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Investigation of Phytochemical Characteristics and Genetic Diversity of Plantago Ovata under Drought Stress

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Abstract

Background:

Plantago is widely used as a medicinal supplement due to its rich content of active polysaccharides. To study the effects of drought stress on phytochemical characteristics (based on genetic variation), a test was performed in two laboratory and greenhouse conditions on P.ovata species.

Methods:

In the laboratory phase, this experiment was conducted in a factorial design with a completely randomized design with three replications, under drought stress conditions. The treatments included, examining the species of P.ovata with accessions (Ilam-Dehloran, Alborz, Central, Bushehr-Dashtestan, Hormozgan -1, Hormozgan -2, South Khorasan-Cain, and South Khorasan-Sarbisheh). Drought stress was considered as an invoice. In the laboratory phase, using electrophoresis, the evaluation of proteins separated in SDS-PAGE indicates the presence of genetic diversity as well as the phytochemical differences between different populations.

Results:

The studies showed that P. ovata species in extreme stress levels reacted and moderate efficiency. The pattern of Dendrogram patterns shows that these populations were grouped in 4 evolutionary clouds (Clade I, II, III, and IV). From the perspective of functional differences, the population of P.ovata Dashtestan (76%) was the highest amount of these proteins. According to the results of the comparison of the mean of P. ovata species, it was found that Ilam-Dehloran oxidation in peroxidase-polyphenol-protein-protein traits was best, and this species is a tolerant species of drought stress.

Conclusion:

According to the laboratory level and the greenhouse of P.ovata species, it has the potential to cultivate in dry and semi-arid regions with stress levels used in this experiment.

Keywords: phytochemical characteristics; genetic diversity; plantago ovata; drought stress; p.lanceolata and p.ovata, biochemical characteristics; genetic diversity.

Introduction:

Many Plantago species, which are found all over the world, are used as herbal medicines. Phytochemical studies of various organs of this plant (leaves, stems, etc.) show their high potential to produce a wide range of biologically active secondary metabolites [1]. This plant is cultivated in some parts of Iran due to its medicinal and commercial advantages [2]. Psyllium seeds and leaves contain Aucubin glycosides [3], tannins [2], and xylene [4]. Psyllium is also rich in mucilage [5]. Its shell powder was widely used as a laxative [6].

The husk mucilage is used for the treatment of constipation and irritation of the digestive tract and it acts as a laxative, anti-diabetic, cholesterol-lowering, and hemorrhoid remedy and is also found to be helpful for weight loss and arthritis treatment [7].

Plantago ovata is a medically and economically important species of the monotypic genus of Plantago. P. ovata is an annual plant whose bark is commonly called psyllium and is a very effective laxative. Other uses for Plantago ovata psyllium include ice cream, cosmetics, printing, and finishing. Consumption of its shell also lowers blood cholesterol levels and is very important commercially [8].

Global agricultural and food production is affected by various environmental stressors, especially drought and salinity [10]. These stressors inhibit plant growth and significantly reduce crop productivity and may even jeopardize overall yield. Currently, salinity affects 25 to 30% of the total arable land and 33 to 50% of irrigated land [11].

It is predicted that this situation will worsen due to the consequences of climate change [12], the need for more irrigation has led to the use of lower quality water, which in turn increases the soil salinity rate [13]. Salinity due to osmotic stress and ion toxicity, impairs plant growth and development, inhibits cell function, and ultimately causes plant death [14]. Salinity stress causes ionic and osmotic imbalances, oxidative stress [15], and also reduced photosynthetic, physiological, metabolic, and molecular changes in plants, seed germination is delayed or completely inhibited, high seedling mortality [16], or a general inhibition of photosynthesis and growth occurs [17]. Most plants are glycophytes and are sensitive to salinity. A small group of them are also halophytes and can complete their life cycle in saline soils [18].

The genus Plantago is particularly interesting for studying the mechanisms of salt tolerance in plants, as it includes halophytes and glycophytes, as well as species that are compatible with xeric environments [19].

In this study, different seeds of 8 populations of Plantago ovata in the Natural Resources Gene Bank of Iran were selected. Phytochemical properties were studied under drought treatment at different concentrations. To compare different seeds, test and control populations were studied under four treatments including: adequate irrigation (95% control), low drought stress (75% field capacity), mild drought stress (55% field capacity), and severe drought stress (35% field capacity) .

For this purpose, changes in osmotic protective solutions (proline and soluble sugars), proteins, relative moisture content (RWC), antioxidant enzymes (peroxidase and polyphenol oxidase), and pigments under stress were studied. The study of ecotypes of psychedelic species by biochemical characteristics makes it possible to identify the genetic diversity of different species.

Methods:

In this study, Plantago ovata were investigated in the following four levels of drought stress:

1- No stress or control (95% of field capacity)
2- Mild stress (75% of field capacity)
3- Moderate stress (55% of field capacity) and
4- Severe stress (35% of field capacity)

The characteristics of the genetic materials, genotype code, plant species, population code, and the locations are shown in Table 1.

Genotype code	Plant species	Location
1	*Plantago ovata*	Ilam-Dehloran
2	*Plantago ovata*	Alborz
3	*Plantago ovata*	Central
4	*Plantago ovata*	Bushehr-Dashtestan
5	*Plantago ovata*	Hormozgan -1
6	*Plantago ovata*	Hormozgan -2
7	*Plantago ovata*	South Khorasan-Qaen
8	*Plantago ovata*	South Khorasan-Sarbisheh

Table 1: Plantago ovata accessions and related locations

In order to evaluate genetic diversity based on biochemical characteristics, 12 accessions (population) of two species of Plantago ovata (8 populations) and Plantago lanceolata (4 populations) of Plantaginaceae were selected from different cities in Iran. Osmotic protective solutions (proline and soluble sugars), protein spectrophotometer, relative water content (RWC), antioxidant enzymes (peroxidase and polyphenol oxidase), and plant pigments under stress were also studied.

To extract the protein extract, 0.5g of fresh plant tissue was ground in a porcelain mortar with liquid nitrogen and then 1 ml of Tris-HCl buffer was added, 0.05 M with pH=7.5. The resulting homogeneity was centrifuged for 15 minutes at 11,000 rpm at 4°C and the supernatant was used to measure enzyme activity

Peroxidase activity was also measured by Kar and Mishra (1976) method (20). 50µl of the protein extract was added to a 2.5 mL extraction buffer containing 100 mM Tris-HCl buffer, 5mM oxygenated water and 10mM Pyrogallol in an ice bath, and the absorption change curve at 425 nm was read.

To measure the activity of polyphenol oxidase enzyme, 0.1M phosphate buffer and 0.02 M Pyrogallol substrate were used. In a cold porcelain mortar, mix 0.5 g of fresh vegetable tissue with 1.5ml of 0.1M phosphate buffer and mash well. The resulting mixture was centrifuged at 4000 rpm for 20 minutes and the supernatant was used as the source of the enzyme for 2 to 4 hours. The supernatant should be stored on ice until the evaluation. Then 2 ml of buffer solution and 50 µg of enzyme extract were mixed well. The cuvette was placed on a spectrophotometer as a blank and the absorbance was read at 420 nm. 100µl of pyruvate solution was added to the cuvette spectrophotometer and mixed well.

The protein content of the samples was also measured by the Bradford method (1976) (21). 0.5g of plant tissue was extracted by crushing with 0.6 ml of buffer and centrifuged for 15 minutes at 11000 rpm and 4 °C. The floating supernatant was then poured into new tubes and centrifuged for 4 minutes at 4,000rpm (albeit for 20 seconds) and finally the supernatant was removed. To measure the amount of protein, 10µl of the extract was added to 5 ml of Bradford solution and 290µl of extraction buffer and the adsorption rate was read at 595 nm.

To measure proline, the leaf sample was removed from the pod area and immediately transferred to the laboratory. First, 0.5g of

healthy leaves without necrotic spots were weighed and ground in porcelain mortar. Then 10 ml of 3% sulfosalicylic acid was added to it and the contents of the mortar were stirred and then the contents of the mortar were filtered. 2 ml of the resulting solution plus 2ml of ninhydrin acid and 2 ml of acetic acid were placed in a boiling bath (100°C) for one hour.

Changes in osmotic protective solutions (proline and soluble sugars), protein spectrophotometer, relative water content (RWC), antioxidant enzymes (peroxidase and polyphenol oxidase) and plant pigments under stress were studied. The protein content of the samples was also measured by Bradford (1976) method. Proline was measured by colorimetric measurement. Relative humidity was measured using the following equation: Rwc= (Fw-Dw)/ (Tw-Dw) ×100

To determine the molecular weight of the bands, a standard marker containing 5 proteins with specific molecular weights was used. Data related to each chemical property are subjected to different treatments (control and test groups at different levels of drought stress including -0.3 -0, -0.6, -0.9, and -1.2 MPa). Statistical analysis was performed based on analysis of variance of factorial design randomly with 2 factors (Factors related to plant species and drought stress, respectively) in 2 species (12 populations in total and 3 replications).

After confirming the significant difference between the genotypes, the comparison of the mean values was performed by Duncan's tests. Correlation coefficients between the mean of traits in each of the treatments and the mean of the total were calculated. Genotypes were identified by cluster analysis and principal component analysis.

Statistical analysis was performed by principal component analysis (PCA). In order to evaluate the genetic diversity based on biochemical and morpho- physiological characteristics, 12 accessions (population) from Plantago ovata (8 populations) from different locations were selected.

Results:

The results of the variety of proteins extracted and separated on polyacrylamide gel from the studied samples are shown in figure 1.

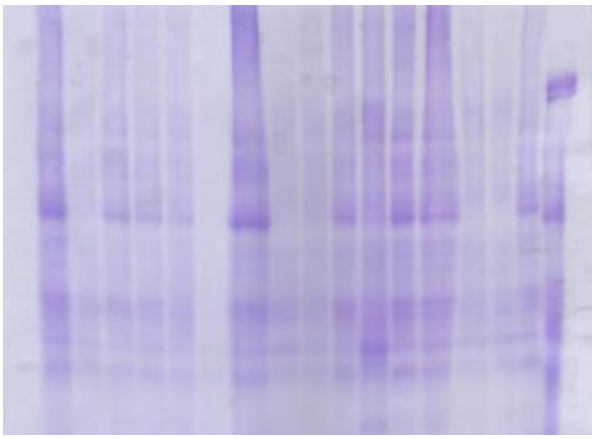

Figure 1: SDS PAGE protein pattern of populations of Plantagoovata.

The results show that the populations related to Bandar Abbas and Karaj had the highest and the populations of Khorramabad and Meshgin shahr the lowest number of alleles of these proteins. The total volume of proteins separated on the polyacrylamide matrix, including structural proteins and functional proteins and peptides among different populations, was investigated.

The population of P. ovata in Dashtestan region has the highest amount (76%) of proteins. Other populations show a homogeneous and similar pattern. The differences between the ecotypes may be due to differences in environmental conditions such as climate, soil quality, light and other biological factors.

The results of analysis of variance regarding the effect of drought stress on physiological characteristics of P.ovata in greenhouse conditions show that the effect of the species was significant in all traits. The effect of drought stress on the levels of peroxidase, polyphenol oxidase, chlorophyll A, carotenoids, chlorophyll A and B, chlorophyll A/B ratio, protein and relative humidity were significant at the level of one and five percent.

Comparison of traits in greenhouse conditions:

Comparison of the mean effect of drought stress on the amount of peroxidase in P. ovata accessions showed that mild stress treatment had the highest rate (9.34) and moderate stress treatment had the lowest rate (3.88), respectively.

Polyphenol Oxidase:

Comparison between different accessions of Plantago ovata for polyphenol oxidase showed that the highest value with an average of 3.98 was observed in Ilam-Dehloran accession. However, there was no statistically significant difference between Bushehr-Dashtestan and Hormozgan-1 accessions. The lowest amount of polyphenol oxidase was observed in Alborz access with an average of 2.39.

Based on the mean squares of drought stress treatment among P.ovata accessions, the highest amount of polyphenol oxidase (3.15) was observed in moderate and the lowest amount (2.63) was observed in severe stress. Likewise, comparing the mean interaction of P.ovata treatment and drought stress showed that “Ilam-Dehloran” accession with mild stress had the highest amount of polyphenol oxidase (with an average of 5.32) and the interactions of “South Khorasan-Sarbisheh” accession with severe stress, they had the lowest rate (the mean value: 2.01).

Carbohydrate:

The mean squares between Plantago ovata accessions for carbohydrate trait showed that the highest value (with an average of 67.11 mg/g fresh weight) for “Bushehr-Dashtestan” and the lowest value (with an average of 34.16 mg/g fresh weight) for “Alborz" were obtained, respectively. The results of analysis of variance for carbohydrate trait showed that P.ovata was not affected by the main drought stress treatment.

Proline:

The results of analysis of variance of drought stress treatment in

proline trait in P.ovata species showed that this trait was not affected by this treatment and the interaction of populations of both species in drought stress had no significant effect on proline trait.

Chlorophyll A:

The mean squares of drought stress treatment on P. ovata species showed that the highest amount of chlorophyll a in mild and moderate drought stress (both with an average of 0.64 mg/g fresh weight) and the lowest amount of chlorophyll a (with the mean value equivalent to 0.51 mg/g fresh weight was observed in the control treatment.

The results of comparing the mean interaction of P. ovata and drought stress showed that "Ilam-Dehloran" accession was higher in mild stress (with an average of 0.82 mg /g fresh weight) and the reciprocal interaction of "Hormozgan-2" access in severe stress (the mean value: 0.34 mg/g fresh weight) had the lowest chlorophyll content.

Chlorophyll B:

Comparison of the mean values between Plantago ovata accessions for chlorophyll B showed that "Hormozgan-1" and "Markazi" accessions had the highest and lowest chlorophyll B (the mean value: 0.27 and 0.2 mg/g fresh weight), respectively. Analysis of variance of drought stress treatment in chlorophyll B trait showed that this trait was not affected by this treatment.

Comparison of the mean interaction of P.ovata species treatment and drought stress showed that "Hormozgan-2" accession in mild stress (with an average of 0.36 mg/g fresh weight) was the highest and the reciprocal effect of "Hormozgan-2" accession in severe stress conditions (With an average of 0.13 mg/g fresh weight) had the lowest amount of chlorophyll B.

Chlorophyll A&B:

Comparison of means between Plantago ovata accessions showed that the highest amount of A&B chlorophyll with an average of 0.98 was observed in "Hormozgan-1" accession and the lowest amount with an average of 0.76 was observed in the "South Khorasan-Sarbisheh".

The comparison of the mean drought stress treatment in P.ovata showed that the average stress with an average of 0.89 had the highest and the control stress (95% of field capacity) with an average of 0.71 had the lowest amount of A&B chlorophyll.

The results of comparing the mean interaction of P. ovata and drought stress showed that Hormozgan accession access (in mild stress with an average of 1.17), the highest and Hormozgan -2 accession effect (in severe stress with an average of 0.47) had the lowest A&B chlorophyll content.

Carotenoid:

The result of comparing the mean values between Plantago ovata accessions showed that the highest and lowest levels of carotenoids were observed with the mean of 0.31 and 0.24 (mg/g fresh weight) in "Hormozgan-1" and "Markazi" accessions, respectively.

The results of comparing the mean interaction of P.ovata species treatment and drought stress showed that Hormozgan-2 access in mild stress (with an average of 0.37mg/g of fresh weight) was the highest and the bilateral interaction of Hormozgan-2 access in severe stress (with an average of 0.16mg/g of fresh weight) had the lowest carotenoid content.

Conclusion and Discussion:

With the increasing desire to use medicinal plants, the demand for the production of these plants has increased. Plantago is one of the most important medicinal plants in the pharmaceutical industry, which has several species, of which P. ovata is of great importance in agriculture and medicine. Domestication and cultivation of this plant is acceptable as an alternative to water-intensive crops such as corn and wheat in marginal crops. This type of medicinal plant is widely used in the food, cosmetics, and medical industries due to its mucilage. Oral application of mucilage of this plant helps to reduce blood cholesterol, also in China, India, and Iran from its seeds to treat respiratory problems, fever, cough, cold, urinary problems, gonorrhea, diabetes, and digestive problems as an alternative to chemical drugs such as Antibiotics are used [22].

As the demand for medicinal plants in traditional medicine and pharmacy increases, some of them are cultivated economically, but water shortage is a serious problem in the cultivation of these plants [23].

Among non-biological stresses, drought and salinity have the greatest effect on medicinal plants [24]. Although the production of secondary metabolites of medicinal plants is usually genotype-dependent, their biosynthesis is affected by environmental factors and changes [25].

Hence, Peroxidase activity increased in spring barley under drought stress conditions [26]. According to the results of this experiment, the reduction of protein in coriander [27] and dill [28] is under dehydration. Under severe stress conditions, chlorophyll a + b and relative water content increased due to lack of stress and moderate drought stress [29].

Carbohydrate content under drought stress increased relative to drought stress in maize [43]. Drought stress under mild stress (0.3 MPa) had little effect on chlorophyll content in alfalfa, but in all alfalfa cultivars at 1 MPa chlorophyll content showed a very sharp decrease. Under drought stress, the amount of carotenoids in soybean increased due to its antioxidant role and protection of photosynthetic pigments and chlorophyll [30].

Moisture stress increased the amount of carotenoids and proline in tomato plants [31]. Drought stress at the level of F25 Fc in safflower reduced chlorophyll a, b and carotenoids [32]. Drought stress decreased chlorophyll a, b and carotenoids while increasing chlorophyll a / b and proline in canola [33]. The amount of carotenoids from normal moisture conditions to severe moisture stress conditions decreased carotenoids and relative water content and increased the ratio of chlorophyll a/b and peroxidase in basil [34].

Drought stress is also one of the most important non-living stressors that causes significant changes in physiological and

biochemical activities (photosynthesis, respiration, transpiration, hormone metabolism and enzyme activity) in most plants [35]. In general, dehydration It has adverse effects on plant physiological processes such as photosynthesis, nutrient uptake, cell development, cell division, accumulation and transport of nutrients [36]. Other traits are also effective: To reduce water stress, tolerant species or cultivars or low-yielding local cultivars can be used [37, 38].

Plants themselves by regulating specific morphological characteristics or regulating growth rate by increasing water uptake, reducing water loss and increasing or decreasing the transition rate from vegetative to reproductive stages, which are ways to avoid drought. As well as having an anti-system Enzymatic oxidant (superoxide dismutase, catalase, peroxidase and ascorbic glutathione acid) and non-enzymatic (secondary metabolites such as flavonoids, total phenols), growth regulators (proline, soluble protein, soluble sugars [39].

Amino acids make up the structure of proteins, including essential and non-essential amino acids (phosphoserine, taurine, phosphoethanolamine, urea, proline, aspartic acid, serine, glutamic acid, sarcosine, alpha-aminoadiproic acid, glycine -Aminoethanol, hydroxyproline, arnitin, 1-methylhistidine, Anserine, Carnosine, arginine, methionine, leucine, etc.) [40].

The production of superoxide or hydroxyl radicals causes the oxidation of amino acids and seriously damages the structure and function of proteins. Oxygen free radicals cause their degradation by degrading enzymes by altering the position of amino acids in protein filaments. In addition, hydrogen peroxide, even at low concentrations, oxidizes and inhibits the sulfhydryl groups of Calvin cycle enzymes such as glycerol aldehyde dehydrogenase and fructose bisphosphatase [41, 42].

Production of soluble proteins the booklet of osmotic regulators is compatible with drought stress, but the reduction of soluble proteins under drought stress can be due to a sharp decrease in photosynthesis under drought stress [43].

Carbohydrates in plants have different functions, in drought stress conditions, they play a role as a molecule compatible with osmotic regulation [44].

In stress conditions, having high photosynthetic potential that helps the growth of plants, expresses the importance of chlorophyll content in plants [45]. Increased leaf chlorophyll in drought stress conditions in drought tolerant cultivars due to increased activities is an enzyme [46].

Carotenoids have a protective role against induced oxidative stress and also play a role in the toxicity of chlorophyll and reduce the toxic effects of free radicals [4]). Drought stress resistance is significant because it plays an important role in regulating many metabolic processes, including ion transport [48].

In plant cells, proline can play an important regulatory role in the activity and function of catalase, peroxidase and polyphenol oxidase enzymes and their participation in the development of metabolic responses to environmental factors [49].

Relative water content Leaves under drought stress as a good indicator of drought tolerance Conservation of relative leaf water content under drought stress has been shown to help maintain relative leaf water content under drought stress due to closed pores and the ability of roots to absorb water when soil water potential is low [50].

Under drought stress conditions, the antioxidant system of plants is activated to deal with oxidative stresses. The activity of the antioxidant system is higher in drought tolerant cultivars than in sensitive cultivars. Under normal environmental conditions, the activity of antioxidants is lower, but when exposed to environmental stress (drought stress), the activity of catalase, Superoxide dismutase (SOD) and peroxidase increases and eliminates the produced hydrogen peroxide radicals (Lumet et al., 2014). Polyphenol oxidases are among the antioxidants that are widely present in plants. The activity of this antioxidant is related to age, species, species, maturity and stress stage in plants [51].

Polyphenol oxidase is a protein enzyme that catalyzes two different reactions that consume molecular oxygen. Peroxidase and polyphenol oxidase increase their activity in response to abiotic stresses [52].

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