

Morphological Evaluation of *Asparagus Azerbaijanensis* Wild Accessions and Drought Tolerance Assessment of Iranian *Asparagus* Under Greenhouse Condition

Atefeh Namaki

University of Zanjan

Zahra Ghahremani (✉ z.ghahremani@znu.ac.ir)

University of Zanjan

Mohamad Ebrahim Ranjbar

University of Zanjan

Research Article

Keywords: *Asparagus azerbaijanensis*, Drought stress, Iranian asparagus, Morphological variation.

Posted Date: September 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-868052/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Some of the most cultivated commercial cultivars of asparagus are susceptible to abiotic stresses. Different species of asparagus plant are distributed in different regions of Iran which could be useful genetic resources for applying in asparagus breeding programs. The purposes of the current study were to evaluate morphological variation of *A. azerbaijanensis* accessions and drought tolerance assessment of Iranian asparagus in order to introduce superior accessions. Two different accessions of *A. azerbaijanensis* species were collected. A number of 30 plants were selected and 22 morphological traits were then evaluated. A factorial experiment based on completely randomized design was performed in order to drought tolerance assessment of 9 Iranian asparagus accessions. The average of spear length and diameter in Ahar and Makou plants were 48.94 cm and 4.70 mm, respectively. Based on results, plants of Ahar accession produced short and thick spears but on the other hand, Makou accession plants produced longer and thinner spears. Score 8 (80% similarity rate) was averagely chosen by panel members in terms of similarity rate of *A. azerbaijanensis* accessions spears taste to that of Mary Washington commercial cultivar. The highest percentage of seed germination was recorded in Baladeh and Gazanak accessions (71.66 and 70.83%, respectively). Baladeh accession showed the highest carotenoid content under drought stress conditions (0.43 mg g Fw⁻¹). Suitable size and delicious taste of spear in *A. azerbaijanensis* species has made it a suitable candidate for commercial cultivation and also improving spear characteristics by using selection method. Baladeh and Gazanak accessions could be introduced as superior accessions with high drought tolerance rate. High resistance rate of these accessions can be related to their ploidy level and distribution areas characteristics.

Introduction

Asparagus is a perennial plant which belongs to Asparagaceae family. Fleshy stem is edible part of asparagus which is called spear (Kanno and Yokoyama, 2011). Some of the most cultivated commercial cultivars of asparagus are susceptible to abiotic stresses such as drought and salt stress (Drost and Wilcox-Lee 1997). *Asparagus officinalis* is the most economically important species and Mary Washington is the most cultivated commercial cultivar of asparagus worldwide (Amian et al. 2018). Drought stress is one of the most important environmental stresses which can seriously affect quality and productivity rate of agriculture crops (Ghahremani et al. 2020). Iran is the second largest country in the Middle East which is climatically part of the Afro-Asian belt of deserts and has a unique plant and animal diversity (Ebrahimi et al., 2021). According to some comprehensive researches which were conducted by Sarabi et al. (2010), Mousavizadeh et al. (2015) and Hamdi and Assadi, (2017), different species of asparagus plant such as *A. azerbaijanensis*, *A. breslerianus*, *A. officinalis*, *A. persicus* and *A. verticillatus* are distributed in different regions of Iran. Mousavizadeh et al. (2015), reported that based on some evaluated morphological and physiological properties of asparagus plants and also climate and soil characteristics of distribution areas, Iranian asparagus could be useful and suitable genetic resource to apply in asparagus breeding programs. Based on reports, plants of *A. breslerianus* species which distributed in dry gypsum hills located in central areas of Iran showed high levels of salt resistance rate (Mousavizadeh et al., 2017). High levels of genetic and morphological variation of Iranian asparagus different accessions were reported by Sarabi et al. (2010). According to their researches, wild populations of Iranian asparagus could be considered as valuable gene pool to extend genetic variation of commercial cultivars.

The main purposes of the current study were to evaluate morphological variation of *A. azerbaijanensis* accessions collected from western regions of Iran, drought tolerance assessment of Mary Washington commercial cultivar under greenhouse condition and also evaluation of drought resistance rate of Iranian asparagus different accessions in order to introduce superior accessions for applying in future asparagus breeding programs.

Materials And Methods

Morphological evaluation of *A. azerbaijanensis* wild accessions

In first experiment, two different accessions of *A. azerbaijanensis* species were identified and collected from Ahar (a city located in East Azerbaijan province, Iran) and Makou (a city located in West Azerbaijan province, Iran) cities (Fig. 1). The locations of distribution regions were detected according to Hamdi and Asadi, (2017) and also based on local people guidance. A number of 30 plants containing male and female plants (in equal numbers) were selected and 22 morphological traits were evaluated according to Mousavizadeh et al. (2015) (Fig. 2). Soil samples were collected from four different locations in distribution areas at a depth of 0 to 60 cm and some of the most important soil characteristics were then measured. The climate characteristics were collected from weather stations located in Ahar and Makou cities and exact geographical location of distribution regions was recorded by using GPS software (Table 1).

Drought tolerance assessment of Iranian asparagus

In second experiment, Iranian asparagus accessions belong to three different species including *A. azerbaijanensis*, *A. officinalis* and *A. verticillatus* were collected from different regions of Iran (distribution areas were identified according to Mousavizadeh et al. (2015) and

Hamdi and Asadi (2017) and also based on local people guidance) (Table 2 & Fig. 3).

Asparagus fruits were harvested from late spring to early summer 2020. Seeds were then separated and stored under cold condition until beginning of greenhouse experiment. Seeds sterilization was carried out by sodium hypochlorite (1.5 % (v/v)) for 10 min and rinsing in distilled water for three times (5 min for each time). Mary Washington commercial cultivar was applied as control. Based on field water capacity (FC), four levels of drought stress including 100% FC (control), 75% FC (mild drought stress), 50% FC (moderate drought stress) and 25% FC (severe drought stress) were simulated. Gravimetric method was used to determine FC. First, the soil (5 kg) was oven dried for 48 h at 103 °C and then weighted. Then, a plastic pot (18×22 cm) was filled with dried soil and then saturated with distilled water. Soil weight was re-calculated after 48 h of drainage. Finally, FC was evaluated by calculating of difference between drained soil weight and dried soil weight. Sterilized seeds were sown at depth of 1 cm. First time irrigation was carried out immediately after sowing and irrigation was then continued at 6-days interval. The greenhouse temperature and relative humidity were adjusted on 27 ± 2 °C and 65%, respectively. Tables 3 and 4 show some important characteristics of applied soil and irrigation water and Fig. 4 shows experimental units and Iranian asparagus seedling grown under greenhouse condition.

Three different groups of asparagus traits including a) seed germination indices b) morphological traits and c) physiological traits were measured to determine drought resistance rate of each accession.

a) Seed germination indices

Seed germination percentage and speed were calculated by using of following formula:

$$(1) \text{ Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \text{ (Czabator 1962)}$$

$$(2) \text{ Germination speed} = n_1/d_1 + n_2/d_2 + n_3/d_3 + \dots \text{ (Czabator 1962)}$$

n = number of germinated seeds

d = number of days

b) Seedling growth characteristics

Plant height, stem diameter, leaf length, leaf diameter, fresh weight of aerial and underground parts of plant and dry weight of aerial and underground parts of plant were measured as seedling growth characteristics. Dry weights were measured after 24 h oven-drying of plant tissues at 65 °C.

c) Physiological traits

Total chlorophyll and carotenoid content, electrolyte leakage and proline content were the evaluated physiological traits. Total chlorophyll and carotenoid content was evaluated according to spectrophotometric method (*spectrophotometer-SAFAS UVmc2*) as described by Witham et al. (1971). Electrolyte leakage was evaluated based on electrical conductivity changes measurement as described by Lutts et al. (1996). Proline content was measured by using method described by Bates (1973). Sulfosacilic acid was used to extract free proline. proline content was determined by a spectrophotometric absorption measurement (*spectrophotometer-SAFAS UVmc2*) at 520 nm and expressed as $\mu\text{mol g}^{-1}$ FW.

Statistical analysis

In order to morphological evaluation of *A. azerbaijanensis* accessions, a number of 30 plants of each accession containing male and female plants (in equal numbers) were selected and 22 morphological traits were then evaluated. Mean comparing was performed based on t test at confidence level of 1%. Maximum, minimum, mean and coefficients of variation of each one of evaluated traits were assessed to determine morphological variation. Correlation between measured morphological characteristics and spear length and diameter was evaluated based on Pearson correlation method. Taste panel analysis was performed by employing of 10 people panel members and taste similarity rate was scored on scale of 0–10.

A factorial experiment based on completely randomized design (with 3 replications) was performed in order to drought tolerance rate assessment of Iranian asparagus accessions. The factors were a) asparagus accessions in 10 levels including nine Iranian asparagus accessions and Mary Washington commercial cultivar as control and b) drought stress in four levels including 100% FC (control), 75% FC (mild drought stress), 50% FC (moderate drought stress) and 25% FC (severe drought stress). Mean comparing was performed based on

Tukey's test at confidence level of 1%. Cluster analysis was performed using Ward's method and Euclidean distance and dendrogram was designed by Minitab 16 software.

Results

Morphological evaluation of *A. azerbaijanensis* wild accessions

Based on obtained results, plant height to first branch, fruit number per plant and seed number per fruit showed the highest coefficient of variation among the evaluated morphological traits (48.09, 42.93 and 40.05% respectively). So, these traits could be recommendable candidates to create morphological variation in asparagus breeding programs. The average of spear length and diameter in Ahar and Makou plants were 48.94 cm and 4.70 mm, respectively. In average, 2.37 seed number per fruit and 159.40 fruit number per plant were counted in Ahar and Makou plants. The average number of cladode in *A. azerbaijanensis* accessions was 10.28. According to mean comparing results, there was significant difference between Ahar and Makou accessions in terms of number of primary and secondary branches, length of secondary branch, scale number, cladode number, length and diameter of leaf and spear diameter. Makou accession plants showed higher value of length and diameter of leaf and spear diameter comparing to Ahar accession plants. Based on results, plants of Ahar accession produced short and thick spears but on the other hand, Makou accession plants produced longer and thinner spears (Table 5).

Results of correlation analysis between evaluated morphological traits and spear properties showed that there is significant correlation between spear length and diameter and number of primary and secondary branches, internode length of secondary branch, leaf length and diameter and scale number (Fig. 5). According to results, spear length increased and spear diameter decreased with increasing the number of primary and secondary branches. In contrast, increasing of leaf length and diameter led to producing shorter and thicker spears in *A. azerbaijanensis* accessions. Among the evaluated properties, number of primary and secondary branches showed the highest correlation with diameter of spear ($r = -0.75$) and leaf length was the highest correlated trait to length of spear ($r = -0.85$) (Table 6).

Taste panel analysis showed that no one of panel members detect bitter taste in spear harvested from *A. azerbaijanensis* accessions plants. In the other hand, all of panel members detected astringent taste in spears harvested from both Ahar and Makou accessions plants. Score 8 (80% similarity rate) was averagely chosen by panel members in terms of similarity rate of *A. azerbaijanensis* accessions spears taste to that of Mary Washington commercial cultivar.

Drought tolerance assessment of Iranian asparagus

a) Seed germination indices and seedling growth characteristics

Based on results of analysis of variance, simple effect of accession was significant on length of leaf. Also, simple effects of accession and drought stress were significant on percentage and speed of germination, plant height and dry weight of aerial and underground parts of plant. Leaf diameter, fresh weight of aerial and underground parts and stem diameter were significantly affected by interaction between accession and drought stress. Mean comparing results showed that the highest number of leaf was recorded in Alborz accession (2.68 cm). There was no significant difference between Baladeh, Chalaki, Shiraz, Taleghan and Gazanak accessions and Mary Washington commercial cultivar in terms of length of leaf. The lowest values of leaf length were recorded in Ahar, Makou and Mahmoudabad accessions (1.68, 1.70 and 1.77 cm, respectively) (Fig. 6).

The highest percentage of seed germination was recorded in Baladeh and Gazanak accessions (71.66 and 70.83%, respectively). There was no significant difference between Ahar and Makou accessions in seed germination percentage and lowest seed germination was recorded in Alborz, Chalaki and Taleghan accessions. Iranian asparagus accessions were divided into two groups in terms of seed germination speed. The first group with high germination speed included Baladeh, Gazanak, Mahmoudabad, Makou, Ahar and Shiraz accessions and the second group with lower values of germination speed included Alborz, Chalaki and Taleghan accessions and Mary Washington commercial cultivar. Results showed that polyploid accessions of asparagus were more successful plants comparing to diploid plants in terms of plant height trait under drought stress condition. Baladeh, Gazanak, Mahmoudabad and Taleghan were the superior accessions with the highest recorded values of plant height and Makou, Ahar and Alborz accessions and Mary Washington commercial cultivar showed the lowest values of plant height under drought stress conditions. Studied asparagus accessions were divided into three groups in terms of dry weight of aerial parts of plant. The first group with high dry weight included Baladeh and Gazanak accessions, the second group with moderate dry weight included Alborz, Chalaki and Taleghan accessions and the third group with low dry weight included Ahar, Makou and Shiraz accessions and Mary Washington cultivar. The highest and lowest values of dry weight of underground parts of plant were recorded in Gazanak accession and Mary Washington cultivar (0.32 and 0.15 g, respectively). Dry weight of underground parts of Gazanak accession plants was 53.13% higher than that of Mary Washington commercial cultivar plants under drought stress conditions (Table 7).

The lowest values of dry weight of aerial and underground parts were recorded in plants grown under highest level of drought stress (0.06 and 0.09 g respectively). Increasing of drought stress severity from 100% FC to 25% FC led to a significant decrease in seed germination percentage and speed. No significant difference was observed between asparagus plants grown under 100, 75 and 50% FC in terms of plant height (Table 8).

Generally, Increasing of drought stress severity significantly reduced leaf diameter in all applied accessions. Baladeh (under control condition) and Ahar (under 25% FC drought stress) accessions showed the highest and lowest values of leaf diameter (0.44 and 0.19 mm, respectively). Increasing of drought stress severity from control condition to 25% FC reduced leaf diameter of Mary Washington asparagus plants by 20.69%. Ahar and Alborz accessions showed the lowest stem diameter under highest level of drought stress (0.25 and 0.35 mm, respectively). Under control condition, the highest values of stem diameter were related to Baladeh, Gazanak, Taleghan and Mahmoudabad accessions, respectively. Most variation of fresh weight of aerials parts was observed under control condition and highest level of drought stress. Increasing of drought stress severity from 75–50% FC had no significant effect on fresh weight of aerials parts in all applied accessions except Ahar accession. Under highest level of drought stress, Baladeh, Gazanak, Taleghan and Mahmoudabad accessions showed the highest values of fresh weight of plant aerial parts and in the other hand, the lowest values of this trait were related to Ahar accession (0.16 g) and Mary Washington commercial cultivar (0.15 g). All applied asparagus accessions divided into three groups in terms of effect of drought stress in highest level on fresh weight of plant underground parts. Balade and Gazanak were the superior accessions, Alborz, Chalaki, Taleghan and Mahmoudabad showed the moderate values and the lowest values of this trait were related to Makou, Ahar and Shiraz accessions and Mary Washington commercial cultivar (Table 9).

b) Physiological characteristics

Simple effects of accession and drought stress were significant on total chlorophyll and carotenoid content and electrolyte leakage. Also, free proline content of asparagus accessions was affected by interaction between accession and drought stress. According to mean comparing results, the highest total chlorophyll content was recorded in Baladeh, Gazanak and Mahmoudabad accessions under different applied levels of drought stress. Makou, Ahar and Shiraz accessions and Mary Washington cultivar were the least valuable plant materials in terms of total chlorophyll content under drought stress conditions. Total chlorophyll content of Mary Washington asparagus plants was 45.86% lower than that of Gazanak accession plants which showed the highest chlorophyll content under drought stress conditions ($1.81 \text{ mg g Fw}^{-1}$) (Fig. 7a). Increasing of drought severity from 100% FC to 75% FC did not cause a significant decrease in total chlorophyll content of asparagus accessions. The lowest asparagus plants chlorophyll content was recorded under stress level of 25% FC ($0.96 \text{ mg g Fw}^{-1}$) (Fig. 7b).

Baladeh accession showed the highest carotenoid content under drought stress conditions ($0.43 \text{ mg g Fw}^{-1}$) and in the other hand, Ahar and makou accessions were the least valuable plant materials in terms of this measured trait. There was no significant difference between Chalaki, Mahmoudabad and Taleghan accessions in total carotenoid content under different levels of drought stress (Fig. 8a). There was no significant difference between asparagus plants grown under stress levels of 100% and 75% FC in terms of total carotenoid content but increasing of drought stress severity to 50% FC led to a significant decrease in this trait. The lowest carotenoid content was recorded in plants grown under highest applied drought stress level ($0.352 \text{ mg g Fw}^{-1}$) (Fig. 8b).

The lowest electrolyte leakage was recorded in Gazanak and Baladeh accessions under drought stress conditions. Electrolyte leakage in Mary Washington asparagus plants (64.86%) was higher than that of Gazanak, Baladeh, Mahmoudabad, Chalaki, Taleghan and Alborz accessions and lower than that of Ahar, Makou and Shiraz accessions (Fig. 9a). According to obtained results, electrolyte leakage of asparagus plants cells increased with increasing of drought stress severity. Electrolyte leakage increased by 27.32% with increasing of drought severity from 75% FC to 50% FC (Fig. 9b).

Gozanak was the superior accession in terms of free proline content under highest applied level of drought stress ($4.5 \text{ } \mu\text{mol g Fw}^{-1}$) and the highest free proline content was recorded in Gazanak, Mahmoudabad and Taleghan accessions under drought stress level of 50% FC (3.66, 2.66 and $2.60 \text{ } \mu\text{mol g Fw}^{-1}$, respectively). Plants of Gazanak and Mahmoudabad accessions showed the highest free proline content produced in the tissue under drought stress level of 75% FC (2.25 and $2.00 \text{ } \mu\text{mol g Fw}^{-1}$, respectively).

Cluster analysis

A cluster analysis was performed in order to group of Iranian asparagus accessions based on measured characteristics under different applied levels of drought stress. Results showed that asparagus accessions were divided into four groups at Euclidean distance of 4. First group included Ahar, Makou and Shiraz accessions and Mary Washington cultivar (which according to drought tolerance assessment showed the low levels of resistance rate to drought stress conditions). Taleghan accession placed in the second group and third group included Alborz and Chalaki accessions. Accessions collected from north of Iran placed in the fourth group which based on drought tolerance assessment showed the highest level of tolerance rate to applied drought stress conditions (Fig. 10).

Discussion

Iran is one of biggest countries located in Middle East. Based on reports, more than 8,000 different plant species are distributed in different regions of Iran. Existence of different climate and soil characteristics in different regions of Iran could be considered as one of main reasons for high plant diversity of this country. Identification, genetic and morpho-physiological evaluation and introducing of superior plant wild accessions could be as first step to introduce desired characteristics into crops (Ebrahimi et al., 2021).

According to researches conducted in recent decades, Iran is one of important regions of asparagus plant distribution. *A. azerbaijanensis*, *A. breslerianus*, *A. bojnurdensis*, *A. khorasanensis*, *A. officinalis*, *A. persicus*, *A. touranensis* and *A. verticillatus* are some of asparagus species distributed in different areas of Iran and these asparagus species can be considered as valuable genetic resources for applying in asparagus plant breeding projects (Sarabi et al., 2010; Mousavizadeh et al., 2015; Hamdi and Asadi, 2017). For instance, *A. breslerianus* was introduced as salt resistant species by Mousavizadeh et al. (2017). Plants of this species were collected from gypsum hills located in central regions of Iran (Semnan province). Results of an *in-vitro* experiment showed that calli of *A. breslerianus* could tolerate NaCl treatment up to 109.4 mM and this species showed a high capacity of sodium accumulation in callus tissues.

Morphological assessment of four Iranian asparagus accessions (*A. breslerianus*, *A. officinalis*, *A. persicus* and *A. verticillatus*) showed that plant height and seed number per fruit were the evaluated traits with highest coefficients of variation (Mousavizadeh et al., 2015) which this result is in line with our obtained results for *A. azerbaijanensis* accessions. Also, the highest values of spear length and diameter were observed in *A. verticillatus* accessions (58.48 cm and 6.30 mm, respectively) which were 16.32% and 25.40% higher than those of *A. azerbaijanensis* accessions. Results of research on asparagus accessions collected from Taleghan mountains (located in Alborz province) showed that there is significant correlation between scale and spear characteristics which is in line with our obtained results for *A. azerbaijanensis* accessions (Sarabi et al., 2010). Based on our study, high morphological variation was detected in *A. azerbaijanensis* plants. So, using of different accessions of this species in asparagus breeding projects with aim of creating asparagus plants with desired vegetative and reproductive characteristics could be recommendable. Also, suitable size and delicious taste of spear in *A. azerbaijanensis* species has made it a suitable candidate for commercial cultivation and also improving spear characteristics by using selection method.

Drought stress is the most damaging environmental stresses in agriculture crops production worldwide (Ghahremani et al., 2020). Most regions of Iran have dry and warm climate and water deficit is most important limiting factor for agriculture and crop production in this country. Using wild dry resistant species in breeding projects with aim of producing resistant cultivars could be an effective method to overcome water deficit problem in dry lands (Ajtahed et al., 2021). For instance, a number of 20 Iranian fenugreek accessions were collected and drought tolerance indices of them were then assessed to introduce superior plant materials for using in future breeding programs. Results showed that Ardestan accession could be considered as a valuable genetic resource for using in fenugreek breeding projects. Also, correlation between grain yield and dry biomass was recommended as indicator for screening of drought tolerance rate in this crop (Sadeghzadeh et al., 2009). Results of our study showed that drought stress levels of 50 and 25% FC can seriously affect seed germination indices, growth parameters and physiological characteristics of asparagus plant. In a similar research, Drost and Wilcox-Lee (1997), reported that increasing of drought stress level from control to -0.5 MPa under greenhouse condition led to a significant decrease in fresh and dry weight of spear, number of nudes and fern and shelf life of spear. Results of a field research conducted by Zinkernagel and Kahlen (2017), showed that water deficit in asparagus cultivation can significantly affect shoot growth, total yield, stomatal conductivity and assimilation rate. Results of our study showed that Baladeh and Gazanak accessions could be as superior accessions with high drought tolerance rate. High resistance rate of these accessions can be related to their ploidy level and distribution areas characteristics. Baladeh and Gazanak are polyploid accessions with high ploidy level (8x) and they are distributed in mountainous areas characterized by rocky soils and low precipitations (Mousavizadeh et al., 2015) (located in Mazandaran province, north of Iran). Studies showed that plants with higher ploidy levels are generally more successful in confronting with biotic and abiotic stresses. Increasing of chromosome sets can lead to a significant increase in metabolites and enzymes production by plant cells under stress condition, so plants can subsequently neutralize adverse effects of stresses with more power (Vandenhout et al., 1995). For instance, results of a research on responses of different wheat accessions (with different ploidy levels: 2x, 4x and 6x) to drought stress showed that the highest amounts of biochemical compounds, enzymes and different proteins effective on stress neutralization were recorded in hexaploid accession under different applied levels of drought stress and polyploid plants (4x and 6x) showed the higher values of morpho-physiological characteristics comparing to diploid plants (2x) (Wang et al., 2017). Also, Chandra and Dubey (2010), reported that activity rate of guaiacol peroxidase enzyme and malon de aldehyd accumulation in diploid (2x) accessions of *Cenchrus* species were higher than those of tetraploid (4x) accessions under drought stress conditions. But in the other hand, free proline content of tetraploid (4x) plants cells was higher than that of diploid (2x) plants.

Conclusion

In conclusion, results of our study showed that wild accessions of *A. azerbaijanensis* species, due to their high morphological variation and desired spear taste and size, are suitable candidates to apply in asparagus breeding programs with the aim of producing hybrids plants with superior vegetative growth. Also, the superiority of Iranian asparagus different accessions (specially Baladeh and Gazanak accessions) over the commercial cultivar in tolerating of water deficit stress, promises the possibility of further using of Iranian asparagus accessions in breeding projects with aim of producing dry resistant cultivars.

Declarations

Funding:

This work was supported by university of Zanjan (grant number 00.01.360).

Conflicts of interest:

We have no conflicts of interest to disclose.

References

1. Ajtahed SS., Rezaei A, Hosseini Tafreshi, SA (2021) Identifying superior drought-tolerant Bermudagrass accessions and their defensive responses to mild and severe drought conditions. *Euphytica* 217: 91. <https://doi.org/10.1007/s10681-021-02821-z>
2. Amian L, Rubio J, Castro P, Gil J, Moreno R (2018) Introgression of wild relative *Asparagus* spp. germplasm into the Spanish landrace 'Morado de Huétor'. *Acta Horti* 1223: 33-38.
3. Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39: 205-207.
4. Chandra A, Dubey A (2010) Effect of ploidy levels on the activities of Δ^1 -pyrroline-5-carboxylate synthetase, superoxide dismutase and peroxidase in *Cenchrus* species grown under water stress. *Plant Physiol Biochem* 48: 27-34.
5. Czabator FJ (1962) Germination value: An index combining speed and completeness of pine seed germination. *For Sci* 8: 386-395.
6. Drost D, Wilcox-Lee D (1997) Soil water deficits and asparagus: I. Shoot, root, and bud growth during two seasons. *Sci Hort* 70: 131-143.
7. Ebrahimi A, Poursalavati A, Mohamadi Esboei M, Rashidi Monfared S (2021) Population and individual multivariate analysis of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry genotypes: applications for breeding, conservation and development. *Euphytica* 217: 152. <https://doi.org/10.1007/s10681-021-02844-6>
8. Ghahremani Z, Mikaealzadeh M, Barzegar T, Ranjbar ME (2020) Foliar application of ascorbic acid and gamma aminobutyric acid can improve important properties of deficit irrigated cucumber plants (*Cucumis sativus* cv. Us), *Gesunde Pflanzen* 73: 77-84. <https://doi.org/10.1007/s10343-020-00530-6>
9. Hamdi SMM, Assadi M (2017) *Asparagus azerbaijanensis*, a new species of *Asparagus* subgen. *Asparagopsis* (Asparagaceae) from Iran. *Phytotaxa*. 297(1): 93-96.
10. Kanno A, Yokoyama J (2011) Asparagus. In *Wild Crop Relatives: Genomic and Breeding Resources*, K. Chittaranjan, ed. (Berlin, Germany: Springer Berlin Heidelberg). pp.23–42.
11. Lutts S, Kinet JM, Bouharmont J (1996) NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann Bot* 8: 389-398.
12. Mousavizadeh SJ, Hassandokht MR, Kashi A (2015) Multivariate analysis of edible *Asparagus* species in Iran by morphological characters. *Euphytica*. 206: 445-457.
13. Mousavizadeh SJ, Hassandokht MR, Kashi A (2017) In vitro response of *Asparagus breslerianus* to NaCl. *J Med Plant By-product* 2: 153-163.
14. Sadeghzadeh Ahari D, Kashi AK, Hassandokht MR, Amri A, Alizadeh K (2009) Assessment of drought tolerance in Iranian fenugreek landraces. *J Food Agric Environ* 7(3&4): 414-419.
15. Sarabi B, Hassandokht MR, Hassani ME, Ramak-Masoumi T, Rich T (2010) Evaluation of genetic diversity among some Iranian wild asparagus populations using morphological characteristics and RAPD markers. *Sci Hort* 126:1-7.
16. Vandenhout H, Ortiz R, Vuylsteke D, Swennen R, Bai KV (1995) Effect of ploidy on stomatal and other quantitative traits in plantain and banana hybrids. *Euphytica* 83: 117-122. <https://doi.org/10.1007/BF01678038>
17. Wang JY, Turner NC, Liu YX, Siddique KHM, Xiong YC (2017) Effects of drought stress on morphological, physiological and biochemical characteristics of wheat species differing in ploidy level. *Funct Plant Biol* 44: 219-234.
18. Witham FH, Blaydes DF, Devlin RM (1971) *Experiments in Plant physiology*. Vannoststrand New York, P, 245.

19. Zinkernagel J, Kahlen K (2017) Water relations of asparagus crop under different water supply and implications for plant-based irrigation scheduling. *Acta Hort* 1150: 385-390.

Tables

Table 1
Climate characteristics, geographical location and soil properties of distribution areas

Species	Sample collection area	Geographical location of collection area		Altitude (m)	Total annual precipitation (mm)	Average of annual temperature (°C)	Average of relative humidity (%)	Soil pH	Soil EC (dS/m)	Climate
		Longitude (East)	Latitude (North)							
<i>A. azerbaijanensis</i>	Makou	44° 24'	39° 21'	1296	164.5	12.3	51	8	1.6	Semi-dry cold
<i>A. azerbaijanensis</i>	Ahar	47° 32'	38° 45'	1331	295.5	9.5	62.9	7.8	1.5	Semi-humid cold

Table 2
Ploidy level and origin of collected accessions and distribution areas climate characteristics

Species	Code	Ploidy level	Type	origin and climate characteristics
<i>A. azerbaijanensis</i>	AH	*	Wild accession	East Azerbaijan, Ahar (Cold and semi-humid)
<i>A. azerbaijanensis</i>	MK	*	Wild accession	West Azerbaijan, Makou (Cold and semi-dry)
<i>A. officinalis</i> L.	MW	2x	Commercial cultivar	Mary Washington
<i>A. officinalis</i> L.	AL	2x	Naturalized accession	Alborz, Karaj (Cold Mediterranean)
<i>A. officinalis</i> L.	TA	4x	Wild accession	Alborz, Taleghan (Cold Mediterranean)
<i>A. officinalis</i> L.	MA	4x	Wild accession	Mazandaran, Mahmoudabad (Wet-warm temperate)
<i>A. officinalis</i> L.	GZ	8x	Wild accession	Mazandaran, Gazanak (Semi-humid Mediterranean)
<i>A. officinalis</i> L.	BL	8x	Wild accession	Mazandaran, Baladeh (Cold temperate)
<i>A. officinalis</i> L.	SH	*	Wild accession	Fars, Shiraz (Warm and Semi-dry)
<i>A. verticillatus</i> L.	CH	2x	Wild species	Golestan, Chalaki (Wet temperate)

Data in the table is based on researches conducted by Mousavizadeh et al., 2015, 2016 and Hamdi and Assadi, 2017

*: There is no valid report of ploidy level determination

Table 3
Characteristics of applied soil

Clay (%)	Silt (%)	Sand (%)	Soil texture	Organic matter (%)	K (g kg ⁻¹)	Na (g kg ⁻¹)	Ca (g kg ⁻¹)	N (%)	EC (ds m ⁻¹)	pH
36	38	26	Clay loam	0.94	0.2	0.12	0.11	0.08	1.43	7.3

Table 4
Characteristics of applied irrigation water

SO ₄ ⁻² (mg.l ⁻¹)	HCO ₃ ⁻ (mg.l ⁻¹)	CO ₃ ⁻² (mg.l ⁻¹)	Cl (mg.l ⁻¹)	Mg (mg.l ⁻¹)	Ca (mg.l ⁻¹)	K (mg.l ⁻¹)	Na (mg.l ⁻¹)	EC (dS.m ⁻¹)	pH
549.02	160	0.01	435.3	240.9	397	2.71	152	1.76	7.2

Table 5
Evaluated morphological traits and mean comparing results

	Unit	Max	Min	Mean	CV %	Makou accession	Ahar accession
Plant height	cm	177	72.50	131.87	18.12	134.34a	129.40a
Plant height to the first branch	cm	112.60	17.80	36.51	48.09	37.14a	35.87a
Diameter of main stem	mm	10.20	1.71	5.75	27.92	5.57a	5.93a
Height of main stem	cm	162	68	111.54	21.13	105.78a	117.31a
Number of primary branches	-	58	20	37.53	30.96	28.86b	46.20a
Number of secondary branches	-	26.66	6.33	14.02	38.42	9.89b	18.15a
Length of primary branches	cm	45.76	19.23	33.42	19.22	32.37a	34.47a
Length of secondary branches	cm	13.63	4.56	9.16	4.56	10.05a	8.27b
Internode length of primary branches	cm	3.36	0.90	2	29.52	2.17a	1.84a
Internode length of secondary branches	cm	1.70	0.40	1.03	34.49	1.25a	0.82b
Diameter of primary branches	mm	1.92	0.75	1.39	21.72	1.46a	1.32a
Diameter of secondary branches	mm	1.11	0.32	0.72	28.92	0.58a	1.86a
Scale number	-	18	7	12.90	24.46	10.93b	14.86a
Cladode number	-	16.33	6	10.28	25.37	9.30b	11.26a
Diameter of leaf	mm	0.36	0.12	0.24	29.92	0.29a	0.18b
Length of leaf	cm	2.23	0.50	1.29	39.03	1.71a	0.87b
Seed diameter	mm	4.83	2.66	4.16	11.91	4.20a	4.11a
Seed number per fruit	-	5	1	2.37	40.05	2.08a	2.66a
Fruit diameter	mm	8.90	5.71	6.92	11.24	6.81a	7.13a
Fruit number per plant	-	362	37	159.4	42.93	155.59a	163.23a
Spear length	cm	60.60	26.32	48.94	26.26	50.42a	46.48b
Spear diameter	mm	6.98	2.80	4.70	24.03	4.38b	5.12a

In each row having at least one common letter are not statistically different at 1% level according to t test

Table 6
Correlation between evaluated morphological traits and spear properties in Makou and Ahar accessions

	Plant height	Plant height to the FB	Diameter of stem	Height of stem	Diameter of SB	Diameter of PB	Number of PB
SL	-0.15ns	-0.12ns	-0.01ns	0.10ns	0.15ns	-0.22ns	0.64**
SD	0.05ns	0.06ns	-0.17ns	-0.27ns	-0.13ns	-0.11ns	-0.75**
	Number of SB	Internode length of PB	Internode length of SB	Length of SB	Length of PB	Length of leaf	Diameter of leaf
SL	0.60**	-0.27ns	-0.67**	-0.33ns	0.08ns	-0.85**	-0.65**
SD	-0.75**	0.24ns	0.55**	0.23ns	-0.18ns	0.74**	0.63**
	Cladode number	Scale number	Fruit number per plant	Fruit diameter	Seed number per fruit	Seed diameter	Spear length
SL	0.24ns	0.50**	0.01ns	0.01ns	0.25ns	0.12ns	1**
SD	-0.39ns	-0.60**	-0.02ns	-0.31ns	-0.32ns	0.05ns	-0.69**

**Significant at 1% level, *Significant at 5% level and ns Non-significant

SL (spear length), SD (spear diameter), FB (first branch), PB (primary branch), SB (secondary branch)

Table 7
Seed germination indices and seedling growth characteristics of Iranian asparagus accessions under drought stress conditions

Accession	Germination percentage	Germination speed	Plant height	Dry weight of aerial parts	Dry weight of underground parts
AL	35e	1.21b	21.53c	0.22ab	0.18ab
AH	39.16de	2.23a	22.77c	0.13b	0.16ab
BL	71.66a	2.77a	90.38a	0.31a	0.21ab
CH	35e	0.98b	26.98bc	0.22ab	0.17ab
SH	48.33cd	2.35a	34.90ab	0.16b	0.24ab
TA	35.83e	1.40b	39.32a	0.24ab	0.21ab
GZ	70.83a	2.87a	41.75a	0.31a	0.32a
MK	39.16de	2.27a	25.64c	0.11b	0.16ab
MA	64.16ab	2.42a	38.98a	0.23ab	0.19ab
MW	58.33bc	1.28b	23.35c	0.15b	0.15b

In each row having at least one common letter are not statistically different at 1% level according to Tukey's test

Accessions code is based on Table 2

Table 8
Effect of drought stress different levels on Seed germination indices and seedling growth characteristics

Drought stress (%FC)	Germination percentage	Germination speed	Plant height	Dry weight of aerial parts	Dry weight of underground parts
100	33a	2.91a	33.48a	0.31a	0.31a
75	23b	2.41b	33.88a	0.25ab	0.21b
50	14.66c	1.64c	33.62a	0.21b	0.29b
25	8.33d	0.95d	25.67b	0.06c	0.09c

In each row having at least one common letter are not statistically different at 1% level according to Tukey's test

Table 9
Effect of interaction between accession and drought stress on measured characteristics

Different accessions of Iranian asparagus										
Leaf diameter (mm)										
Drought stress (%FC)	AL	AH	BL	CH	SH	TA	GZ	MK	MA	MW
100	0.367a-f	0.28c-i	0.44a	0.33a-h	0.30b-i	0.39a-d	0.42ab	0.29b-i	0.361a-g	0.29b-i
75	0.31a-i	0.24e-i	0.41abc	0.29b-i	0.30b-i	0.31a-i	0.40a-d	0.27d-i	0.35a-g	0.28c-i
50	0.30b-i	0.20hi	0.38a-d	0.29b-i	0.28c-i	0.28c-i	0.40a-d	0.23e-i	0.33a-h	0.24e-i
25	0.28c-i	0.19i	0.34a-g	0.20h-i	0.233f-i	0.27c-i	0.37a-e	0.22ghi	0.31a-i	0.236e-i
Stem diameter (mm)										
	AL	AH	BL	CH	SH	TA	GZ	MK	MA	MW
100	0.99a-i	0.76d-j	1.6a	0.85b-j	0.65f-j	1.46abc	1.50ab	0.79c-j	1.36a-d	0.79c-j
75	0.89b-j	0.71d-j	1.34a-e	0.85b-j	0.62g-j	1.32a-f	1.07a-h	0.70d-j	1.12a-h	0.78c-j
50	0.86b-j	0.63g-j	1.30a-g	0.81b-j	0.61g-j	1.21a-h	0.74d-j	0.67e-j	1.10a-h	0.75d-j
25	0.35i-j	0.25j	0.73d-j	0.65f-j	0.57hij	0.69d-j	0.74d-j	0.63g-j	0.68d-j	0.67e-j
Fresh weight of aerial part (g)										
	AL	AH	BL	CH	SH	TA	GZ	MK	MA	MW
100	0.86abc	0.63abc	1.83a	1.43abc	0.60abc	1.72ab	1.90a	0.56abc	1.74ab	0.90abc
75	0.51abc	0.57abc	1.36abc	1.24abc	0.52abc	1.36abc	1.28abc	0.48abc	0.79abc	0.83abc
50	0.50abc	0.37bc	1.00abc	0.90abc	0.48abc	1.01abc	0.93abc	0.46abc	0.79abc	0.70abc
25	0.23c	0.16c	0.40bc	0.19c	0.28c	0.45bc	0.70abc	0.30c	0.42bc	0.15c
Fresh weight of underground part (g)										
	AL	AH	BL	CH	SH	TA	GZ	MK	MA	MW
100	1.50abc	1.32abc	1.69ab	0.83abc	1.32abc	1.44abc	1.95a	0.86abc	1.35abc	1.29abc
75	0.88abc	0.76abc	1.47abc	0.80abc	0.83abc	0.87abc	0.86abc	0.86abc	0.80abc	0.70abc
50	0.70abc	0.62abc	1.24abc	0.49bc	0.65abc	0.69abc	0.77abc	0.30bc	0.64abc	0.51abc
25	0.26bc	0.16c	0.71abc	0.41bc	0.22c	0.25bc	0.77abc	0.20c	0.24bc	0.16c

In each row and column having at least one common letter are not statistically different at 1% level according to Tukey's test

Accessions code is based on Table 2

Table 10
Effect of interaction between accession and drought stress on free proline content

Different accessions of Iranian asparagus										
Free proline content ($\mu\text{mol g Fw}^{-1}$)										
Drought stress (%FC)	AL	AH	BL	CH	SH	TA	GZ	MK	MA	MW
100	0.79e-h	0.23h	1.18d-h	0.70fgh	0.11h	1.16d-h	1.97c-g	0.20h	1.35d-h	1.50d-h
75	1.18d-h	0.45gh	1.94d-g	1.17d-h	0.48gh	1.72d-h	2.25c-f	0.41gh	2.00c-g	1.70d-h
50	2.08c-g	1.70d-h	2.11c-g	1.97c-g	1.19d-h	2.60bcd	3.66a-b	1.70d-h	2.66bcd	2.36b-f
25	2.34b-f	2.11c-g	3.15abc	2.35b-f	1.2d-h	3.03abc	4.50a	2.08c-g	3.00abc	2.60bcd

In each row and column having at least one common letter are not statistically different at 1% level according to Tukey's test

Accessions code is based on Table 2

Figures



Figure 1

Distribution regions of *A. azerbaijanensis* wild accessions



Figure 2

a & b: Ahar accession plants c & d: Makou accession plants e: aerial part of plant (Makou accession) f: cladode and leaves (Ahar accession)
g: fruits (Ahar accession)

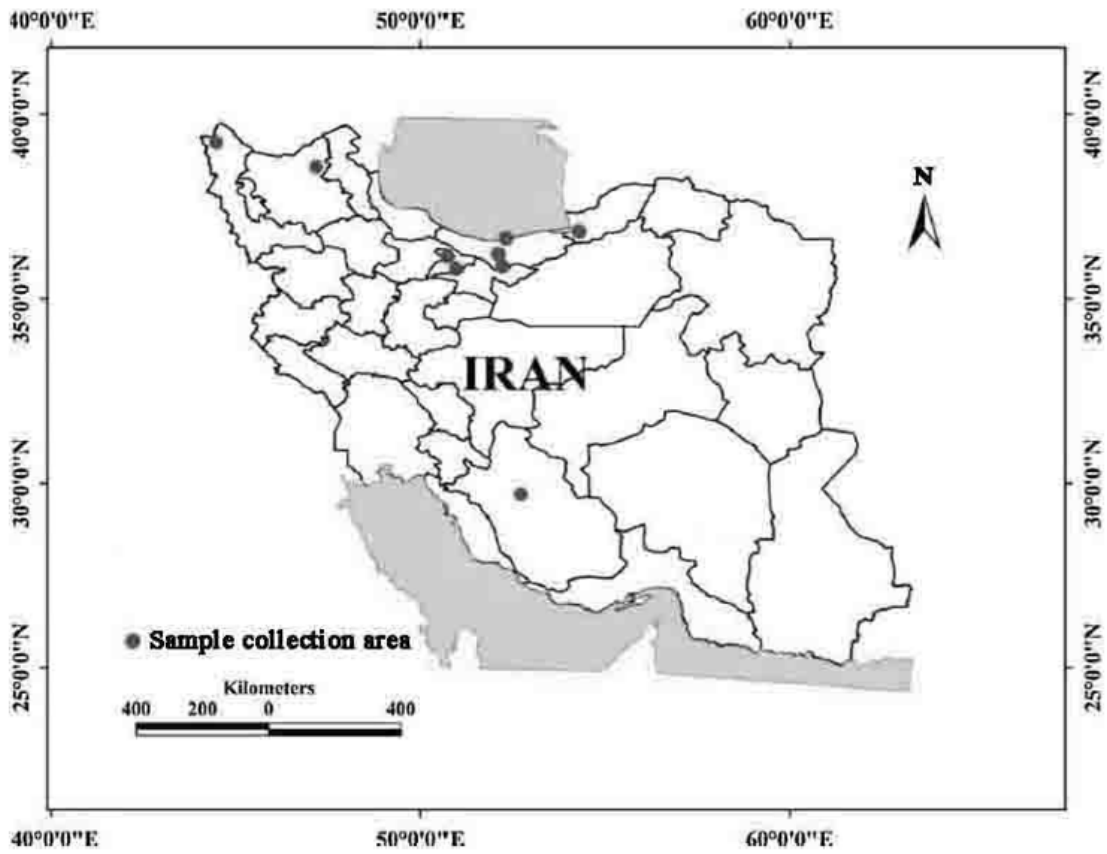


Figure 3

Locations of asparagus plants distribution areas

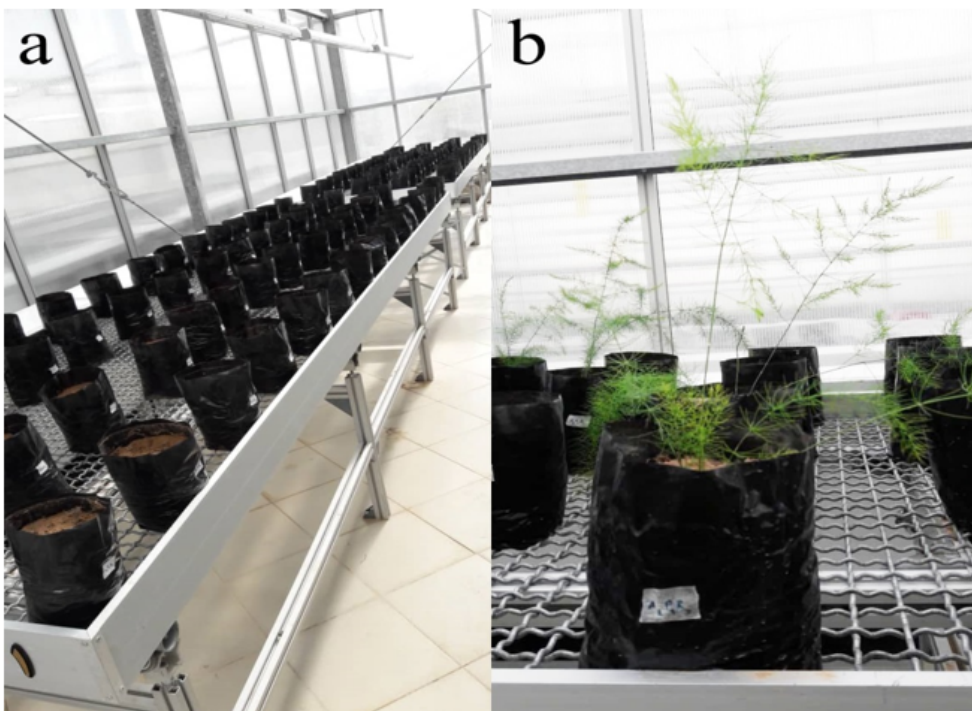


Figure 4

a: experimental unites b: Gazanak accession seedling

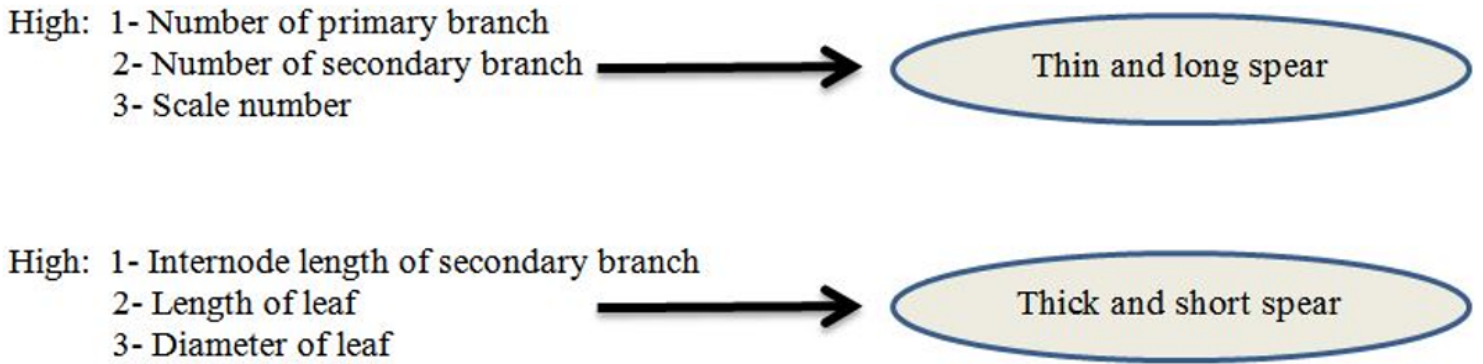


Figure 5

Correlation between evaluated morphological traits and length and diameter of spear

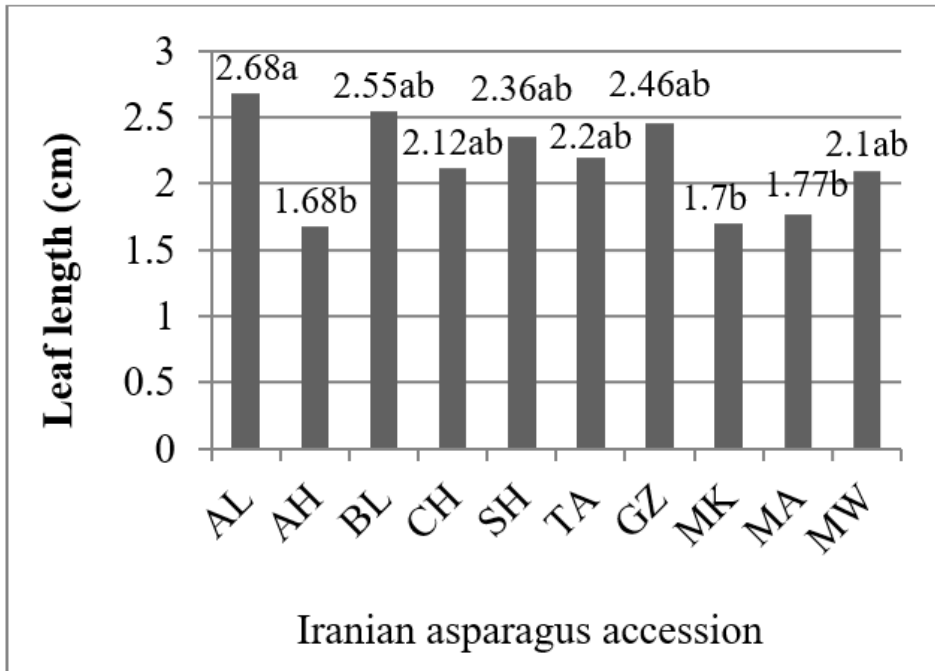


Figure 6

Effect of accession on length of leaf (Accessions code is based on Table 2)

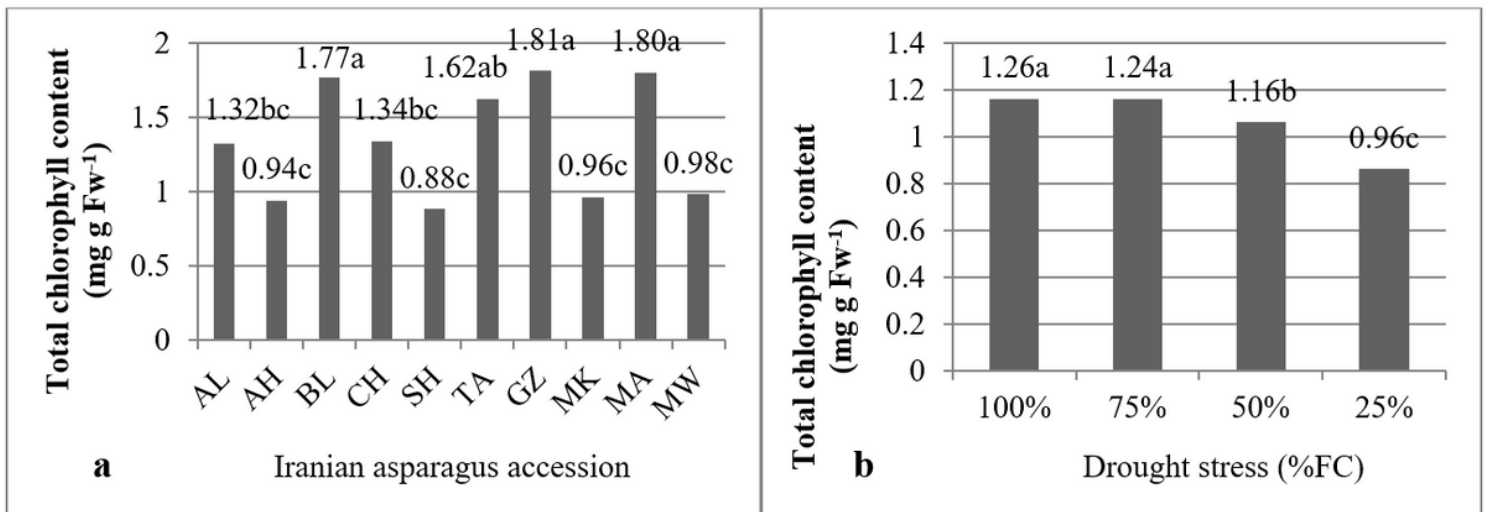


Figure 7

Effect of accession (a) and drought stress (b) on total chlorophyll content of asparagus plants

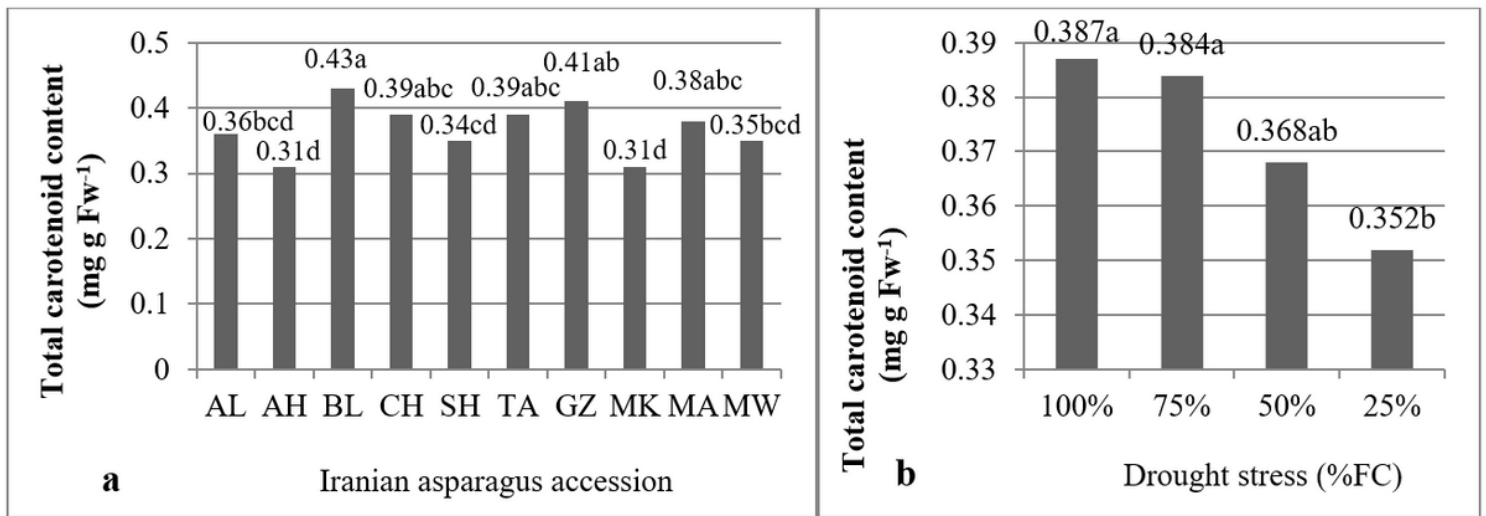


Figure 8

Effect of accession (a) and drought stress (b) on total carotenoid content of asparagus plants (Accessions code is based on Table 2)

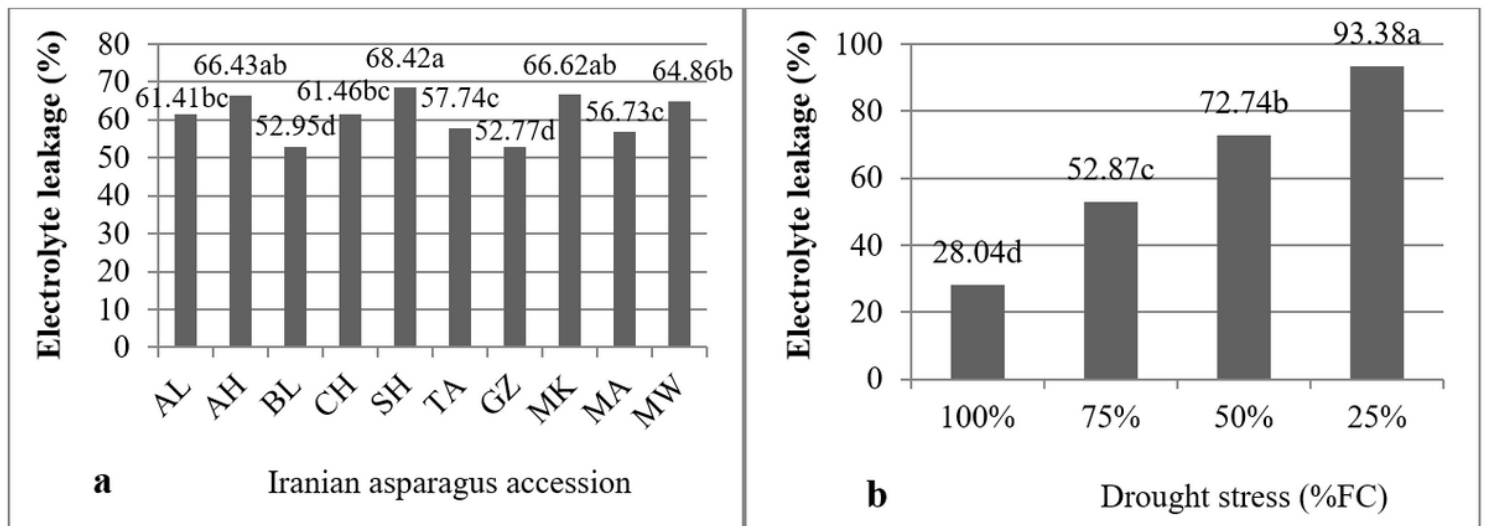


Figure 9

Effect of accession (a) and drought stress (b) on electrolyte leakage percentage (Accessions code is based on Table 2)

Dendrogram using Ward Method

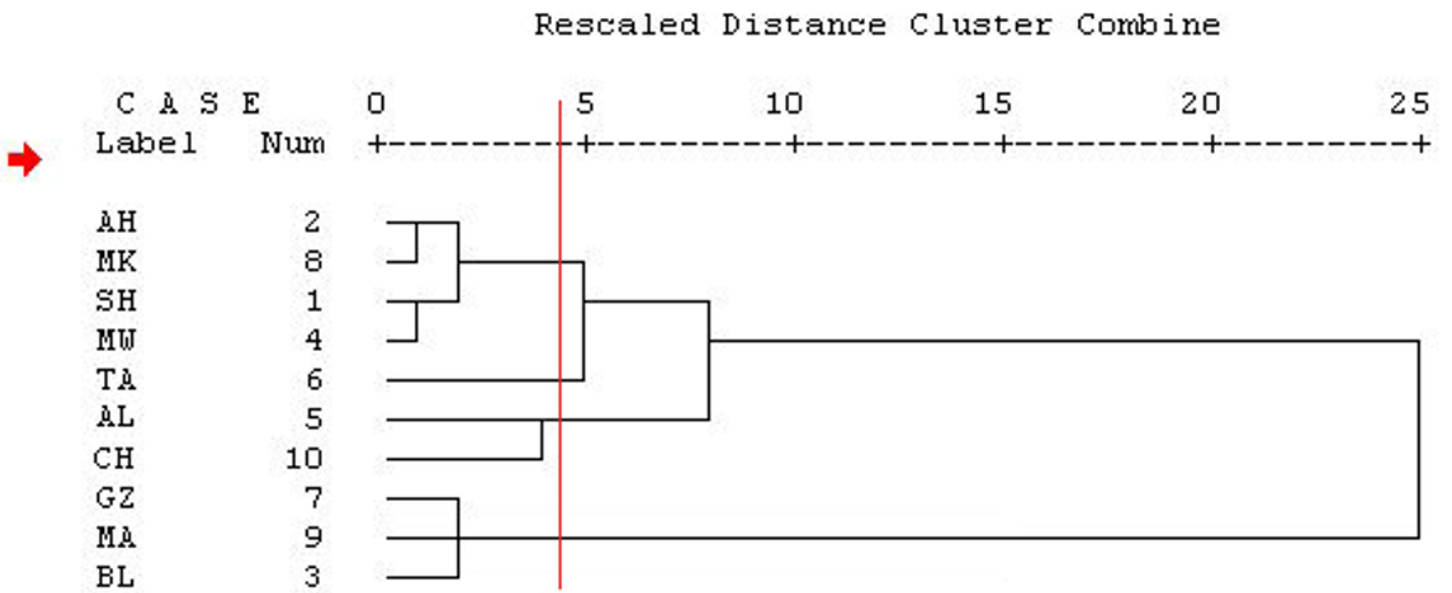


Figure 10

Cluster analysis based on measured characteristics under drought stress conditions (Accessions code is based on Table 2)