsar (7.1 cm). The widest leaves was (4.83 cm) in Rudsar, and narrowest (0.5 cm) in Kivi, Yazdabad and Boyer-Ahmad. Ecotype Rudsar showed the maximum leaf area (34.33 cm^2), while minimum (1.63 cm^2) was noted in Yazdabad, followed by France 1499 (1.65 cm^2), Kivi (2.18 cm^2), Dehloran and Spain 2 (2.5 cm^2). Largest number of leaves and branches (175 and 14.33, respectively) was recorded for Kivi, Rudsar and Rasht, and smallest number (14.66 and 4.33, respectively) was observed in Dehloran. The thickest shoot (9.23 cm) was in Rudsar and thinnest (2.48 cm) in France 1499. Kivi showed the highest fresh and dry weights (161.07 g and 27.72 g, respectively) and France 1499 the lowest (3.74 g and 0.64 g, respectively), followed by Dehloran (8.53 g and 1.49 g, respectively). The Kivi ecotype showed the highest seed weight (2.91 g) and The lowest (0.076 g) was observed for France 1499, followed by Dehloran (0.16 g) **(Figure 2B)**.

3.2 Biochemcal parameters

***A. retroflexus*:** The highest chlorophyll a content (5.21 mg g^{-1} FW) was detected in ecotype Ardabil, which was equal with ecotype France (5.12 mg g^{-1} FW) and the minimum (2.06 mg g^{-1} FW) in Zarand. The ecotype Rasht had the highest chlorophyll b content (3.11 mg g^{-1} FW) and the lowest (0.28 mg g^{-1} FW) was found for Ardabil. The highest total chlorophyll content (7.69 mg g^{-1} FW) was recorded in Gilan, which was equal to Rudsar (7.61 mg g^{-1} FW), while it was at lowest (2.82 mg g^{-1} FW) in Kerman. The Ardabil had the highest total carotenoids content (1.95 mg g^{-1} FW) The lowest (0.71 mg g^{-1} FW) was in Shahr-e-Ray. The maximum total soluble protein content (1.17 mg g^{-1} FW) was recorded in Ardabil, followed by France (1.16 mg g^{-1} FW) and the lowest (0.11 mg g^{-1} FW) was recorded in Hamedan, followed by Kerman (0.16 mg g^{-1} FW). The highest CAT activity (1.65 units mg^{-1} protein min^{-1}) was detected in Ardabil, and lowest (0.85 units mg^{-1} protein min^{-1}) in Hamedan, followed by Kerman (0.88 units mg^{-1} protein min^{-1}). The highest POD activity (1.14 units mg^{-1} protein min^{-1}) was recorded in Bojnurd followed by Ilam (1.12 units mg^{-1} protein min^{-1}) and the lowest (0.77 units mg^{-1} protein min^{-1}) in Shahr-e-Ray followed by Moghan (0.81 units mg^{-1} protein min^{-1}) and Gorgan (0.82 units mg^{-1} protein min^{-1}). The highest PPO activity (1.78 units mg^{-1} protein min^{-1}) was recorded in Ilam, and the lowest (1.52 units mg^{-1} protein min^{-1}) in Shahr - e- Ray followed by Gorgan (1.53 units mg^{-1} protein min^{-1}) **(Figure 2A)**.

C. album: The largest concentration chlorophyll a (4.79 mg g^{-1} FW) was recorded in ecotype Yazdabad and the lowest (1.98 mg g^{-1} FW) in Spain 2 followed by Ardabil (2 mg g^{-1} FW). The Boyer Ahmad had the highest chlorophyll b and total chlorophyll content (2.75 mg g^{-1} FW and 7.46 mg g^{-1} FW respectively), while lowest (0.66 mg g^{-1} FW and 2.7 mg g^{-1} FW respectively) was found in Kivi. The highest total carotenoids (2.09 mg g^{-1} FW) was recorded in Yazdabad and the lowest was detected in Spain 2 (0.56 mg g^{-1} FW). The Yazdabad had the highest total soluble protein content (1.1 mg g^{-1} FW), followed by Yazdabad (1.08 mg g^{-1} FW). The lowest was found (0.13 mg g^{-1} FW) in Ardabil, followed by Spain 2 (0.14 mg g^{-1} FW). The highest CAT activity (1.64 Units mg^{-1} protein min^{-1}) was measured in the ecotype Shahr-e-Ray and the lowest in the ecotypes Spain 2 and Kivi (0.8 Units mg^{-1} protein min^{-1})

followed by France 1499 and Ardabil (0.83 Units mg^{-1} protein min^{-1}). The Boyer Ahmad, Yazd Abad and Shahr-e-Ray had the highest (1.1 Units mg^{-1} protein min^{-1}) POD activity, while lowest (0.77 Units mg^{-1} protein min^{-1}) was in Ardabil, followed by Kivi and Moghan (0.8 Units mg^{-1} protein min^{-1}). The highest PPO activities (1.7 Units mg^{-1} protein min^{-1}) in Shahr-e-Ray and Yazdabad, and the lowest in Kive and Tehran (1.51 Units mg^{-1} protein min^{-1}) **(Figure 2B)**.

3.3 Correlation among measured traits

***A. retroflexus*:** The correlations coefficient among the morphological and biochemical ecotypes presented in **Table 3A**. Plant height showed significant positive correlation with the leaf area (r=0.8), diameter of stem (r=0.87), fresh weight (r=0.9), and seed weight (r=0.9). Inflorescence length was significantly negatively correlated with the number of leaves (r=−0.69), number of branches (r=−0.74). Leaf length showed significantly positively correlated with leaf area (r=0.98), diameter of stem (r=0.78), fresh weight (r=0.69) and seed weight (r=0.63). The leaf area was positively correlated with diameter of stem (r=0.83), fresh weight (r=0.73), and seed weight (r=0.68). The number of leaves was positively correlated with the number of branches (r=0.69). Diameter of stem showed highly significant positive correlated with fresh weight (r=0.87), but had negative correlation with seed weight (r=−0.85).

Chlorophyll a content showed highly significant positively correlation with total chlorophyll content (r=0.87), carotenoid (r=0.79), total protein (r=0.93), and highly significant negative correlation with CAT (r=-0.78), POD (r=-0.73) and PPO activity (r=-0.64). Chlorophyll b content was significantly positively correlated with total chlorophyll content (r=0.67). Carotenoid content showed significant positively correlation with total chlorophyll content (r=0.65) and significant negative correlation with CAT (r=-0.55). Total chlorophyll content showed positive correlation with carotenoid (r=0.77), total protein (r=0.75), and negatively correlation with POD (r=-0.5). Total soluble protein content was significantly negatively correlated with CAT (r=-0.87), POD (r=-0.82) and PPO (r=-0.77) activity. CAT activity was positively correlated with POD (r=0.86) and PPO (r=0.8) activity. POD activity was positively correlated with PPO (r=0.88) activity **(Table 3A)**.

C. album: Plant height was positively correlated with leaf area (r=0.49), number of leaves (r=0.76), number of branches (r=0.63), diameter of stem (0.74), fresh weight (r=0.85), and seed weight (r=0.89). In addition, inflorescence length was significantly negatively correlated with leaf width (r= 0.49). Leaf area was positively correlated with the number of branches (r=0.58) and diameter of stem (0.58). The number of leaves showed positive correlated with number of branches (r=0.56), diameter of stem (0.48), fresh weight (r=0.97) and seed weight (r=0.94). Number of branches was significantly positive correlated with fresh weight (r=0.57) and seed weight (r=0.65). Diameter of stem was positively correlated with fresh weight (r=0.55), seed weight (r=0.64), chlorophyll a content (r= 0.57), total chlorophyll content (r=0.49), and total protein (r=0.55).

Chlorophyll a content was significantly negatively correlated with the CAT activity (r=-0.62) while a positive correlation with chlorophyll b content (r=0.92), total chlorophyll content (r=0.99), carotenoid (r=0.85) and total protein (r=0.9). Chlorophyll b content showed negative correlation with CAT activity (r=-0.54), while a positive correlation with total chlorophyll content (r=0.96), carotenoid (r= 0.82) and total protein (r=0.92). Carotenoid was significantly positive correlated with total protein (r=0.86), but negative correlated with CAT (r=-0.55) and POD (r=-0.5) activity. Total soluble protein content was significantly negative correlated with CAT (r=-0.67) and POD (r=-0.53) activity. CAT activity was positively correlated with POD (r=0.86) and PPO (r=0.7) activity. POD activity was positively correlated with PPO (r=0.82) activity (**Table 3B).**

3.4 Principal component analysis (PCA)

***A. retroflexus*:** In this evaluation, effective traits were divided into 4 components accounting for 88.22% of the total observed variance. Factor loading values higher than 0.5 were considered significant as suggested by Wu et al. (2016). Four principal components (PC1, PC2, PC3, PC4) explained together more than 88.22% of the total variation (**Table 4A**). PC1 related with plant height, leaf length, leaf width, leaf area, diameter of stem, fresh and dry weight and seed weight explained 35.92% of the total variability. Component PC2 was associated with chlorophyll a, carotenoids, total protein content, CAT, POD and PPO activity and accounted for 26.83% of the total variability. Component PC3 was mainly associated with inflorescence length, number of leaves and branches and accounted for 15.39% of the total variability. Component PC4 showed the integration with chlorophyll b, total chlorophyll and explained 10.06% of total variability. Hence, the morphological and biochemical parameters could effectively explain the existing variability.

A scatter plot based on the first two components explained the morphological and biochemical diversity among the measured traits **(Figure 3A)**. Four distinct groups are determined: group I consists in total protein, chlorophyll a, total chlorophyll and carotenoids; group II consists in leaf length, leaf area, leaf width, seed weight, plant height, diameter of stem, fresh weight, dry weight, number of branches, number of leaves and chlorophyll b; group III consists in Cat, POD and PPO; group IV consists in inflorescence length.

C. album: A PCA demonstrated that the first four principal components accounted for 89.01% of the total variance (**Table 4B**). PC1, which explained 30.5% of the total variability, was highly correlated with plant height, leaf length, number of leaves and branches, diameter of stem, fresh and dry weight and seed weight. PC2 was highly correlated with chlorophyll a and b, total chlorophyll, carotenoids and total protein content, explaining 30.06% of the total variability. PC3 was highly correlated with the leaf width, leaf area, and explained 14.97% of the total variability. PC4 was associated with CAT, POD and PPO activity and accounted for 13.46% of the total variability.

A scatter plot based on first two factor analysis of ecotypes demonstrated four distinct groups **(Figure 3B)**: group I consists in total protein, chlorophyll a, chlorophyll b, total chlorophyll, leaf length, number of branches and carotenoids; group II consists in leaf area, leaf length, leaf width, diameter of stem, plant height, seed weight, dry weight and fresh weight; group III consists in CAT, POD and PPO; group IV consists in inflorescence length.

3.5 Cluster analysis

***A. retroflexus*:** Cluster analysis was carried out with the Ward method, based on morphological and biochemical parameters. Generally, ecotypes were divided into two main clusters (**Figure 4A**). With a decrease in the Squared Euclidean distance, the ecotypes were divided into four main sub-clusters: first sub-cluster (Hamedan, Sari and Moghan ecotypes), second sub-cluster (Gorgan, Shahr-e-Rey, Zarand and Bojnurd ecotypes), third sub-cluster (Rasht, Rudsar, Yazd, Spain 1 and Spain 3 ecotypes), fourth sub-cluster (Ilam, France, Ardabil and Spain 2 ecotypes). The results of canonical detection function analysis to determine the best cut-off point showed more differentiation with 4 groups (**Table 5**).

C. album: Ecotypes were divided into two main clusters and four sub-clusters which was confirmed with canonical detection function analysis (**Figure 4B and Table 5**): first sub-cluster (Rudan, France 1617, France 1499, Tehran, Dehloran, Moghan, Hamedan and Spain 1 ecotypes), second sub-cluster (Boyer-Ahmad, Shahr-e-Ray, Mashhad and Yazdabad ecotypes), third sub-cluster (Rudsar and Rasht ecotypes), fourth sub-cluster (Ardabil, Kivi and Spain 2 ecotypes).

3.6 Canonical correlation analysis

Since 99% of trait-related changes are justified by Köppen climate classification, therefore, this function was used to interpret the correlation of two sets of variables in *A. retroflexus and C. album*.

***A. retroflexus*:** According to results, Cfa and Bwk climate provided relatively positive correlation with PH, FL, BN, FW, DW, SW, Ca, Car and antioxidant enzymes and negative correlation with LL, LW, LA, LN, SD, Cb, TC and TP. In Csa and Bsa climate, the results were the opposite of the above. The traits were not very affected by the Dsa climate (**Table 6A).**

C. album: Results showed positive correlations between Bsh, Bsk and Bwh climate and FL, SD, TP and leaf photosynthetic pigments, moreover negative correlations with PH, LL, LW, LA, LN, BN, FW, DW, SW and antioxidant enzymes. In Csa and Cfa climate, the results were the opposite of the above (**Table 6B**).

4. Discussion

We set out to understand the morphological and biochemical traits of wild weed populations for two main reasons. The first is that by characterizing these traits from populations collected from different locations we better understand the weeds capacity to have adapted to different climates. As these populations were grown under common garden conditions, differences in the traits measured will be driven by heritable differences in the populations. Moreover, by understanding the variability within and between the ecotypes, it is possible to get an accurate measure of how variable these traits can be within a species. The second reason to study these traits is that well-characterized collections of wild populations of weeds are a useful resource for plant breeders as they provide information to guide crop improvement through gene introgression, cultivar selection, and conventional breeding practices (Sagnard et al., 2011; Adamczyk-Chauvat et al., 2017; Neve, 2018). Since the genetic resources of weeds remain largely unexplored, understanding the variability of their morphological and biochemical traits will act as a primary effort to simplify improvement of vegetable plants (Andini et al., 2013).

This work measured 11 morphological and 8 biochemical traits of 16 *A. retroflexus* L and 17 *C. album* L. ecotypes grown in a common garden experiment that were collected from several contrasting environments. Morphological traits differed significantly within the species, such as number of branches, fresh and dry weight, number of leaves, leaf area and diameter of stem in *A. retroflexus* L. and inflorescence length, leaf area, fresh and dry weight and number of leaves in *C. album* L.. Moreover; biochemical traits such as total protein content, peroxidase activity and chlorophyll a in *A. retroflexus* L. and total protein content, carotenoid content, catalase and peroxidase activity in *C. album* L. demonstrated a high coefficient of variation, therefore, high diversity among ecotypes. PCA of these data indicated that a combination of plant height, leaf length, leaf width, leaf area, diameter of stem, fresh and dry weight and seed weight explained the most variability of *A. retroflexus* while plant height, leaf length, number of leaves and branches, diameter of stem, fresh and dry weight and seed weight drove the variability of *C. album*. Canonical correlation analysis suggested that areas classified as Cfa and Bwk climates according to the Köppen climate classification system had more value of PPO, POD and Car, on the other hand, Bsk and Csa had more values of SD and LL in *A. retroflexus* L. and showed Bwh, Bsk and Bsh had more value of FL and Car , besides Cfa and Csa had more value of PPO, POD and CAT in *C. album*. Hamedan and Moghan moreover, Ardabil and Spain 2 consistently cluster together in both species, but they are classified in

different climate conditions. So, measured values among ecotypes showed different results in similar climate classification from which they were collected.

Scatter plot based on first two component indicated that group I reflected photosynthetic pigments whereas group III represented enzymatic activity. Group II may indicate morphological traits. Inflorescence length was in Group IV that were found to be effective parameters for explaining the natural variability among the studied *A. retroflexus* and *C. album* ecotypes.

Based on the morphological and biochemical traits, cluster analysis established the phylogenetic relationship among the *A. retroflexus* and *C. album* ecotypes. The dendrogram revealed no separate group among ecotypes according to Köppen climate classification which suggest high level of morphological and biochemical diversity among them.

Variability observed among ecotypes is not surprising since a high level of genetic heterogeneity is expected in plant species that are able to grow in a wide range of environmental conditions. Morphological differences have been reported in ecotypes and populations of many weeds (Van Etten et al., 2017; Bajwa et al., 2017; Le et al., 2020). A higher level of variability in morphological parameters is maintained in many of the weedy or wild relatives of crop plants (Pickersgill, 1981; Hubner et al., 2003). In fact, identification of weed species based solely on their morphological traits can be difficult (Khaing et al., 2013; Sammour et al., 2012) as weeds can exhibit a large number of morphs depending on the environment in which they are grown. The observed variation in morphological appearance might be explained in three possible ways: 1) naturally existing variations (Chan and Sun, 1997); 2) mixed mating system that may facilitate the natural introgression process; (3) polyploidy, leading to gene combination, might have resulted in higher morphological variation (Andini et al., 2013). Weedy plants are regarded as rich sources of variation and a repository of genetic diversity. These amaranths are known to be able to survive in a large variety of habitats (Frankton and Mulligan, 1987) and the ecotypes studied were collected from a variety of locations across their range, therefore, it is unsurprising that the different selection pressures they faced in their past have shaped the morphologies they adopt in a common garden experiment. Although self-pollination is more likely to occur, amaranths can also cross pollinate through wind, with mean outcrossing rates ranging from 4 to 34% (Kulakow and Hauptli, 1994); therefore amaranths have the capacity to maintain beneficial traits as well as accumulate new ones. Polyploidy is common among plant species and recent large-scale transcriptomics indicates that whole-genome duplications have occurred repeatedly throughout flowering plants evolution (Leebens-Mack et al., 2019).

This research suggests that these heritable morphological and biochemical traits vary significantly between ecotypes from similar climate and suggests the local environments they have adapted to have affected the way the trait was selected. Our data are similar to other studies done with amaranths. Andini et al. (2013) assessed the variations in morphology of Indonesian amaranths and compared them with the worldwide variation. They proposed high levels of genetic variability for most morphological traits. Thapa and Blair (2018) evaluated the morphological diversity of close to 300 cultivated grain amaranths and their wild relatives from two gene banks through field assessments of leaf, flower and grain characteristics. They concluded that the amaranth collection was a source of diversity traits and adaptation traits. Some other studies have showed that the variability of morphological traits is affected by a combination of species, climate, and soil factors (Reich et al., 2007; Han et al., 2011; Liu et al., 2012; Li et al., 2018).

In the present investigation, correlation matrix of traits showed significant and positive correlations. Traits such as the leaf width with seed weight and fresh and dry weight were correlated in *A. retroflexus* L.. Likewise, diameter of stem with total chlorophyll and fresh and dry weight in *C. album* L. had the lowest and highest positive correlations in *A. retroflexus* L., respectively. Also, chlorophyll a with total protein content and total chlorophyll correlated with

peroxidase activity in *A. retroflexus* L. along with inflorescence length with leaf width and total protein content with catalase activity had the lowest and highest negative correlations, respectively.

Biochemical parameters, namely leaf photosynthetic pigments and antioxidant enzymes, were found to differ among the ecotypes of these weed species. Weed species overcome stress more easily than cultivated plants by activating various metabolic and biochemical processes (Pavlović et al., 2014). Chlorophylls are essential for photosynthesis and their amounts can directly influence plant photosynthetic ability and biomass (Curran et al., 1990; Filella et al., 1995). Besides chlorophylls, carotenoids are also essential for the photosynthesis process (Ong and Tee, 1992) protecting chlorophylls from photo-oxidative destruction (Giri et al., 2013). In this study, wide variations of leaf photosynthetic pigments were measured in the *A. retroflexus* and *C. album* ecotypes. This study has identified photosynthetically efficient cultivars which could be used in improvement programs (Hussain and Reigosa, 2015; Zhang et al., 2016)

We also detect a significant variation in antioxidant enzyme activities among the studied various *A. retroflexus* and *C album* ecotypes. Factors such as season, area, sampling site, water and soil nutrients affect protein content (Sigua et al., 2012). The antioxidant enzyme activities decrease reactive oxygen species (ROS) and protect plant cells from oxidative damage under stressful conditions (Chaves and Oliveira, 2004). The disparate antioxidant potential of the *A. retroflexus* and *C. album* ecotypes could alter their biotic and abiotic stress tolerance or resistance. According to Slabbert and Kruger (2014), greenhouse screening for leaf antioxidative enzymes production in amaranth demonstrated ecotype variation.

Our results suggest that when chlorophylls, carotenoids and soluble protein contents were reduced in different ecotypes, the activities of antioxidant enzymes were increased. Even under favorable conditions, ROS production is carried out in as the result of different metabolic processes and toxic oxygen derivatives are produced as a result of different stresses. Plants adopt effective systems for scavenging active oxygen species that support them against destructive oxidative reactions (Foyer et al., 1994). Antioxidant enzymes act as key elements in the defense mechanisms. Many changes have been observed in the activities of antioxidant enzymes in different ecotypes of plants (Aziz and Larher, 1998).

Generally, total chlorophyll concentrations showed a significant negative correlation with the level of antioxidant activities. The reaction centers of photosystem I and photosystem II are the major sites of ROS generation in the chloroplast thylakoids (Asada, 2006). One of the key factors that affect the balance between the damage and restoration of the photosynthetic activity is the relationship between the stability of the oxidative stress and the activity of the antioxidant system (Kreslavski et al., 2009). The reduced electron acceptors accumulation may increase the generation of ROS and lead to oxidative injuries. These injuries could enhance chlorophyll b degradation or the prevention of its biosynthesis, damage PSII components and inactivate chloroplast enzymes (Cui et al., 2006).

In conclusions, ecotypes differed significantly in morphological and biochemical traits which are expected to affect the ability of specific ecotypes to compete with other plants and respond to herbicides, biotic, and abiotic stresses. The assessment of morphological and biochemical traits plays an essential role in crop improvement for providing information for propagation, domestication, and breeding programs as well as conservation of genetic resources for plant species (Pickersgill, 1981). Our finding may help to inform breeding programs that aim to combine the superior characteristics of weedy-types into elite crop cultivars via genetic recombination and selection of wild/weedy types (Andini, 2013). The existing diversity could further add new genetic information in global gene pool of weedy species. In addition, the results showed that many field traits have promise for genome analysis in the future, where combining molecular marker data with agro-morphology can identify genes for amaranth control.

5. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6. Author Contributions

Shiva Hamidzadeh Moghadam performed the experiments and data collection, data analysis, and figure preparation, writing of the manuscript. Mohammad Taghi Alebrahim conceived the original idea and formulated the research plan, oversaw the research and writing of the manuscript. Ahmad Tobeh, Mehdi Mohebodini, Danièle Werck-Reichhart and Dana MacGregor contributed to data analysis and writing of the manuscript.

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9. References

Adamczyk-Chauvat, K., Delaunay, S., Vannier A., François, C., Thomas, G., Eber, F., et al. (2017). gene introgression in weeds depends on initial gene location in the crop: *Brassica napus-Raphanus raphanistrum* model. *Genetics*. 206 (3), 1361-1372. doi: 10.1534/genetics.117.201715

Alebrahim. M.T., Rashed Mohassel, M.H., Wilcockson, S., Baghestani, M.A., Ghorbani. (2011). Evaluatin of 6 unregistered herbicides efficacy in Iran potato fields and herbicide relation to cytochromes P450 mono-oxygenase enzyme. Ph. D. Thesis. Ferdowsi. University of Mashhad, Iran.(In Persian).

Alebrahim. M.T., Majd, R., Rashed Mohassel, M.H., Wilcockson, S., Baghestani, M.A., Ghorbani, R., and Kudsk, P. (2012). Evaluating the efficacy of pre-and post-emergence herbicides for controlling Amaranthus retroflexus L. and Chenopodium album L. in potato. Plant protect. 42, 345-350.

Andini, R., Yoshida, S., Yoshida, Y., and Ohsawa, R. (2013). *Amaranthus* genetic resources in Indonesia: morphological and protein content assessment in comparison with worldwide amaranths. *Genet Resour Crop Evol*. 60, 2115-2128. doi: 10.1007/s10722-013-9979-y

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol*. 141, 391-396. doi: 10.1104/pp.106.082040

Aziz, A., and Larher, F. (1998). Osmotic stress induced changes in lipid composition and peroxidation in leaf discs of *Brassica napus* L. *J. Plant Physiol*. 153 (5), 754-762. doi: 10.1016/S0176-1617(98)80231-9

Bajwa, A.A., Chauhan, B.S., and Adkins, S. (2017). Morphological, physiological and biochemical responses of two Australian biotypes of *Parthenium hysterophorus* to different soil moisture regimes. *Environ Sci Pollut Res*. 24 (19), 16186-16194. Doi: 10.1007/s11356-017-9176-1

Bajwa, A.A., Zulfiqar, U., Sadia, S., Bhowmik, P., and Chauhan, B.S. (2019). A global perspective on the biology, impact and management of *Chenopodium album* and *Chenopodium murale*: two troublesome agricultural and environmental weeds. *Environ Sci Pollut Res*. 26, 5357-5371. https://doi.org/10.1007/s11356-018-04104-y

Baskin, J.M., and Baskin C.C. (1978). A discussion of the growth and competitive ability of C_3 and C_4 plants. *Castanea*. 43 (2), 71-76.

Bhattacharjee, SK. (2001). Handbook of medicinal plant. Jaipur, Pointer publishers.

Bond, W., Davies, G., and Turner, R. (2007). The biology and non-chemical control of Common Amaranth (*Amaranthus retroflexus* L.). http://www.gardenorganic.org.uk/ organicweeds/ downloads/ amaranthus [Accessed November 21, 2011].

Bradford, M.M. (1976). Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 72, 248-254. doi: 10.1016/0003-2697(76)90527-3

Brimhall, P.B., Chamberlain, E.W., and Alley, H.P. (1967). Competition of annual weeds and sugarbeets. *Weed Sci*. 13 (1), 33–35. doi: 10.2307/4041092

Buchanan, G.A., Crowley, R.H., Street, J.E. and McGuire, J.M. (1980). Competition of sicklepod (*Cassia obtusifolia* L.) and redroot pigweed (*Amaranthus retroflexus* L.) with cotton (*Gossypium hirsutum* L.). *Weed Sci*. 28, 258–262. doi: 10.1017/S0043174500055259

Chan, K.F., and Sun, M. (1997). Genetic diversity and relationships detected by isozyme and RAPD analysis of crop and wild species of *Amaranthus*. *Theor Appl Genet*. 95, 865–873. doi: 10.1007/s001220050637

Chance, B., and Maehly, A.C. (1955). "Assay of catalases and peroxidases," in Methods in Enzymology ,ed. N.P Colowick and N.P Kaplan (New York: Academic Press), 764-775.

Chaves, M.M., and Oliveira, M.M. (2004). Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J. Exp. Bot*. 55, 2365-2384. doi: 10.1093/jxb/erh269

Clapham, A.R., Tutin, T.G., and Moore, D.M. (1987). Flora of the British Isles, 3rd edition, UK: Cambridge University Press.

Costea, M., Weaver, S.E., and Tardif, F.J. (2003). The biology of Canadian weeds. 130. *Amaranthus retroflexus* L., *A. powellii* S. Watson and *A. hybridus* L. *Can. J. Plant Sci*. 84, 631-668

Cui, L.; Li, J.; Fan, Y.; Xu, S.; and Zhang, Z. (2006). High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility. *Bot. Stud*. 47, 61-69.

Curran, P.J., Dungan, J.L., and Gholz, H.L. (1990). Exploring the relationship between reflectance red edge and chlorophyll content in slash pine. *Tree Physiol*. 7, 33-48. doi: 10.1093/treephys/15.3.203

Dieleman, A., Hamil, A.S., Weise, S.F., and Swanton, C.J. (1995). Empirical models of pigweed (*Amaranthus* spp.) interference in soybean (*Glycine max*). *Weed Sci*. 43, 612-618. doi: 10.1017/S0043174500081728

Filella, I., Serrano, I., Serra, J., and Penuelas, J. (1995). Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. *Crop Sci*. 35, 1400–1405. doi: 10.2135/cropsci1995.0011183X003500050023x

Foyer, C.H., Lelandais, M., and Kunert, K.J. (1994). Photooxidative stress in plants. *Physiol. Plant*. 92 (4), 696-717. doi: 10.1111/j.1399-3054.1994.tb03042.x

Frankton, C., and Mulligan, G.A. (1987). Weeds of Canada (Publication 948). Toronto: NC Press.

Gambino, P., and Vilela, A. (2011). Morphological traits and allocation patterns related to stress-tolerance and seed yield in wild and domesticated evening primrose (*Oenothera* L. Onagraceae). *Ind Crops Prod*. 34, 1269-1276. doi: org/10.1016/j.indcrop.2010.07.014

Garbari, F., and Pedulla, M.L. (2001). *Alternanthera philoxeroides* (Mart.) Griseb. (*Amaranthaceae*), a new species for the exotic flora of Italy. *Webbia*. 56 (1), 139-143. doi: 10.1080/00837792.2001.10670707

Geleta, N., Labuschagne, M.T., and Viljoen, C.D. (2006). Genetic diversity analysis in sorghum germplasm as estimated by AFLP, SSR and morpho-agronomical markers. *Biodivers. Conserv*. 15, 3251–3265. doi: 10.1007/s10531-005-0313-7

Giri, S., Shrivastava, D., Deshmukh, K., and Dubey, P. (2013). Effect of air pollution on chlorophyll content of leaves. *Curr. Agric. Res. J*. 1 (2), 93–98. doi: 10.12944/CARJ.1.2.04

Han, W.X., Fang, J.Y., Reich, P.B., Woodward, F., and Wang, Z. (2011). Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecol. Lett*. 14, 788-796. doi: 10.1111/j.1461-0248.2011.01641.x

Horak, M.J., Loughin, T.M. (2000). Growth analysis of four *Amaranthus* species. *Weed Sci*. 48, 347-355. doi: 10.1614/0043-1745(2000)048[0347:GAOFAS]2.0.CO;2

Hubner, R., Fykse, H., Hurle, K., and Klemsdal, S.S. (2003). Morphological differences, molecular characterization, and herbicide sensitivity of catchweed bedstraw (*Galium aparine*) population. *Weed Sci*. 51, 214-225. doi: 10.1614/0043-1745(2003)051[0214:MDMCAH]2.0.CO;2

Hussain, M.I., Reigosa, M.J. (2015) Characterization of xanthophyll pigments, photosynthetic performance, photon energy dissipation, reactive oxygen species generation and carbon isotope discrimination during artemisinin-induced stress in *Arabidopsis thaliana*. *PLoS ONE*. 10 (1), e0114826. doi: org/10.1371/journal.pone.0114826

Jabran, K., Mahmood, K., Melander, M., Bajwa, A.A., and Kudsk, P. (2017) .Weed dynamics and management in wheat. *Adv Agron*. 145, 97-166. doi: 10.1016/bs.agron.2017.05.002

Jannatabadi, A.A., Talebi, R., Armin, M., Jamalabadi, J.G., and Baghebani, N. (2014). Genetic diversity of Iranian landrace chickpea (*Cicer arietinum* L.) accessions from different geographical origins as revealed by morphological and sequence tagged microsatellite markers. *J. Plant Biochem. Biol*. 23, 225-229. doi: 10.1007/s13562-013-0206-x

٥٧٢ Kar, M., and Mishra, D. (1976). Catalase, Peroxidase, and Polyphenol oxidase activities during Rice leaf
٥٧٣ senescence. *Plant Physiol*. 57 (2), 315-319. doi: 10.1104/pp.57.2.315
٥٧٤ Kenzevic, S.Z.,Weise, S.F., and Swanton, C.J. (1995). Comparison of empirical models depicting density of
٥٧٥ redroot pigweed (*Amaranthus retroflexus* L.) and relative leaf area as predictors of yield loss in maize (*Zea*
٥٧٦ *mays* L.). *Weed Res*. 35, 207-214. doi: 10.1111/j.1365-3180.1995.tb01783.x
٥٧٧ Khaing, A.A., Moe, K.T., Chung, J.W., Baek, H.J., and Park, Y.J. (2013). Genetic diversity and population
٥٧٨ structure of the selected core set in *Amaranthus* using SSR markers. *Plant Breed*. 132, 165-173. doi:
٥٧٩ 10.1111/pbr.12027
٥٨٠ Kreslavski, V.D., Lyubimov, V.Y., Shabnova, N.I., Balakhnina, T.I., and Kosobryukhov, A.A. (2009). Heat-
٥٨١ induced impairments and recovery of photosynthetic machinery in wheat seedlings. Role of light and
٥٨٢ prooxidant-antioxidant balance. *Physiol. Mol. Biol. Plants*. 15, 115-122. doi: 10.1007/s12298-009-0013-y
٥٨٣ Le, V., Ahderom, S., Apopei, B., and Alameh, K. (2020). A novel method for detecting morphologically similar
٥٨٤ crops and weeds based on the combination of contour masks and filtered Local Binary Pattern operators.
٥٨٥ *GigaScience*. 9 (3), giaa017. doi: 10.1093/gigascience/giaa017
٥٨٦ Leebens-Mack, J.H., Barker, M.S., Carpenter, E.J., Deyholos, M.K., Gitzendanner, M.A., Graham, S.W. et al.
٥٨٧ (2019). One thousand plant transcriptomes and the phylogenomics of green plants. *Nature*. 574, 679-685. doi:
٥٨٨ 10.1038/s41586-019-1693-2
٥٨٩ Li, Y., He, N., Hou, J., Xu, L., Liu, C., Zhang, J. et al. (2018). Factors influencing leaf chlorophyll content in
٥٩٠ natural forests at the biome scale. *Front. Ecol. Evol*. 6:64. doi: 10.3389/fevo.2018.00064
٥٩١ Lichtenthaler, H.K., and Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of
٥٩٢ leaf extracts in different solvents. *Biochem. Soc. Trans*. 11, 591-592. doi.org/10.1042/bst0110591
٥٩٣ Liu, C., Wang, X., Wu, X., Dai, S., He, J., and Yin, W. (2012). Relative effects of phylogeny, biological characters
٥٩٤ and environments on leaf traits in shrub biomes across central Inner Mongolia, China. *J. Plant Ecol*. 6, 220-
٥٩٥ 231. doi: 10.1093/jpe/rts028
٥٩٦ Ma, X., Wu, H., Jiang, W., Ma, Y., and Ma, Y. (2015). Interference between Redroot Pigweed (*Amaranthus*
٥٩٧ *retroflexus* L.) and Cotton (*Gossypium hirsutum* L.): Growth Analysis. *PLoS ONE*. 10 (6), e0130475. doi:
٥٩٨ 10.1371/journal.pone.0130475
٥٩٩ MacGregor, D.R. Deak, K.I., Ingram, P.A, Malamy, J.E. (2008) Root system architecture in *Arabidopsis* grown
٦٠٠ in culture is regulated by sucrose uptake in the aerial tissues. *The Plant Cell* 20 (10) 2643-2660; DOI:
٦٠١ 10.1105/tpc.107.055475
٦٠٢ Mandák, B., Zákravský, P., Dostál, P., and Placková, I. (2011). Population genetic structure of the noxious weed
٦٠٣ *Amaranthus retroflexus* in Central Europe. *Flora*. 206, 697-703. doi: 10.1016/j.flora.2011.01.010
٦٠٤ Mondini, L., Noorani, A., and Pagnotta, M.A. (2009). Assessing plant genetic diversity by molecular tools.
٦٠٥ *Diversity*. 1 (1), 19-35. doi: 10.3390/d1010019
٦٠٦ Manly, B.F.J. (2005). Multivariate statistical methods, A Primer. Third edition. London – New York: Chapman
٦٠٧ and Hall/ CRC.
٦٠٨ Neve, P. (2018). Gene drive systems: do they have a place in agricultural weed management?. *Pest Manag Sci*.
٦٠٩ 74 (12), 2671-2679. doi: 10.1002/ps.5137
٦١٠ Ong, A.S.H., and Tee, E.S. (1992). Natural sources of carotenoids from plants and oils. *Method Enzymol*. 213,
٦١١ 142-167. doi: 10.1016/0076-6879(92)13118-H
٦١٢ Pavlović, D., Nikolić, B., Đurović, S., Waisi, H., Anđelković, A., and Marisavljević, D. (2014). Chlorophyll as a
٦١٣ measure of plant health: Agroecological aspects. Pestic. Phytomed. (Belgrade), 29 (1), 21–34. doi:
٦١٤ 10.2298/PIF1401021P
٦١٥ Pickersgill, B. (1981). Biosystematic of crop-weed complexes. *Die Kulturpflanze*. 29, 377-388. doi:
٦١٦ 10.1007/BF02014766
٦١٧ Poonia, A., and Upadhayay, A. (2015). *Chenopodium album* Linn: Review of nutritive value and biological
٦١٨ properties. *J Food Sci Technol*. 52, 3977-3985. doi: 10.1007/s13197-014-1553-x
٦١٩ Reich, P.B., Wright, I.J., and Lusk, C.H. (2007). Predicting leaf physiology from simple plant and climate
٦٢٠ attributes: a global glopnet analysis. *Ecol. Appl*. 17, 1982-1988. doi: 10.1890/06-1803.1
٦٢١ Rodenburg, J., Riches, C.R., and Kayeke, J.M. (2010). Addressing current and future problems of parasitic weeds
٦٢٢ in rice. *Crop Prot*. 29: 210-221. doi: 10.1016/j.cropro.2009.10.015
٦٢٣ Sagnard, F., Deu, M., Dembe´le´, D., Leblois, R., Toure´, L., Diakite´, M. et al. (2011). Genetic diversity,
٦٢٤ structure, gene flow and evolutionary relationships within the *Sorghum bicolor* wild-weedy-crop complex in
٦٢٥ a western African region. *Theor Appl Genet*. 123 (7), 1231-1246. doi: 10.1007/s00122-011-1662-0
٦٢٦ Sammour, R.H., Radwan, S.A., and Mira, M. (2012). Genetic diversity in genus *Amaranthus*: From morphology
٦٢٧ to genomic DNA. *Res Rev Biosci*. 6, 351-360.
٦٢٨ Sarabi, V., Mahallati, M.N., Nezami, A., Mohassel, M.H.R. (2013). Effects of common lambsquarters
٦٢٩ (*Chenopodium album* L.) emergence time and density on growth and competition of maize (*Zea mays* L.).
٦٣٠ *Aust J Crop Sci*. 7, 532-537

٦٣١ Sigua, G.C., Williams, M., Grabowski, J., Chase, C., and Kongchum, M. (2012). Effect of flooding duration and
٦٣٢ nitrogen fertilization on yield and protein content of three forage species. *Agron J*. 104 (3), 791-798.
٦٣٣ doi:10.2134/agronj2011.0364
٦٣٤ Slabbert, M.M., and Krüger, G.H.J. (2014). Antioxidant enzyme activity, proline accumulation, leaf area and cell
٦٣٥ membrane stability in water stressed *Amaranthus* leaves. *S Afr J Bot*. 95, 123-128. doi:
٦٣٦ 10.1016/j.sajb.2014.08.008
٦٣٧ Stace, C. (1997). New Flora of the British Isles. 2rd edition., UK: Cambridge University Press.
٦٣٨ Sudhakar, S., Li, Y., Katz, M.S., and Elango, N. (2001). Translational regulation is a control point in
٦٣٩ RUNX2/Cbfa1 gene expression. Biochem. *Biophys. Res. Commun*. 289: 616-22. doi: 10.1006/bbrc.2001.6033
٦٤٠ Teichmann, and T., Muhr, M. (2015). Shaping plant architecture. *Front. Plant Sci*. 6, 233. doi:
٦٤١ 10.3389/fpls.2015.00233
٦٤٢ Thapa, R., and W. Blair M. (2018). Morphological Assessment of Cultivated and Wild Amaranth Species
٦٤٣ Diversity. *Agron*. 8 (11), 272. doi: 10.3390/agronomy8110272
٦٤٤ Thompson, J.D. (1999). Population differentiation in Mediterranean plants: insights into colonization history and
٦٤٥ the evolution and conservation of endemic species. *Heredity*. 82, 229-236. doi: 10.1038/sj.hdy.6885040.
٦٤٦ Urbas, P., and Zobel, K. (2000). Adaptive and inevitable morphological plasticity of three herbaceous species in
٦٤٧ a multi-species community: field experiment with manipulated nutrients and light. *Acta Oecologica*. 21 (2),
٦٤٨ 139-147. doi: 10.1016/S1146-609X(00)00115-6
٦٤٩ Van Etten, M.L., Conner, J.K., Chang, S.M., and Baucom, R.S. (2017). Not all weeds are created equal: A
٦٥٠ database approach uncovers differences in the sexual system of native and introduced weeds. *Ecol Evol*. 7 (8),
٦٥١ 2636-2642. doi: 10.1002/ece3.2820
٦٥٢ Weaver, S.E. (1991). Size-dependent economic thresholds for three broadleaf weed species in soybeans. *Weed*
٦٥٣ *Technol*. 5, 674-679. doi: 10.1017/S0890037X00027536
٦٥٤ Williams, J.T. (1963): Biological flora of the British Isles. *Chenopodium album* L. *J. Ecol*. 51, 711-725.
٦٥٥ Wu, J., Zhang, C., Chen, J., Cai, C., Wang, L., Fu, D., and Ou, L. (2016). Morphological diversity within litchi
٦٥٦ (*Litchi chinensis* Sonn.) based on leaf and branch traits. *Sci. Hortic*. 207, 21-27. doi:
٦٥٧ 10.1016/j.scienta.2016.05.004
٦٥٨ Zhang, C.J., Lim, S.H., Kim, J.W., Nah, G., Fischer, A., and Kim, D.S. (2016). Leaf chlorophyll fluorescence
٦٥٩ discriminates herbicide resistance in *Echinochloa* species. *Weed Res*. 56 (6). doi:10.1111/wre.12226
٦٦٠

Table 1. Region name, country of origin, geographical coordinates and Köppen climate classification of *A. retroflexus* and *C. album* ecotypes used herein

A. retroflexus				
No.	Region name	Origin	Coordinate	Köppen climate classification
1	Rasht	Iran	37°16'05 N 49°35'20 E	Humid subtropical climate (Cfa)
2	Gorgan	Iran	36°45'06 N 54°21'40 E	Hot summer mediterranean climate (Csa)
3	Rudsar	Iran	37°08'16 N 50°17'10 E	Humid subtropical climate (Cfa)
4	Sari	Iran	36°33'57 N 53°03'31 E	Hot summer mediterranean climate (Csa)
5	Shahr-e-Rey	Iran	35°34'37 N 51°27'44 E	Cold semi-arid climate (Bsk)
6	Ilam	Iran	33°38'05N 46°24'54 E	Hot summer mediterranean climate (Csa)
7	Yazd	Iran	31°10'97 N 53°11'97 E	Cold desert climate (Bwk)
8	Bojnurd	Iran	37°53'74 N 57°24'96 E	Cold semi-arid climate (Bsk)
9	Zarand	Iran	30°47'27 N 56°50'10 E	Cold desert climate (Bwk)
10	Hamedan	Iran	34°47'50 N 48°30'45 E	Hot summer mediterranean climate (Csa)
11	Ardabil	Iran	38°14'54 N 48°17'03 E	Hot-summer humid continental climate (Dsa)
12	Moghan	Iran	39°13'00 N 47°33'53 E	Humid subtropical climate (Cfa)
13	France	France	47°19'20 N 5°2'28 E	Humid subtropical climate (Cfa)
14	Spain 1	Spain	37°53'18 N 4°46'38 W	Hot summer mediterranean climate (Csa)
15	Spain 2	Spain	37° 53'15 N 4° 46'35 W	Hot summer mediterranean climate (Csa)
16	Spain 3	Spain	37° 53'14 N 4° 46'45 W	Hot summer mediterranean climate (Csa)
			C. album	
1	Rudsar	Iran	37°08'13 N 50°16'52 E	Humid subtropical climate (Cfa)
2	Rasht	Iran	37°16'03 N 49°35'08 E	Humid subtropical climate (Cfa)
3	Boyer-Ahmad	Iran	30°53'47 N 51°24'96 E	Hot semi-arid climate (Bsh)
4	Rudan	Iran	27°25'44 N 57°10'45 E	Hot desert climate (Bwh)
5	Moghan	Iran	39°12'03 N 47°34'24 E	Humid subtropical climate (Cfa)
6	Kivi	Iran	37'41'02 N 48°20'53 E	Hot summer mediterranean climate (Csa)
7	Ardabil	Iran	38°12'44 N 48°17'38 E	Hot-summer humid continental climate (Dsa)
8	Yazdabad	Iran	32°39'41 N 51°41'21 E	Cold semi-arid climate (Bsk)
9	Shahr-e-Ray	Iran	35°34'22 N 51°27' 44 E	Cold semi-arid climate (Bsk)
10	Tehran	Iran	35°41'13 N 51°26'22 E	Cold semi-arid climate (Bsk)
11	Dehloran	Iran	32°41'49 N 47°16'05 E	Hot semi-arid climate (Bsh)
12	Hamadan	Iran	34°49'46 N 48°19' 47 E	Hot summer mediterranean climate (Csa)
13	Mashhad	Iran	36°16'24 N 59°38'16 E	Cold semi-arid climate (Bsk)
14	Spain 1	Spain	37°53' 15 N 4°46'35 W	Hot summer mediterranean climate (Csa)
15	Spain 2	Spain	37°53' 14 N 4°46'45 W	Hot summer mediterranean climate (Csa)
16	France 1617	France	47°19'20 N 5°2'28 E	Humid subtropical climate (Cfa)
17	France 1499	France	47°19'29 N 5°2'22 E	Humid subtropical climate (Cfa)

Table 2) Variance analysis of the evaluated traits in *A: retroflexus* **(A)** and *C. album* **(B)** ecotypes

(A)

Source of variation	Degrees of freedom	Mean squares										
		PH	FL	LL	LW	LA	LN	BN	SD	FW	DW	SW
Replication	2	3^{ns}	0.41^{ns}	1.38^{ns}	0.36^{**}	33.17^{**}	159.5^{**}	1.75^{ns}	0.36^{**}	31.4^{ns}	0.94^{ns}	0.001^{ns}
Ecotype	15	1302.4^{**}	163.2^{**}	21.1^{**}	4.4^{**}	786.62^{**}	1470.61^{**}	10.3^{**}	18.07^{**}	1455.67^{**}	50.16^{**}	0.57^{**}
Error	30	5.68	0.696	0.3	0.05	5.18	66.25	0.77	0.33	41.42	1.42	0.005
CV		4.8	8.3	9.84	8.06	11.66	11.83	12.22	10.26	11.99	12.09	7.1

Source of variation	Degrees of freedom	Mean squares							
		Ca	Cb	TC	Car	TP	CAT	POD	PPO
Replication	2	0.003^{ns}	0.0008^{ns}	0.003^{ns}	0.0002^{ns}	0.0016^{ns}	0.0039^{**}	0.001^{ns}	0.0007^{ns}
Ecotype	15	4.21^{**}	1.86^{**}	7.38^{**}	0.4^{**}	0.4^{**}	0.17^{**}	0.044^{**}	0.018^{**}
Error	30	0.01	0.0004	0.011	0.0002	0.004	0.0006	0.001	0.0004
CV		2.87	1.32	1.95	1.21	8.73	1.84	3.28	1.29

(B)

Source of variation	Degrees of freedom	Mean squares										
		PH	FL	LL	LW	LA	LN	BN	SD	FW	DW	SW
Replication	2	74.43^{**}	5.34^{*}	0.05^{ns}	0.04^{ns}	2.12^{ns}	142.82^{ns}	1.11^{ns}	0.011^{ns}	13.47^{ns}	0.03^{ns}	0.002^{ns}
Ecotype	16	1567.97^{**}	47.8^{**}	6.55^{**}	4.46^{**}	209.75^{**}	4829.2^{**}	30.34^{**}	11.03^{**}	5735.64^{**}	169.8^{**}	2.09^{**}
Error	32	6.16	1.58	0.06	0.019	0.98	56.55	0.47	0.13	28.46	0.87	0.006
CV		4.26	14.34	6.56	7.99	12.73	10.88	7.58	7.21	10.44	10.58	8.29

Source of variation	Degrees of freedom	Mean squares							
		Ca	Cb	TC	Car	TP	CAT	POD	PPO
Replication	2	0.0002^{ns}	0.0017^{ns}	0.0017^{ns}	0.003^{*}	0.00007^{ns}	0.001^{ns}	0.004^{ns}	0.0002^{ns}
Ecotype	16	4.58^{**}	1.26^{**}	11.23^{**}	0.71^{**}	0.43^{**}	0.26^{**}	0.047^{**}	0.19^{**}
Error	32	0.0006	0.0005	0.001	0.0008	0.0013	0.0006	0.0003	0.0002
CV		0.85	1.88	0.78	2.27	7.41	2.41	2.11	0.97

PH: Plant Height, FL: Inflorescence length, LL: Leaf Length, LW: Leaf Width, LA: Leaf Area, LN: Number of leaves, BN: Number of Branches, SD: Diameter of stem, FW: Fresh weight, DW: Dry Weight, SW: Seed Weight, Ca: Chlorophyll a, Cb: Chlorophyll b, TC: Total Chlorophyll, Car: Carotenoid content, TP: Total Protein content, CAT: Catalase activity, POD: Peroxidase activity, PPO: Polyphenol oxidase

Table 3) Correlation matrices for the morphological and biochemical traits in *A. retroflexus* **(A)** and *C. album* **(B)**

(A)

Characteristics	PH	FL	LL	LW	LA	LN	BN	SD	FW	DW	SW	Ca	Cb	TC	Car	TP	CAT	POD	PPO
PH	1																		
FL	0.169	1																	
LL	0.736	0.125	1																
LW	0.759	0.245	0.893	1															
LA	0.803	0.152	0.986	0.913	1														
LM	-0.286	-0.69	-0.167	-0.339	-0.199	1													
BM	0.109	-0.748	0.051	-0.15	0.012	0.695	1												
SD	0.874	0.053	0.781	0.765	0.83	-0.076	0.164	1											
FW	0.901	0.054	0.691	0.664	0.73	0.028	0.349	0.877	1										
DW	0.894	0.044	0.671	0.654	0.709	0.03	0.357	0.869	0.998	1									
SW	0.908	0.05	0.637	0.628	0.681	-0.03	0.339	-0.858	0.984	0.989	1								
Ca	0.027	-0.186	0.181	-0.038	0.118	0.196	0.168	-0.048	-0.008	-0.036	-0.015	1							
Cb	-0.134	-0.323	-0.001	-0.031	-0.025	-0.259	0.231	-0.02	-0.059	-0.054	-0.077	0.231	1						
TC	-0.047	-0.303	0.136	0.045	0.076	0.279	0.243	-0.046	-0.036	-0.054	-0.05	0.872	0.677	1					
Car	0.099	-0.347	0.061	-0.08	0.019	0.196	0.451	0.29	0.117	0.107	0.158	0.79	0.348	0.773	1				
TP	0.008	-0.174	0.226	-0.027	0.145	0.223	0.134	-0.014	0.008	-0.018	-0.011	-0.934	-0.093	0.753	0.659	1			
CAT	-0.252	0.067	-0.301	-0.14	-0.255	-0.078	-0.072	-0.214	-0.223	-0.199	-0.233	-0.781	0.273	-0.453	-0.551	-0.873	1		
POD	-0.318	0.49	-0.437	-0.279	-0.416	-0.115	0.017	-0.3	-0.243	-0.212	-0.2	-0.73	0.088	-0.508	-0.43	-0.825	0.865	1	
PPO	-0.283	-0.154	-0.47	-0.3	-0.428	0.086	0.195	-0.224	-0.182	-0.162	-0.183	-0.648	0.191	-0.394	-0.332	-0.779	0.801	0.88	1

(B)

Characteristics	PH	FL	LL	LW	LA	LN	BN	SD	FW	DW	SW	Ca	Cb	TC	Car	TP	CAT	POD	PPO
PH	1																		
FL	0.176	1																	
LL	0.669	0.265	1																
LW	0.349	-0.491	0.456	1															
LA	0.496	-0.374	0.665	0.941	1														
LM	0.762	-0.016	0.333	0.233	0.318	1													
BM	0.636	-0.241	0.396	0.578	0.568	0.562	1												
SD	0.746	0.07	0.765	0.583	0.714	0.485	0.479	1											
FW	0.855	0.058	0.447	0.234	0.361	0.975	0.575	0.556	1										
DW	0.856	0.064	0.454	0.233	0.362	0.974	0.571	0.556	0.99	1									
SW	0.892	-0.028	0.495	0.323	0.446	0.941	0.652	0.644	0.976	0.974	1								
Ca	0.317	0.21	0.609	0.1	0.304	-0.033	-0.015	0.573	0.04	0.041	0.136	1							
Cb	0.189	0.445	0.519	-0.152	0.041	-0.126	-0.183	0.376	-0.056	-0.052	-0.006	0.921	1						
TC	0.279	0.296	0.589	0.014	0.218	-0.065	-0.073	0.49	-0.008	0.01	0.089	0.991	0.965	1					
Car	0.024	0.253	0.444	-0.077	0.097	-0.153	-0.33	0.351	-0.11	-0.109	-0.062	0.859	0.826	0.836	1				
TP	0.286	0.257	0.584	0.05	0.259	-0.035	-0.056	0.559	0.026	0.028	0.108	0.992	0.928	0.988	0.863	1			
CAT	-0.04	-0.129	-0.314	-0.108	-0.218	0.104	0.048	-0.436	0.116	0.111	0.034	-0.626	-0.542	-0.609	-0.552	-0.672	1		
POD	0.088	-0.142	-0.319	0.057	-0.135	0.131	0.041	-0.273	0.189	0.182	0.135	-0.473	-0.41	-0.46	-0.502	-0.53	0.866	1	
PPO	0.146	-0.294	-0.152	0.13	0.51	-0.305	0.085	-0.027	0.179	0.172	0.202	-0.243	0.25	0.25	-0.284	-0.308	0.701	0.823	1

Table 4) Principal component analysis of morphological and biochemical traits in *A. retroflexus* (A) and *C. album* **(B)** ecotypes

A)

	Principal component			
Characteristics	1	2	3	4
PH	**0.897**	0.262	0.16	0.084
FL	0.177	0.313	**-0.682**	-0.166
LL	**0.504**	0.602	0.32	-0.156
LW	0.215	0.027	**0.942**	-0.02
LA	0.345	0.225	**0.876**	-0.066
LN	**0.947**	-0.114	0.033	0.02
BN	**0.625**	-0.153	0.523	-0.056
SD	**0.595**	0.516	0.449	-0.152
FW	**0.981**	-0.018	0.034	0.096
DW	**0.982**	-0.016	0.031	0.089
SW	**0.963**	0.057	0.151	0.086
Ca	0.066	**0.964**	0.087	-0.17
Cb	-0.014	**0.946**	-0.191	-0.125
TC	0.04	**0.975**	-0.008	-0.158
Car	-0.12	**0.882**	-0.087	-0.183
TP	0.058	**0.952**	0.032	-0.238
CAT	0.052	-0.493	-0.115	**0.781**
POD	0.107	-0.338	-0.071	**0.896**
PPO	0.085	-0.099	0.158	**0.934**
Eigen variance	5.79	5.71	2.84	2.55
Percentage of variance	30.5	30.06	14.976	13.46
Cumulative percentage	30.5	60.56	75.54	89.01

B)

	Principal component			
Characteristics	1	2	3	4
PH	**0937**	0.093	-0.078	-0.081
FL	0.097	-0.06	**-0.831**	-0.19
LL	**0.844**	0.253	-0.208	0.128
LW	**0.842**	0.046	-0.364	0.102
LA	**0.855**	0.194	-0.228	0.098
LN	-0.126	0.099	**0.832**	0.54
BN	0.27	0.004	**0.934**	0.105
SD	**0.94**	0.034	0.016	-0.023
FW	**0.948**	0.03	0.191	-0.094
DW	**0.943**	0.003	0.203	-0.098
SW	**0.928**	0.022	0.189	-0.109
Ca	-0.051	**0.901**	0.137	0.335
Cb	-0.028	-0.083	0.194	**0.941**
TC	-0.053	0.64	0.201	**0.726**
Car	0.038	**0.616**	0.382	0.423
TP	-0.042	**0.962**	0.122	0.147
CAT	-0.162	**-0.933**	-0.075	0.214
POD	-0.247	**-0.9**	0.031	0.054
PPO	-0.218	**-0.869**	0.242	0.117
Eigen variance	6.82	5.09	2.92	1.91
Percentage of variance	35.92	26.83	15.39	10.06
Cumulative percentage	35.92	62.76	78.163	88.22

Eigen values are significant ≥0.5 which are indicated by bold letters.

Table 5) Discriminant analysis to determine the cut-off point dendrogram of cluster analysis in *A. retroflexus* (A) and *C. album* (B) ecotypes

(A)

Number of groups	Wilks' Lambda	Chi-square	Significance level
2	.007	53.843	.000
3	.093	26.128	.000
4	.428	9.344	.009

(B)

Number of groups	Wilks' Lambda	Chi-square	Significance level
2	.000	138.831	.000
3	.004	55.988	.000
4	.095	23.531	.001

Table 6) Canonical correlations between Köppen climate classification and morphological and biochemical traits in *A. retroflexus* **(A)** and *C. album* **(B)** ecotypes

(A)

First function correlation									0.999										
Climate classification			Cfa			Csa			Bsk			Bwk			Dsa				
Function 1			-0.323			0.269			0.679			-0.731			0.096				
Traits	PH	FL	LL	LW	LA	LN	BN	SD	FW	DW	SW	Ca	Cb	TC	Car	TP	CAT	POD	PPO
Function 1	-0.115	-0.023	0.115	0.07	0.081	0.042	-0.021	0.22	-0.1	-0.08	-0.069	-0.02	0.11	0.03	-0.15	0.08	-0.093	-0.16	-0.2

(B)

First function correlation										0.999									
Köppen climate classification			Cfa			Csa			Bsk			Dsa			Bsh			Bwh	
Function 1			0.59			0.25			-0.28			0.05			-0.25			-0.8	
Traits	PH	FL	LL	LW	LA	LN	BN	SD	FW	DW	SW	Ca	Cb	TC	Car	TP	CAT	POD	PPO
Function 1	0.17	-0.6	0.006	0.36	0.3	0.29	0.44	-0.02	0.26	0.25	0.32	-0.14	-0.2	-0.16	-0.33	-0.19	0.43	0.4	0.62

Figure 1) Map of the sample collection regions for *A. retroflexus* **(A)** and *C. album* **(B)** ecotypes

Figure 2 (A) Mean values ± standard error of each morphological and biochemical traits in *A. retroflexus* ecotypes

Figure 2 (B) Mean values ± standard error of each morphological and biochemical traits in *C. album* ecotypes

Figure 3) Scatter plot based on first two component analysis of 19 traits for the *A. retroflexus* **(A)** and *C. album* **(B)** ecotypes

Figure 4) Dendrogram of *A. retroflexus* **(A)** and *C. album* **(B)** ecotypes

Figure 1.JPEG

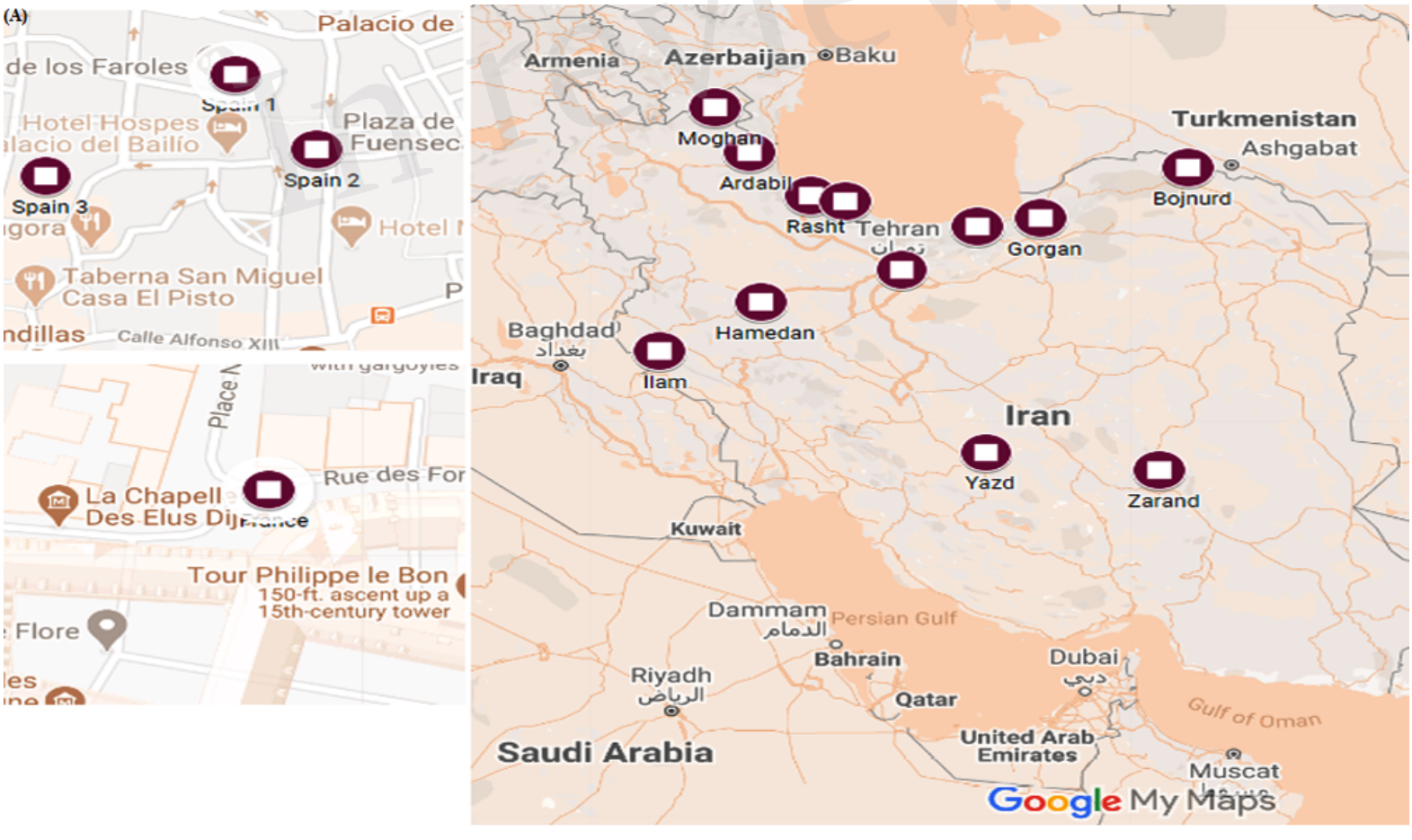

Figure 2.JPEG

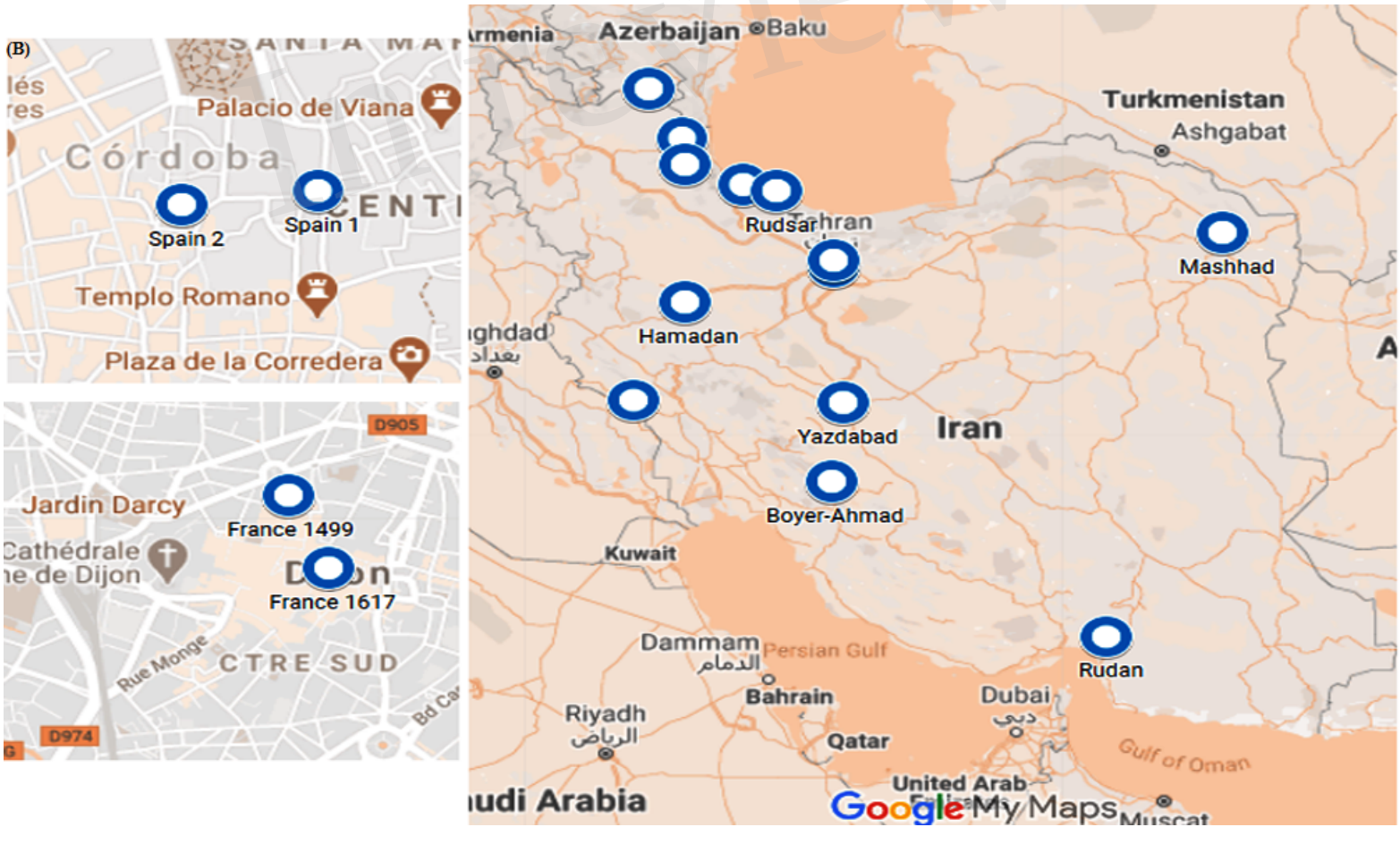

Figure 3.JPEG

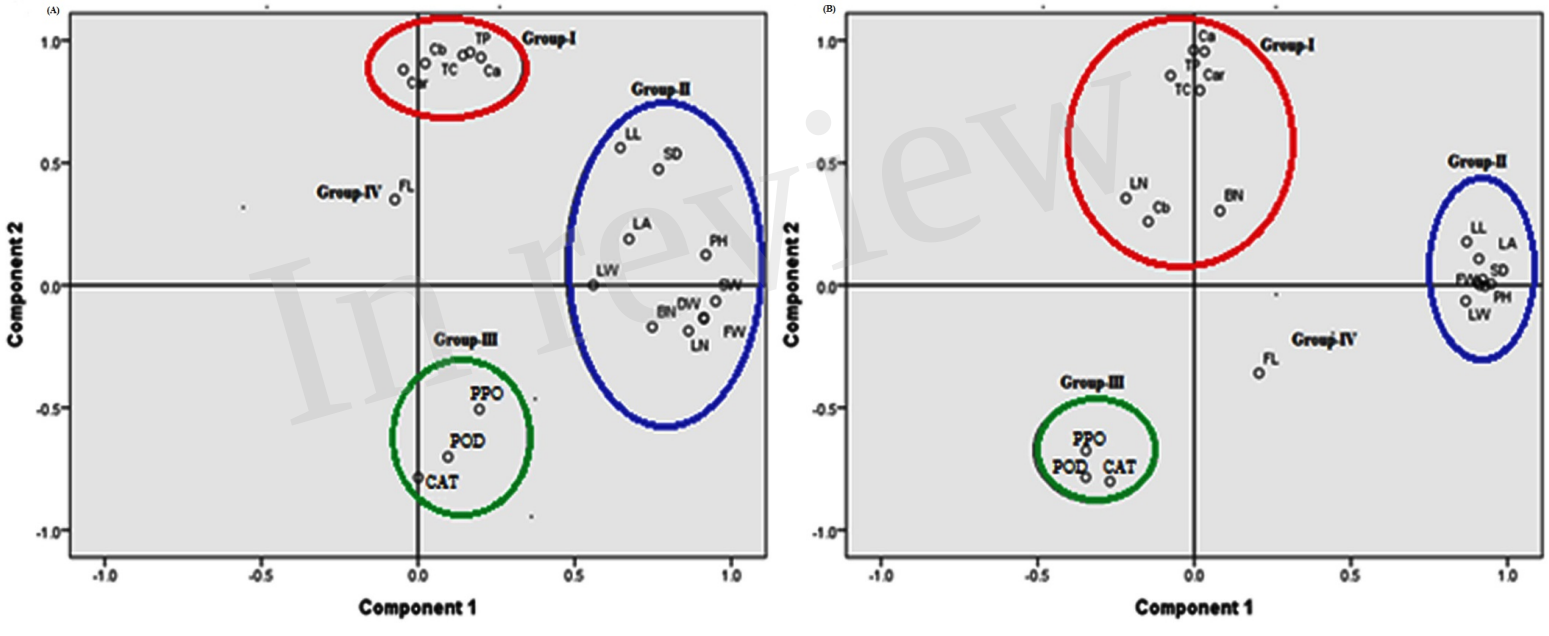

Figure 4.JPEG