Journal of Nuts

Journal homepage: ijnrs.damghaniau.ac.ir

ORIGINAL ARTICLE

Evaluation of Application of the Asparagine and Glutamine Amino Acids on Improving the Biochemical Properties and Yield under Drought Sterss Conditions on Pistachio cv. Shahpsand

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A R T I C L E I N F O

Keywords:

Drought stress;
Enzyme;
Pistachio vera;
Proline;
Protein;
Yield

A B S T R A C T

This experiment was performed to investigate the effect of foliar application of amino acids on improving biochemical properties and yield evaluation of the effect of drought stress on pistachio, cv. Shahpsand in Damghan city during 2019 and 2020. Statistical analysis was conducted as a factorial split-plot based on a randomized complete block design (RCBD) with three replications. The main factor was irrigation intervals (35, 55, and 75 days) and sub-factors were including asparagine and glutamine amino acids at three levels (0, 150 and 300 mg L^{-1}). The results showed that the irrigation interval had a significant effect on catalase, peroxidase, polyphenol oxidase, sodium, chlorine, potassium, proline, protein, relative water content (RWC), and yield at the level of 1% probability and the maximum content of catalase, peroxidase, polyphenol oxidase, chlorine, proline, and RWC were observed at 75 days irrigation interval but the highest yield and potassium were obtained at 35 days irrigation interval. Mineral concentrations of sodium (Na) and chloride (Cl), in leaves increased with increasing irrigation cycle however, the Potassium (K) concentration in leaves decreased. The highest Na concentrations and Cl were observed in 75 days, and the lowest was in 35days. The correlation coefficient between catalase and peroxidase was positive and the correlation between sodium and potassium was Negative, but the correlation between chlorine and protein was negative. Foliar application of amino acids increased plant yield and resistance to drought stress.

Introduction

Pistachio is a perennial, dioecious, diploid plant (x2=n2=30) and belongs to the Anacardiaceae family. This family consists of 75 genera and 600 species. Pistachio is the most important and widely grown commercial nut tree in Iran (Norozi et al., 2019; Sharifkhah et al., 2020). The Shahpasand cultivar an early and tasty cultivar is very famous and compatible with most pistachio growing areas in Iran (Mansoobi, 2020). Drought and salinity are considered as the major abiotic stresses that severely affect pistachio crop yield (Shamshiri and Hasani et al., 2015; Alipour, 2018). Due to the severe restrictions of water resources in most parts of Iran, drought stress has been introduced as the important stress affecting the yield of crops (Hosseini et

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Received: 4 July 2021; Received in revised form: 6 September 2021; Accepted: 26 October 2021
DOI: 10.22034/jon.2021.1934462.1116

al, 2022; Kafi *et al*., 2014). Drought alone has caused a 45% reduction in agronomical and horticultural crop yields (Belhassen, 1997). Glutamate in controlling the antioxidant defense mechanism through glutathione biosynthesis which is the active part of the antioxidant system (Lu, 2013). In addition due to its role in carbon and nitrogen metabolism, glutamate is also involved in the synthesis of chlorophyll and vitamin B9, increasing the leaf chlorophyll content, and increasing the yield by affecting plant photosynthetic activity (Hanson and Gregory, 2011). The glutamate involved in calcium signaling is an indicator of primary root growth but had did not affect lateral root growth (Hirel and Lea, 2001). Aspartic acid is involved in seed germination and amino acid metabolism. Aspartic acid acts as a precursor in the metabolic pathway of the aspartate family and biosynthesis of amino acids, such as isoleucine, methionine, and threonine in many plant species (Rawia *et al*., 2011). In plant cell plastids, aspartate acts as a precursor in arginine production and many metabolic reactions for the biosynthesis of aspartate and arginine (Katoh *et al*., 2006; Zrenner *et al*., 2006). Also, aspartate acts as a buffer to maintain cellular pH.Saeed *et al*., (2005) reported that aspartate acid is involved in the regulation of many processes, including the biosynthesis of chlorophyll, protein, and many plant pigments. This amino acid also promotes the biosynthesis of bio-osmolytes and their storage, including antioxidants, vitamins, and cofactors, which play a role in the cellular homeostasis and defense mechanisms (Azevedo *et al*., 2006). Under stress conditions, aspartate acid is catalyzed to other amino acids, including asparagine, threonine, leucine, isoleucine, and methionine. Foliar application of aspartic acid in plants under water stress conditions improved many growth parameters as well as increased enzymatic activities including POD, POX, CAT, which led to increased plant resistance (Akladious and Abbas, 2013). Therefore, this experiment was performed to investigate the effect of the asparagine and glutamine amino acids in reducing drought stress effects on biochemical traits and yield of Shahpsand cultivar of pistachio.

Materials and Methods

This experiment was performed to investigate the effect of foliar application of amino acids on improving biochemical properties and yield for reducing the effect of drought stress on Pistachio, cv. Shahpsand during the 2019 and 2020 crop years. The 25-years-old trees were grafted on 'Shahpsand' rootstock, at the spacing of 4×5 m using standard commercial practices of irrigation (30 days- control), soil fertilization, as well as pest and weed control scheduling during the experiment. The experiments were conducted as a factorial split based on a randomized complete block design (RCBD) with three replications. The main factor was irrigation intervals (35, 55, and 75 days) and sub-factors were including asparagine and glutamine amino acids at three levels (0, 150, and 300 mg L^{-1}). The experiment was performed in one of the pistachio gardens of Damghan city with the latitude of 34°15′and a longitude of 53°42′ at 1155.4 m above of sea. The flood irrigation method was done once every 30 days and all operations in the garden were done equally for the trees. These treatments were applied separately at the two stages of the millet stage and the bony skin hardened. The variables of catalase, peroxidase, polyphenol oxidase, sodium, chlorine, potassium (Walinga *et al*., 1989), proline, protein (Aaron *et al*., 2007), relative water content (RWC), and yield were measured at the end of the growing season in the laboratory of Islamic Azad University, Damghan Branch. After collecting information and organizing the end season, the analysis of variance was performed by SAS statistical software and the comparison of means was conducted based on Duncan's multiple range test at 1% probability level and Pearson correlation coefficient was used to determine the relationships between variables.

Enzyme extraction

For SOD, CAT, and POD extraction, leaf samples (0.5 g) were homogenized in 10 mL ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000 g. The supernatant was used for enzyme activity assay.

Peroxidase extraction

Peroxidase (POD, EC 1.11.1.7) activity was based on the method described by Chance and Maehly (1955). The reaction mixture contained 3,3'-diaminobenzidine-tetra hydrochloride dehydrate solution containing 0.1% (w v^{-1}) gelatin, 150 mM Na-phosphate-citrate buffer (pH 4.4), and 0.6% H_2O_2. The increase of absorbance was followed for 5 min at 465 nm by a spectrophotometer Elisa (PowerWave XS, BioTek, USA). A unit of POD activity was defined as μM H_2O_2 decomposed ml^{-1} min^{-1} mg^{-1} protein.

Superoxide dismutase extraction

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by recording the decrease in absorbance of superoxide nitro-blue tetrazolium complex by the enzyme (Sen Gupta *et al*., 1993). About 3 mL of reaction mixture containing 0.1 mL of 200 mM methionine, 0.01 mL of 2.25 mM nitrobluetetrazolium (NBT), 0.1 mL of 3 mM EDTA, 1.5 mL of 100 mM potassium phosphate buffer, 1 mL distilled water, and 0.05 mL of enzyme extraction were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL riboflavin (60 μM) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tubes with black cloth.

Tubes without enzyme developed maximal color. A non-irradiated complete reaction mixture that did not develop color served as blank. Absorbance was recorded spectrophotometrically at 560 nm by Elisa (PowerWave XS, BioTek, USA) and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

Catalase extraction

Catalase (CAT, EC 1.11.1.6) activity in the leaves was measured according to Sinha (1972). This method uses H2O2 as the substrate. The reaction mixture of 1.5 mL consisted of 1 mL phosphate buffer (0.01 M, pH 7.0), 0.4 mL distilled water, and 0.1 mL of centrifugation supernatant. Reaction was started by adding 0.5 mL H_2O_2 (320 mM), incubated at 25°C for different time intervals and the reaction was stopped by the addition of 2 mL of dichromate: acetic acid reagent (1:3 ratio). The tubes were immediately placed and kept in a boiling water bath for 20 min and were then centrifuged for 15 min (1500 g). The green color developed during the reaction was read at 570 nm in a spectrophotometer set. Control tubes, devoid of enzyme, were also processed in parallel. The enzyme activity is expressed as nmol H_2O_2 consumed min^{-1} mg^{-1} protein.

Proline extraction

Proline was determined according to the method described by Bates and coworkers (Bates *et al*., 1973). Briefly, one-hundred milligram of frozen plant material was homogenized in 1.5 mL of 3% sulphosalicylic acid and the residues were removed by centrifugation. Then, 100 μL of the extract was reacted with 2mL of glacial acetic acid (GAA) and 2mL of ninhydrin (1.25g ninhydrin dissolved in 30mL GAA and 20mL of 6 M phosphoric acid) for 1 hour at 100ºC. To the reaction

mixture, 1mL toluene was added and the toluene containing chromophore was warmed up to room temperature and its absorbance was determined by a spectrophotometer at 520 nm using standard curves.

Protein extraction

The contents of soluble protein were assayed as described by Bradford (1976); bovine serum albumin (BSA, Sigma chemical) was used as the standard. To determine total soluble proteins, 50 mg of leaf fresh matter was incubated in 5 mL of extraction buffer including Tris-HCL at 25 mM (pH 7.6). The mixture was centrifuged at 2000g for 15 min. Finally, total soluble proteins were read spectrophotometrically at 595 nm by Elisa (PowerWave XS, BioTek, USA).

***Relative Water Content** (RWC)*

In order to calculate RWC, leaf fresh weight samples were weighed, then were submerged in distilled water and finally were dried at 70 °C for 48 h and were weighed again.

RWC (%) = [(W-DW) / (TW-DW)] x 100

W – Sample fresh weight

TW – Sample turgid weight

DW – Sample dry weight.

Potassium and sodium extraction

The concentration of Na and K was measured in the leaves. About 0.1 g milled leaves were weighed from each sample and transferred to the Kjeldahl digestive tubes. Then, 10 ml of concentrated nitric acid (65%) was added to each tube. Two tubes without plant samples and only nitric acid were used as control. Samples were stored overnight (12 hours) at room temperature. Then, the samples were kept in the digested oven for 3–4 hours at 110°C until the sample's color was clarified (yellow amber). About 20 ml of distilled water was added to each tube and filtered by a filter paper and the final volume reached 100 ml in the balloon. The obtained extract was used to measure Sodium and Potassium amounts. The amount of Potassium and Sodium in the samples was read by a Flame Photometer apparatus. The potassium to sodium ratio was calculated. The ratio of potassium to sodium can be considered as resistant to the salinity criterion (Behzadi *et al*., 2021).

Chlorine extraction

The Cl concentration was measured according to the method of Chapman (1961). To measure chlorine ion, the extract was double distilled with water at 100°C and titrated with silver nitrate.

Results

The effect of irrigation on catalase, peroxidase, polyphenol oxidase, sodium, chlorine, potassium, proline, protein, RWC, and yield was significant at 1% probability. The effect of glutamine and asparagine amino acids on catalase, peroxidase, polyphenol oxidase, sodium, chlorine, potassium, proline, protein, and RWC were considerable at 1% and on the yield at 5%. The interaction effect of irrigation interval and glutamine and asparagine amino acids and also the triple interaction effect were significant at 1% except on the yield. The interaction effect of irrigation interval and asparagine amino acid on all characterized was considerable at 1% (Table 1).

Catalase content

The effect of irrigation interval showed that the highest level of catalase was at 75 days interval with 1.71 units/ mg protein and the lowest amount was 1.1 units/ mg protein with 35 days interval. The effect of asparagine amino acid indicated that the maximum was 1.62 units/ mg protein at 300 mg L^{-1} level of the asparagine amino acid and the minimum value was 1.1

units/ mg protein at control condition. The effect of glutamine amino acid appeared that the maximum was 1.53 units/ mg protein at 150 mg L^{-1} and the lowest was 1.22 units/ mg protein at control condition (Table 2).

The interaction effect of irrigation interval on asparagine and gultamin amino acids revealed that the maximum catalase content was 1.901 units/ mg protein at 300 mg L^{-1} of asparagine and 1.958 units/ mg protein at 300 mg L^{-1} glutamine amino acid at 75 days of irrigation interval and the minimum was 1.035 units/ mg protein at 150 mg L^{-1} asparagin and 1.016 units/ mg protein at the control in glutamine treatments at 35 days of irrigation interval (Table 3 and 4). The intraction asparagine and glutamine amino acids indicated that the maximum and minimum were 1.082 units/ mg protein at 150 mg L^{-1} of glutamine and 300 mg L^{-1} of asparagin, and 1.059 units/ mg protein at 0 mg L^{-1} glutamin and 150 mg L^{-1} asparagin amino acid respectively (Table 5).

The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum at 75 days irrigation interval, 300 mg L^{-1} of glutamine amino acid and 0 mg L^{-1} of asparagine amino acid with the amount of 2.143 units/ mg protein, and the lowest ones was 0.724 units/ mg protein at 35 days irrigation interval without asparagine and glutamine amino acids (Table 6).

Therefore, the results showed that with increasing the irrigation interval, the amount of catalase increases under the influence of the asparagine and glutamine amino acids to protect the plant against the negative effects of drought stress.The correlation between catalase and peroxidase was positive and significant at 1% probability ($r=0.62^{**}$). Therefore, with increasing the amount of catalase, the amount of peroxidase also increases and vice versa (Table 7).

Table 1. Analysis of variance of the effect of drought stress, amino acid asparagine and glutamine on biochemical traits and yield of pistachio cultivar Shahpsand.

S.V.O	df	Mean of squares									
		Catalase (Units/ mg protein)	Peroxidase (Units/ mg protein)	Polyphenol oxidase (Units/ mg protein)	Sodium Meq lit^{-1}	Chlorine Meq lit^{-1}	Potassium (percent)	Proline (µg g^{-1} FW)	Protein (mg g^{-1} FW)	RWC (percent)	Yeild (kg ha^{-1})
Block	2	0.005**	0.001*	0.000 ns	0.03 ns	0.03*	0.03 ns	0.00 ns	0.00 ns	2099.66**	89252244 ns
Irrigation period	2	4.968**	0.827**	0.334**	7.05**	4.53**	4.42**	1.09**	0.20**	73.44**	56104802 **
The main error	4	0.001	0.003	0.002	0.001	0.001	0.01	0.00	0.04	2.28	280929.8
Glutamine	2	1.532**	0.335**	0.374**	0.05**	21.26**	0.22**	3.02**	0.73**	95.79**	4678213.2*
Asparagine	2	1.774**	0.078**	0.612**	0.87**	0.29**	1.51**	1.23**	0.21**	534.03**	3855126.8*
Glutamine ✻ stress	4	0.401**	0.853**	1.456**	1.26**	5.45**	0.65**	1.36**	0.15**	277.26**	1467752.1 ns
Asparagine ✻ stress	4	0.747**	0.257**	0.712**	0.30**	3.82**	0.48**	1.54**	0.17**	40.89**	8714152.6**
Glutamine ✻ Asparagine	4	0.474**	0.276**	1.249**	0.43**	4.19**	0.39**	0.70**	0.16**	496.72**	2735653.7*
Asparagine ✻ Stress ✻Glutamine	8	0.286**	0.197**	0.541**	0.57**	7.47**	0.19**	0.34**	0.13**	248.83**	2344775.3*
Sub-error	48	0.002	0.001	0.0001	0.01	0.02	0.01	0.0001	0.01	1.74	1089668

ns,* and**: Non significant, significant at 5% and 1% levels respectively.

Table 2. Comparison of the mean of the main effect of drought stress, amino acid asparagine and glutamine on biochemical properties and yield of Shahpasand cultivar.

Drought stress	Catalase (Units/ mg protein)	Peroxidase (Units/ mg protein)	Polyphenol oxidase (Units/ mg protein)	Sodium Meq lit^{-1}	Chlorine Meq lit^{-1}	Potassium (percent)	Proline (µg g^{-1} FW)	Protein (mg g^{-1} FW)	RWC (percent)	Yeild (kg ha^{-1})
35day	1.10 c	1.27 c	0.72 a	0.72 c	1.69 c	1.10 a	0.78 c	1.24 b	35.57 b	5945.59 a
55day	1.45 b	1.28 b	0.59 b	1.15 b	1.89 b	1.06 a	0.88 b	1.36 a	36.36 b	4075.05 b
75day	1.71 a	1.49 a	0.73 a	1.43 a	2.26 a	0.59 c	1.06 a	1.27 ab	37.86 a	4308.5 b
amino acid asparagine										
0	1.38 b	1.31 c	0.57 b	1.21 a	1.97 b	0.77b	0.87 b	1.22 c	38.1 b	4528.43 b
150mg	1.26 c	1.38 a	0.79 a	0.96 b	2.00 a	1.10a	1.08 a	1.31 b	32.98 c	4741.28 a
300mg	1.62 a	1.36 b	0.67 c	1.12 a	1.86 c	0.88 a	0.78 c	1.34 a	38.71 a	5059.42 a
amino acid glutamine										
0	1.22 b	1.27 c	0.69 b	1.11 b	2.03 a	0.84c	94b.0	1.20 b	35.26 c	4437.37 b
150mg	1.53 a	1.36 b	0.76 a	1.07 c	2.53 a	0.96a	1.13 a	1.25 a	36.62 b	4925.51 ab
300mg	1.5 a	1.42 a	0.59 c	1.12 a	1.28 b	0.95 b	0.66 c	1.42 a	37.92 a	4966.25 a

According to Duncan's multiple range test, the numbers with the same letters in each column are not significantly different.

Table 3. Comparison of the mean interaction of drought stress and amino acid asparagine on biochemical properties and yield of pistachio cultivar Shahpsand.

Asparagine * stress	Catalase (Units/ mg protein)	Peroxidase (Units/ mg protein)	Polyphenol oxidase (Units/ mg protein)	Sodium Meq lit^{-1}	Chlorine Meq lit^{-1}	Potassium (percent)	Proline (µg g^{-1} FW)	Protein (mg g^{-1} FW)	RWC (percent)	Yeild (kg ha^{-1})
d35-a0	1.108 bc	1.302 ab	0.67 a	1.373 ab	2.3 a	1.412 a	0.511 e	1.091 c	35.79 abc	4800.69 b
d35-a150	1.035 c	1.371 ab	0.838 a	1.018 cd	1.473 ab	0.939 cb	1.064 bc	1.264 abc	33.7 abc	6435.10 a
d35-a300	1.163 bc	1.151 b	0.667 a	1.063 c	1.885 ab	0.827 cd	0.767 cde	1.37 a	37.21 abc	6600.97 a
d55-a0	1.179 bc	1.158 b	0.236 b	1.537 a	1.243 b	1.211 ab	0.958 bcd	1.39 a	38.63 abc	4199.07 bc
d55-a150	1.37 b	1.25 ab	0.79 a	1.197 cd	2.233 ab	0.906 cd	0.754 cde	1.311 ab	32.46 c	3664.77 c
d55-a300	1.787 a	1.431 ab	0.736 a	1.568 a	1.585 ab	1.193 ab	0.937 bcd	1.37 a	38 abc	4361.3 bc
d75-a0	1.84 a	1.46 a	0.817 a	0.732 de	2.357 a	0.677 cde	1.132 ab	1.172 bc	39.88 ab	4585.52 bc
d75-a150	1.381 b	1.522 a	0.733 a	0.676 e	2.303 a	0.472 e	1.409 a	1.357 a	32.79 bc	4123.98 bc
d75-a300	1.901 a	1.492 a	0.617 a	0.741 ed	2.113 ab	0.613 de	0.642 de	1.265 abc	40.92 a	4215.99 bc

According to Duncan's multiple range test, the numbers with the same letters in each column are not significantly different.

Table 4. Comparison of the mean interaction of drought stress and amino acid glutamine on biochemical properties and yield of pistachio cultivar Shahpsand.

Glutamine* stress	Catalase (Units/ mg protein)	Peroxidase (Units/ mg protein)	Polyphenol oxidase (Units/ mg protein)	Sodium Meq lit^{-1}	Chlorine Meq lit^{-1}	Potassium (percent)	Proline (µg g^{-1} FW)	Protein (mg g^{-1} FW)	RWC (percent)	Yeild (kg ha^{-1})
d35-g0	1.016 e	1.367 bc	0.993 a	1.292 bc	1.844 bc	1.308 a	0.877 bc	1.056 e	33.51 ab	5905.6 a
d35-g150	1.218 cde	1.274 bc	0.71 abc	1.114 cd	2.493 b	0.838 cde	0.894 bc	1.234 cd	39.63 ab	5884.9 a
d35-g300	1.073 de	1.182 bc	0.473 bc	1.048 cde	1.32 c	1.032 abc	0.57 c	1.435 ab	33.56 ab	6046.3 a
d55-g0	1.315 bcd	1.128 c	0.657 abc	1.624 a	2.286 b	0.972 bcd	0.868 bc	1.262 bcd	32.82 b	3533.2 c
d55-g150	1.548 b	1.44 b	0.737 ab	1.164 cd	1.658 bc	1.218 ab	0.968 b	1.362 abc	35.74 ab	4518.3 bc
d55-g300	1.473 bc	1.272 bc	0.369 c	1.513 ab	1.118 c	1.12 abc	0.813 bc	1.446 a	40.54 a	4173.7 bc
d75-g0	1.342 bcd	1.305 bc	0.407 bc	0.422 f	1.947 bc	0.586 ef	b۰.۹۲۹	1.269 bcd	39.44 ab	3873.4 bc
d75-g150	1.822 a	1.351 bc	0.826 a	0.924 de	3.427 a	0.475 f	1.629 a	1.15 de	34.49 ab	4373.3 bc
d75-g300	1.958 a	1.818 a	0.933 a	0.803 e	1.4 c	0.7 def	0.584 c	1.375 abc	39.66 ab	4678.8 b

According to Duncan's multiple range test, the numbers with the same letters in each column are not significantly different.

Table 5. Comparison of the mean interaction of asparagine and glutamine amino acids on biochemical properties and yield of pistachio cultivar Shahpsand.

Glutamine* Asparagine	Catalase (Units/ mg protein)	Peroxidase (Units/ mg protein)	Polyphenol oxidase (Units/ mg protein)	Sodium Meq lit^{-1}	Chlorine Meq lit^{-1}	Potassium (percent)	Proline (µg g^{-1} FW)	Protein (mg g^{-1} FW)	RWC (percent)	Yeild (kg ha^{-1})
g0-a 0	1.076 c	1.179 b	0.330 c	1.328 a	2.002 bcd	1.308 a	1.024 bc	1.057 c	39.6 ab	3899.81 b
g0-a 150	1.059 c	1.309 ab	0.724 ab	0.935 ab	1.887 bcd	0.673 bc	1.494 a	1.312 b	32.28 cd	4284.27 b
g0-a 300	1.538 ab	1.313 ab	0.871 a	1.074 ab	2.188 abc	0.884 bc	0.856 bcd	1.218 b	33.89 bcd	5128.03 a
g150-a 0	1.537 ab	1.435 ab	0.463 bc	1.113 ab	2.744 ab	0.903 bc	0.907 bcd	1.263 b	34.34 bcd	4579.08 b
g150-a 150	1.248 bc	1.251 b	0.99 a	1.093 ab	3.014 a	0.742 bc	1.143 b	1.238 b	29.91 d	5163.99 a
g150-a 300	1.802 a	1.378 ab	0.82 a	0.996 ab	1.82 cd	0.886 bc	0.781 cd	1.245 b	45.61 a	5033.46 a
g300-a 0	1.513 ab	1.307 ab	0.799 ab	1.2 ab	1.154 d	1.088 ab	0.669 d	1.333 b	40.37 ab	5106.40 a
g300-a150	1.48 ab	1.582 a	0.647 abc	0.863 b	1.108 d	0.902 bc	0.59 d	1.382 b	36.76 bc	4775.59 ab
g300-a 300	1.511 ab	1.383 ab	0.461 bc	1.301 a	1.575 cd	0.863 bc	0.708 cd	1.542 a	36.63 bc	5016.77 a

Mean followed by similar letters in each column are not significantly different.**g= Glutamine , a=Asparagine**

Table 6. Comparison of the mean interaction effect of drought stress, amino acid asparagine and glutamine on biochemical traits and yield of pistachio cultivar Shahpsand.

* stress * Asparagine Glutamine	Catalase (Units/ mg protein)	Peroxidase (Units/ mg protein)	Polyphenol oxidase (Units/ mg protein)	Sodium Meq lit^{-1}	Chlorine Meq lit^{-1}	Potassium (percent)	Proline (µg g^{-1} FW)	Protein (mg g^{-1} FW)	RWC (percent)	Yeild (kg ha^{-1})
d35-g0-a0	0.724 j	1.275 b-f	0.697 b-f	1.528 abc	3.465 ab	1.79 a	0.516 ghi	0.706 i	37.02 b-g	4150.98 efg
d35-g0-a150	1.193 f-i	1.663 a -c	1.082 abc	1.425 a-d	0.9 fg	1.13 b-f	1.291 bcd	1.167 e-h	29.49 fgi	6125.94 a-d
d35-g0-a300	1.133 f-j	f-1.165 d	1.199 ab	0.923 e-i	1.168 d-g	1.005 b-h	0.823 d-h	1.295 b-h	34.02 c--i	7439.73 a
d35-g150-a0	1.237 f-i	1.388 b-f	0.601 c-f	1.163 c-f	2.413 b-e	0.94 c-i	0.731 f-i	1.183 d-h	35.2 b-i	4409.22 d-g
d35-g150-a150	0.948 hij	1.101 d-f	0.995 a-d	1.063 c-g	2.443 bcd	0.748 e-i	1.319 bc	1.295 b-h	39.34 b-g	6732.33 ab
d35-g150-a300	1.468 c-f	1.332 b-f	0.534 def	1.118 c-f	2.625 bc	0.825 d-i	0.633 f-i	1.223 c-h	44.36 abc	6513.26 abc
d35-g300-a0	1.363 e-h	1.243 c-f	0.712 b-f	1.43 a-d	1.023 fg	1.505 ab	0.285 i	1.383 a-g	35.16 b-i	5841.89 a-e
d35-g300-a150	0.965 hij	1.349 b-f	0.439 ef	0.565 hij	1.075efg	0.94 c-i	0.581 f-i	1.329 a-h	32.28 efgi	6447.04 abc
d35-g300-a300	0.89 ij	0.955 f	0.268 f	1.148 c-f	1.863 c-g	0.65 f-i	0.844 c-h	1.593 a	33.25 c-i	5849.93 a-e
d55-g0-a0	1.093 f-j	0.98 ef	0.161 g	1.843 a	1.47 c-g	1.253 b-e	0.9 c-g	1.259 b-h	39.81 b-f	3301.55 g
d55-g0-a150	1.101 f-j	0.953 f	0.576 c-f	1.225 c-f	3.35 ab	0.525 hij	0.766 e-i	1.223 c-h	30.33 fgi	3312.51 g
d55-g0-a300	1.75 a-e	1.443 a-e	1.234 ab	1.805 ab	2.038 c-g	1.138 b-f	0.94 c-g	1.306 a-h	28.31 gi	3985.47efg
d55-g150-a0	1.41 d-g	1.525 a-d	0.271 f	1.445 a-d	1.52 c-g	1.288 bcd	1.058 c-f	1.47 a-d	30.15 fgi	5047.36 b-g
d55-g150-a150	1.358 e-h	1.274 b-f	1.224 ab	0.9 e-i	2.2 b-f	0.995 b-h	0.864 c-h	1.306 a-h	24.03 i	3795.67 g
d55-g150-a300	1.875 abc	1.52 a-d	0.717 b-f	1.147 c-f	1.255 d-g	1.373 abc	0.982 c-g	1.311 a-h	53.02 a	4711.87 c-g
d55-g300-a0	1.033 g-j	0.963 f	0.277 f	1.323 b-e	0.74 g	1.093 b-f	0.916 c-g	1.441 a-e	45.92 ab	4248.28 efg
d55-g300-a150	1.653 b-e	1.523 a-d	0.571 c-f	1.465 a-d	1.15 d-g	1.198 b-e	0.633 f-i	1.405 a-g	43.03 a-e	3886.13 fg
d55-g300-a300	1.735 a-e	1.33 b-f	0.258 f	1.753 ab	1.463 c-g	1.07 b-g	0.889 c-g	1.493 abc	32.68 d-i	4386.55 d-g
d75-g0-a0	1.411 d-g	1.273 b-f	0.525 def	0.615 g-j	1.07 efg	0.883 c-i	1.658 b	1.206 c-h	41.96 b-e	4246.9 efg
d75-g0-a150	0.882 ij	1.312b-f	0.515 def	0.155 j	1.41 c-g	0.365 j	2.425 a	1.547 ab	37.03b-g	3414.35 fg
d75-g0-a300	1.733 a-e	1.331 b-f	0.181 g	0.495 ij	3.36 ab	0.51 hij	0.806 d-h	1.055 h	39.34 b-g	3958.87 fg
d75-g150-a0	1.965 ab	1.393 b-f	0.519 def	0.733 f-i	4.3 a	0.483 hij	0.931 c-g	1.136 fgh	37.67 b-g	4280.65 efg
d75-g150-a150	1.437 d-g	1.378 b-f	0.753 b-f	1.315 b-e	4.4 a	0.483 hij	1.246 b-e	1.114 gh	26.36 i	4963.98 b-g
d75-g150-a300	2.063 ab	1.281 b-f	1.208 ab	0.725 f-i	1.58 c-g	0.46 ij	0.729 f-i	1.2 c-h	39.46 b-g	3875.25 fg
d75-g300-a0	2.143 a	1.715 ab	1.408 a	0.848 e-i	1.7 c-g	0.665 f-i	0.807 d-h	1.175 e-h	40.02 b-f	5229.02 b-f
d75-g300-a150	1.824 a-d	1.875 a	0.931 a-e	0.558 hij	1.1 d-g	0.568 g-j	0.556 ghi	1.411 a-f	34.98 b-i	3993.6 efg
d75-g300-a300	1.908 ab	1.864 a	0.462def	1.004 d-h	1.4 c-g	0.868 c-j	0.39 hi	1.539 ab	43.96 a-d	4813.84 c-g

Mean followed by similar letters in each column are not significantly different. d=Day,g= Glutamine , a=Asparagine

Table 7. Correlation coefficient of the effect of drought stress, amino acid asparagine and glutamine on biochemical traits and yield of Shahpasand pistachio.

Adjective	Catalase	Peroxidase	Polyphenol oxidase	sodium	Chlorine	Potassium	Proline	Protein	RWC	Yeild
Catalase	1.00									
Peroxidase	.62**	1.00								
Chlorine	0.27	0.30	1.00							
sodium	-0.11	-0.17	-0.10	1.00						
Chlorine	0.02	-0.16	-0.05	-0.01	1.00					
Potassium	-0.24	-0.01	-0.06	-0.66**	-0.32	1.00				
Proline	-0.26	-0.14	-0.02	-0.32	-0.03	-0.34	1.00			
Protein	0.04	0.04	-0.24	-0.05	-.59**	0.15	0.14	1.00		
RWC	0.24	0.07	-0.25	-0.14	-0.25	0.11	-0.06	0.04	1.00	
Yeild	-0.21	0.01	0.21	-0.02	-0.17	0.11	-0.14	0.08	-0.03	1.00

**. Correlation is significant at the 0.01 level (2-tailed).

Peroxidase content

The effect of irrigation interval showed that the maximum level of peroxidase was at 75 days irrigation interval with 1.49 units/ mg protein and the minimum was 1.27 units/ mg protein at 35 days irrigation interval. The effect of asparagine amino acid indicated that the maximum was 1.38 units/ mg protein at 150 mg L^{-1} level and the minimum value was 1.31 units/ mg protein at control condition. The effect of glutamine amino acid appears that the maximum was 1.42 units/ mg protein at 300 mg L^{-1} and the minimum was 1.27 units/ mg protein at the control condition (Table 2).

The interaction effect of irrigation interval on asparagine amino acid revealed that the maximum was 1.522 units/ mg protein at 150 mg L^{-1} and 75 days of irrigation interval and the minimum was 1.151 units/ mg protein at 300 mg L^{-1} and 35 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum was 1.818 units/ mg protein at 300 mg L^{-1} at 75 days of irrigation interval and the minimum was 1.128 units/ mg protein at the control and 55 days of irrigation interval (Table 4). The interaction of asparagine and glutamine amino acids revealed that the maximum was 1.582 units/ mg protein at 300 mg L^{-1} of glutamine amino acid and 150 mg L^{-1} of asparagine amino acid and the minimum was 1.179 units/ mg protein at without glutamine and asparagine amino acids conditions (Table 5).

The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum at 75 days irrigation interval, 300 mg L^{-1} of glutamine amino acid and 150 mg L^{-1} of asparagine amino acid with the amount of 1.875 units/ mg protein and the minimum was 0.953 units/ mg protein at 55 days irrigation interval, 150 mg L^{-1} asparagine amino acid and without glutamine amino acid (Table 6). Therefore, the results showed that with increasing the irrigation interval, the amount of peroxidase increases under the influence of the asparagine and glutamine amino acids to protect the plant against the negative effects of drought stress.The correlation between catalase and peroxidase was positive and significant at 1% probability ($r=0.62^{**}$). Therefore, with increasing the amount of peroxidase, the amount of peroxidase also increases and vice versa (Table 7).

Polyphenol oxidase content

The effect of irrigation interval showed that the maximum was at 75 days irrigation interval with 0.73 units/ mg protein and the minimum was 0.59 units/ mg protein at 55 days irrigation interval.The effect of asparagine amino acid revealed that the maximum was 0.79 units/ mg protein at 150 mg L^{-1} of the asparagine amino acid and the minimum was 0.57 units/ mg protein at the control condition. The effect of glutamine amino acid revealed that the maximum was 0.76 units/ mg protein at150 mg L^{-1} and the minimum was 0.57 units/ mg protein at the control condition (Table 2).

The interaction effect of irrigation interval and asparagine amino acid revealed that the maximum was 0.838 units/ mg protein at 150 mg L^{-1} at 35 days of irrigation interval and the minimum was 0.236 units/ mg protein at 0 mg L^{-1} and 55 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum was 0.993 units/ mg protein at 0 mg L^{-1} at 35 days of irrigation interval and the minimum was 0.369 units/ mg protein at 300 mg L^{-1} at days of irrigation interval (Table 4). The interaction of asparagine and glutamine amino acids revealed that the maximum was 0.99 units/ mg protein at 150 mg L^{-1} of glutamine amino acid and 150 mg L^{-1} of asparagine amino acid and the minimum was 0.330 units/ mg protein at without glutamine and asparagine amino acids conditions (Table 5).

The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum at 75 days irrigation interval, 300 mg L^{-1} of glutamine amino acid and 0 mg L^{-1} of asparagine amino acid with the amount of 1.408 units/ mg protein, and the minimum was 0.181 units/ mg protein at 75 days irrigation interval, 300 mg L^{-1} asparagine amino acid and without glutamine amino acid (Table 6). Therefore, the results showed that with increasing the irrigation interval, the amount of polyphenol oxidase increases under the influence of the asparagine and glutamine amino acids to protect the plant against the negative effects of drought stress.

Sodium content

The effect of irrigation interval showed that the maximum sodium content was at 75 days irrigation interval with 1.43 meq L^{-1} and the minimum content was 0.72 meq L^{-1} at 35 days irrigation interval. The effect of asparagine amino acid revealed that the maximum sodium content was 1.21 meq L^{-1} at 0 mg L^{-1} and the minimum was 0.96 meq L^{-1} at 150 mg L^{-1}. The effect of glutamine amino acid revealed the maximum sodium content was 1.12 meq L^{-1} at 300 mg L^{-1} and the minimum was 1.07 meq L^{-1} at 150 mg L^{-1} (Table 2). The interaction effect of irrigation interval and asparagine amino acid revealed that the maximum sodium content was 1.568 meq L^{-1} at 300 mg L^{-1} at 55 days of irrigation interval and the minimum was 0.676 meq L^{-1} at 150 mg L^{-1} at 75 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum sodium content was 1.624 meq L^{-1} at 0 mg L^{-1} at 55 days of irrigation interval and the minimum was 0.422 meq L^{-1} at 0 mg L^{-1} at 75 days of irrigation interval (Table 4).The interaction of asparagine and glutamine amino acids revealed that the maximum sodium content was 1.328 meq L^{-1} at 0 mg L^{-1} of glutamine amino acid and 0 mg L^{-1} of asparagine amino acid and the minimum was 0.863 meq L^{-1} at 300 mg L^{-1} glutamine and 150 mg L^{-1} asparagine amino acids (Table 5). The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum sodium content at 55 days irrigation interval, 0 mg L^{-1} of glutamine amino acid and 0 mg L^{-1} of asparagine amino acid with the amount of 1.843 meq L^{-1} and the minimum was 0.565 meq L^{-1} at 35 days irrigation interval, 150 mg L^{-1} asparagine amino acid and 300 mg L^{-1} glutamine amino acid (Table 6). The correlation between sodium and potassium was positive

and significant at 1% probability ($r=0.66^{**}$). Therefore, with increasing the amount of sodium, the amount of potassium also increases and vice versa (Table 7).

Chlorine content

The effect of irrigation interval showed that the maximum chlorine content was at 75 days irrigation interval with 2.26 meq L^{-1} and the minimum was 1.69 meq L^{-1} at 35 days irrigation interval. The effect of asparagine amino acid revealed that the maximum chlorine content was 2.00 meq L^{-1} at 150 mg L^{-1} asparagine amino acid and the minimum was 1.86 meq L^{-1} at 300 mg L^{-1} asparagine amino acid. The effect of glutamine amino acid revealed that the maximum chlorine content was 2.53 meq L^{-1} at 300 mg L^{-1} glutamine amino acid and the minimum was 1.28 meq L^{-1} at 300 mg L^{-1} glutamine amino acid (Table 2). The interaction effect of irrigation interval and asparagine amino acid revealed that the maximum chlorine content was 2.357 meq L^{-1} at 0 mg L^{-1} at 75 days of irrigation interval and the minimum was 1.243 meq L^{-1} at 0 mg L^{-1} at 55 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum chlorine content was 3.427 meq L^{-1} at 150 mg L^{-1} at 75 days of irrigation interval and the minimum was 1.32 meq L^{-1} at 300 mg L^{-1} at 35 days of irrigation interval (Table 4). The interaction of asparagine and glutamine amino acids revealed that the maximum chlorine content was 3.014 meq L^{-1} at 150 mg L^{-1} of glutamine and asparagine amino acids and the minimum was 1.108 meq L^{-1} at300 mg L^{-1} glutamine and 150 mg L^{-1} asparagine amino acids (Table 5). The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum chlorine content at 75 days irrigation interval, 150 mg L^{-1} of glutamine and asparagine amino acids with the amount of 4.4 meq $L^{-1,}$ and the minimum was 0.74 meq L^{-1} at 55 days irrigation interval, 0 mg L^{-1} asparagine amino acid and 300 mg L^{-1} glutamine amino acid (Table 6). The correlation between chlorine and protein was negative and significant at 1% probability ($r=-0.59^{**}$). Therefore, with increasing the amount of chlorine, the amount of protein decreases and vice versa (Table 7).

Potassium content

The effect of irrigation interval showed that the maximum potassium content was at 35 days irrigation interval with 1.10% and the minimum was 0.59% at 75 days irrigation interval. The effect of asparagine amino acid revealed that the maximum potassium content was 1.10% at 150 mg L^{-1} and the minimum was 0.77% at 0 mg L^{-1}. The effect of glutamine amino acid revealed that the maximum content of potassium was 0.96% at 150 mg L^{-1} and the minimum was 0.84% at 0 mg L^{-1} (Table 2). The interaction effect of irrigation interval and asparagine amino acid revealed that the maximum potassium content was 1.412% at 0 mg L^{-1} at 35 days of irrigation interval and the minimum was 0.472% at 150 mg L^{-1} at 75 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum potassium content was 1.308% at 0 mg L^{-1} at 35 days of irrigation interval and the minimum was 0.475% at 150 mg L^{-1} at 75 days of irrigation interval (Table 4). The interaction of asparagine and glutamine amino acids revealed that the maximum potassium content was 1.308% at 0 mg L^{-1} of glutamine amino acid and 0 mg L^{-1} of asparagine amino acid and the minimum was 0.673% at 0 mg L^{-1} glutamine and 150 mg L^{-1} asparagine amino acids (Table 5). The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum potassium content of at 35 days irrigation interval, 0 mg L^{-1} of glutamine amino acid and 0 mg L^{-1} of asparagine amino acid with the amount of 1.79% and the minimum potassium content was 0.46% at 75 days irrigation interval, 300 mg L^{-1} asparagine amino acid and 150 mg L^{-1} glutamine amino acid (Table 6).

Proline content

The effect of irrigation interval showed that the maximum proline content was at 75 days irrigation interval with 1.06 µg g^{-1} FW and minimum content was 0.78 µg g^{-1} FW at 35 days irrigation interval. The effect of asparagine amino acid revealed that the maximum content of proline was 1.08 µg g^{-1} FW at 150 mg L^{-1} and the minimum was 0.78 µg g^{-1} FW at 300 mg L^{-1}. The effect of glutamine amino acid revealed that the maximum proline content was 1.13 µg g^{-1} FW at 150 mg L^{-1} and the minimum was 0.66 µg g^{-1} FW at 300 mg L^{-1} (Table 2). The interaction effect of irrigation interval and asparagine amino acid revealed that the maximum proline content was1.409 µg g^{-1} FW at 150 mg L^{-1} and 75 days of irrigation interval and the minimum was 0.511 µg g^{-1} FW at 0 mg L^{-1} and 35 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum proline content was 1.629 µg g^{-1} FW at 150 mg L^{-1} with 75 days of irrigation interval and the minimum was 0.57 µg g^{-1} FW at 300 mg L^{-1} and 35 days of irrigation interval (Table 4). The interaction of asparagine and glutamine amino acids revealed that the maximum prolin content was 1.494 µg g^{-1} FW at 0 mg L^{-1} of glutamine amino acid and 150 mg L-1 of asparagine amino acid and the minimum was 0.59 µg g^{-1} FW at300 mg L^{-1} glutamine and 150 mg L^{-1} asparagine amino acids (Table 5). The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum proline content at 75 days irrigation interval, 0 mg L^{-1} of glutamine amino acid and 150 mg L^{-1} of asparagine amino acid with the amount of 2.42 µg g^{-1} FW and the minimum proline content was 0.285 µg g^{-1} FW at 35 days irrigation interval, 0 mg L^{-1} asparagine amino acid and 300 mg L^{-1} glutamine amino acid (Table 6).

Leaf protein content

The effect of irrigation interval showed that the maximum leaf protein content was at 55 days irrigation interval with 1.36 mg g^{-1} FW and the minimum content was 1.24 mg g^{-1} FW at 35 days irrigation interval. The effect of asparagine amino acid revealed that the maximum leaf protein content was 1.34 mg g^{-1} FW at 300 mg L^{-1} and the minimum was 1.22 mg g^{-1} FW at 0 mg L^{-1}. The effect of glutamine amino acid revealed that the maximum leaf protein content was 1.42 mg g^{-1} FW at 300 mg L^{-1} glutamine amino acid and the minimum was 1.20 mg g^{-1} FW at 0 mg L^{-1} (Table 2). The interaction effect of irrigation interval and asparagine amino acid revealed that the maximum leaf protein content was 1.39 mg g^{-1} FW at 0 mg L^{-1} with 55 days of irrigation interval and the minimum was 1.091 mg g^{-1} FW at 0 mg L^{-1} at 35 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum leaf protein content was 1.446 mg g^{-1} FW at 300 mg L^{-1} and 55 days of irrigation interval and the minimum was 1.056 mg g^{-1} FW at 0 mg L^{-1} at 35 days of irrigation interval (Table 4). The interaction of asparagine and glutamine amino acids revealed that the maximum leaf protein content was 1.542 mg g^{-1} FW at 300 mg L^{-1} of glutamine amino acid and 300 mg L^{-1} of asparagine amino acid and the minimum was 1.057 mg g^{-1} FW at0 mg L^{-1} glutamine and asparagine amino acids (Table 5). The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum leaf protein content at 35 days irrigation interval, 300 mg L^{-1} of glutamine amino acid and 300 mg L^{-1} of asparagine amino acid with the amount of 1.593 mg g^{-1} FW and the minimum leaf protein content of was 0.706 mg g^{-1} FW at 55 days irrigation interval, 0 mg L^{-1} asparagine and 0 mg L^{-1} glutamine amino acids (Table 6).

RWC content

The effect of irrigation interval showed that the maximum RWC content was at 75 days irrigation interval with 37.86% and the minimum was 35.57% at 35 days irrigation interval.The effect of asparagine amino acid revealed that the maximum RWC content was 38.71% at 300 mg L^{-1} and the minimum content was 32.98% at150 mg L^{-1}. The effect of glutamine amino acid revealed that the maximum RWC content was 37.92% at300 mg L^{-1} and the minimum was 35.26% at 0 mg L^{-1} (Table 2). The interaction effect of irrigation interval and asparagine amino acid revealed that the maximum RWC content was 40.92% at 300 mg L^{-1} at 75 days of irrigation interval and the minimum was 32.46% at 150 mg L^{-1} at 55 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum RWC content was 40.45% at 300 mg L^{-1} at 55 days of irrigation interval and the minimum was 32.82% at 0 mg L^{-1} g at 55 days of irrigation interval (Table 4). The interaction of asparagine and glutamine amino acids revealed that the mximum RWC content was 45.61% at 150 mg L^{-1} of glutamine amino acid and 300 mg L^{-1} of asparagine amino acid and the minimum was 29.91% at 150 mg L^{-1} glutamine and asparagine amino acids (Table 5). The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum RWC content at 55 days irrigation interval, 150 mg L^{-1} of glutamine amino acid and 300 mg L^{-1} of asparagine amino acid with the amount of 53.02% and the minimum RWC content was 24.03% at 55 days irrigation interval, 150 mg L^{-1} asparagine amino acid and 150 mg L^{-1} glutamine amino acid (Table 6).

Yield

The effect of irrigation interval showed that the maximum of yield was at 35 days irrigation interval with 5945.59 kg ha^{-1} and the minimum was 4308.5 kg ha^{-1} at 55 days irrigation interval.The effect of asparagine amino acid revealed that the maximum of yield was 5059.42 kg ha^{-1} at 300 mg L^{-1} and the minimum was 4528.43 kg ha^{-1} at 0 mg L^{-1}.The effect of glutamine amino acid revealed that the maximum of yield was 4966.25 kg ha^{-1} at 300 mg L^{-1} and the minimum content was 4437.37 kg ha^{-1} at 0 mg L^{-1} (Table 2). The interaction effect of irrigation interval and asparagine amino acid revealed that the maximum of yield was 6600.97 kg ha^{-1} at 300 mg L^{-1} at 35 days of irrigation interval and the minimum was 3664.77 kg ha^{-1} at 150 mg L^{-1} and 55 days of irrigation interval (Table 3). The interaction of irrigation interval and glutamine amino acid revealed that the maximum of yield was 6046.3 kg ha^{-1} at 300 mg L^{-1} at 35 days of irrigation interval and the minimum was 3533.2 kg ha^{-1} at 0 mg L^{-1} at 55 days of irrigation interval (Table 4).The interaction of asparagine and glutamine amino acids revealed that the maximum of yield was 5163.99 kg ha^{-1} at 150 mg L^{-1} of glutamine amino acid and 150 mg L^{-1} of asparagine amino acid and the minimum was 3899.81 kg ha^{-1} at 0 mg L^{-1} glutamine and 0 mg L^{-1} asparagine amino acids (Table 5 and Fig. 1). The interaction of irrigation interval, asparagine, and glutamine amino acids revealed the maximum of yield at 35 days irrigation interval, 0 mg L^{-1} of glutamine amino acid and 300 mg L^{-1} of asparagine amino acid with the amount of 74393.73 kg ha^{-1} and the minimum of yield was 3795.67 kg ha^{-1} at 55 days irrigation interval, 150 mg L^{-1} asparagine amino acid and 150 mg L^{-1} glutamine amino acid (Table 6).

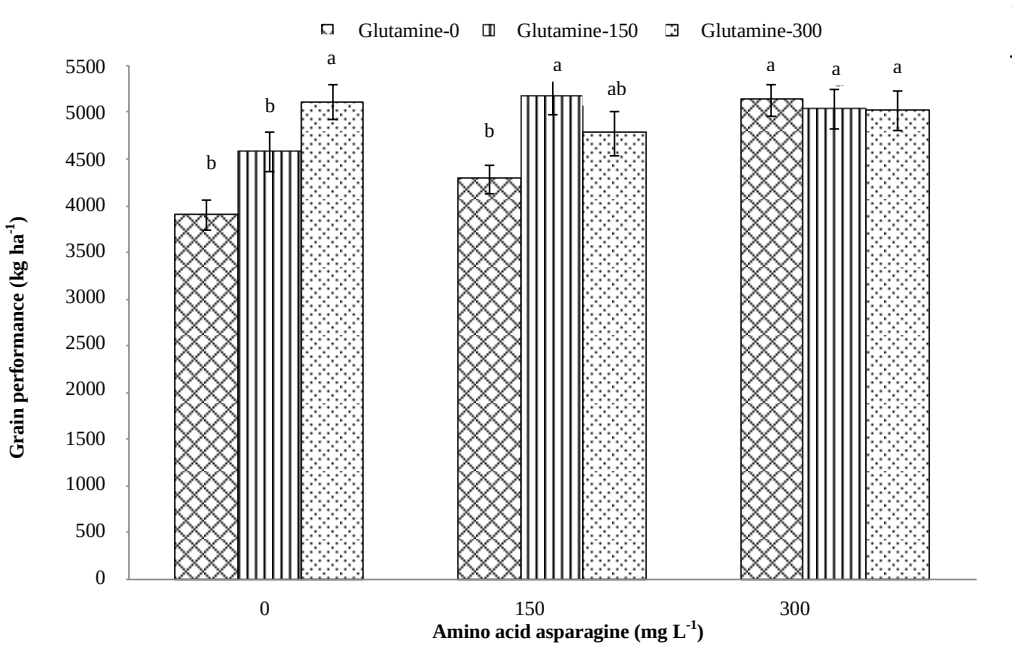

Fig. 1. Interaction of amino acid asparagine and glutamine on Pistachio yield of shahpsand cultivar

Discussion

The most important cellular components in plant and animal cells are proteins. Proteins are made up of different amino acids, in other words, amino acids make up proteins in plant cells. Proteins play a vital role in DNA replication and many molecular and biochemical reactions (Lotfi *et al.*, 2019). In this study, amino acids and proteins showed a fundamental role in reducing the effect of drought stress on yield, which was consistent with the Bastam *et al.* (2013) results. This is because when plants are exposed to stress, they protect the plant. Proteins play an essential role in photosynthesis (Ferguson *et al*., 2005). Plants use photosynthesis to convert water and nutrients (mainly carbohydrates) into amino acids and proteins using complex reactions (Boler K, 1998). Numerous experiments have shown that the yield and productivity of different plants are highly dependent on the amount and quality of amino acids (Salari, 2005). Increasing the quality and quantity of agronomical and horticultural crops, increasing germination, flowering, and fruit growth, increasing plant resistance to drought stress, increasing the activity of beneficial soil microorganisms, the chelating ability of micronutrients such as iron, and increasing their uptake by plants, increasing the uptake of chemical fertilizers if used simultaneously, increasing plant resistance to salinity and water deficit, facilitating plant transpiration and strengthening the root system are the benefits of using amino acid fertilizers (Behrooz *et al.*, 2019; Bernal *et al.*, 2007). In this study, amino acids increased drought stress resistance and yield by increasing the amount of compounds (Crane *et al*., 1977). Other researchers such as Blandino *et al*., (2009) Arena *et al*., (2007), and Apaydin *et al*., (2007) also used foliar application including foliar application of nutrients, foliar application of carbohydrates, foliar application of organic materials. Foliar application of fertilizers containing amino acids in pistachio trees at specific intervals has more effects on increasing the yield. It seems that amino acids and organic compounds reduce the rapid drying of nutrient solution droplets on

the leaf surface, which can play a role in the further absorption of nutrients. These results are similar to our results. On the other hand, foliar application of amino acids in the long-term causes more accumulation of photosynthetic materials and makes them more efficient, which plays a positive role in the quality of the product by increasing the amount of sugar and reducing decay (Bernal *et al*., 2007). Also, S.I. *et al*., showed that consumption of amino acids in trees reduces energy consumption. In other words, when amino acids are given to trees through the foliar application, trees can store more energy, and energy consumption in the plant is significantly reduced. In this study also foliar application of amino acids increased yield. Slocum (2005) said that the expression of glutamate has more importance because it has different roles in various metabolic activities, not only in natural growth conditions but also in specific stressful conditions. The most important role of glutamate is that it is the precursor of many other essential amino acids such as arginine, ornithine, and lysine and is a well-known amino acid in proline biosynthesis. Thus, it indirectly regulates many metabolic activities.

Recently, it has been shown that glutamate is an important signaling molecule that is produced under different environmental conditions in many plant species (Kan *et al*., 2017). It is a source for nitrogen and carbon metabolism that plays a role in the conversion of glutamate to α-ketoglutarate by the glutamate dehydrogenase enzyme (GHD) (Forde and Lea, 2007). Glutamate is also involved in cellular osmotic regulation, especially in stomatal guard cells, and regulates the closing of stoma (Dinu *et al*., 2011). Glutamate is involved in controlling the antioxidant defense mechanism through glutathione biosynthesis, which is an active part of the antioxidant system (Lu, 2013). In addition to its role in carbon and nitrogen metabolism, glutamate is also involved in the synthesis of chlorophyll and vitamin B9, increasing leaf chlorophyll content, and increasing yield by affecting plant photosynthetic activity (Hanson and Gregory, 2011). Aspartic acid is involved in amino acid metabolism. In many plant species, aspartate plays a role in the biosynthesis of amino acids, such as isoleucine, methionine, threonine, and as a precursor in the metabolism of the aspartate family (Rawia *et al*., 2011). In plant cell plastids, aspartate acts as a precursor to arginin production in many metabolic reactions such as aspartate and arginine biosynthesis (Katoh *et al*., 2006; Zrenner *et al*., 2006). Aspartate acts as a buffer to maintain cellular pH. Saeed *et al*., (2005) reported that aspartate acid is involved in the regulation of many processes, including the biosynthesis of chlorophyll, protein, and many plant pigments. This amino acid also promotes the biosynthesis of bio-osmolytes and their storage, including antioxidants, vitamins, and cofactors, which play a role in the cellular homeostasis and defense mechanisms (Azevedo *et al*., 2006, Behzadi Rad *et al.*, 2021). Under stress conditions, foliar application of aspartate acid in plants under water stress conditions improved many growth parameters as well as increased enzymatic activities including POD, POX, and CAT, which led to increased plant resistance. Similar results were obtained in this study and are consistent with the results of Akladious and Abbas (2013). The use of amino acids before, after, and during stress conditions provides amino acids that are directly related to the physiology of stress, thus preventing the negative effects of stress on plants (Mehrnezhad and Javanshah, 2010). Foliar application of nutrients in this study causes its favorable effects on vegetative characteristics, yield and fruit quality, which is consistent with the results of Movahedi Dehnavi *et al* (2006), Tida *et al* (2009) and Goli *et al* (2005). Therefore, due to the severe limitations of water resources in most parts of Iran, drought stress has been introduced as the most important stress affecting the yield of agronomical and horticultural crops (Kafi *et al*., 2014). Considering the

45% reduction in yield of agronomical and horticultural crops due to drought stress and the high concern about the stability of agricultural systems, the use of natural compounds (including amino acids) to regulate growth and biosynthesis, and yield of pistachios in drought stress conditions is recommended.

Acknowledgements

Thanks from the Pistachio Research Center of the Islamic Azad University, Damghan Branch, for its cooperation and for providing laboratory facilities and equipment.

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