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RESEARCH

Hormetic effect of gamma irradiation under salt stress condition in *Phaseolus vulgaris*

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is widely grown around the world and sensitive to stress conditions. Accumulation and degradation of amino acids are informative indicators of plant tolerance. In this study, the vegetative growth and amino acid profiles of two common bean cultivars (Öz Ayşe and F16) under salt stress (50, 100, 150, 200 mM NaCl) were investigated to determine the hormetic effect of low-dose gamma rays (10, 20, 30, 40 Gy ^{60}Co). The irradiated mature embryos of seeds were regenerated in vitro using embryo cultures. The effects of gamma rays on vegetative growth and amino acid profile under stress and non-stress conditions differed. In terms of vegetative growth, 10 Gy for 'Öz Ayşe' and 20 Gy for 'F16' had hormetic effects and stimulated plant growth under non-stress conditions. Under salt stress conditions, the effect of gamma rays varied according to the severity of the stress. 30 Gy for 'Öz Ayşe' and 'F16' at 100 mM salt stress had hormetic effects on vegetative growth. The combined effects of salt stress and gamma rays on the amino acid profile are supported by the vegetative growth results of the plants. Amino acid accumulation occurred at 100 mM at 30 Gy for 'Öz Ayşe' and 'F16'. The most accumulated amino acids were glutamic acid (127.63 mg kg^{-1}), alanine (173.07 mg kg^{-1}), glutamine (188.96 mg kg^{-1}), proline (124.50 mg kg^{-1}), tyrosine (29.23 mg kg^{-1}) in 'Öz Ayşe' and tyrosine (29.88 mg kg^{-1}) in 'F16' under stress. This study shows that low-dose gamma application under moderate stress conditions has a hormetic effect, not under severe salt stress.

Key words: Amino acid, common bean, embryo culture, hormesis, low-dose gamma.

INTRODUCTION

Hormesis is a term used to express the promoting effect of low doses of toxic substances and the suppressive effect of high doses (Calabrese, 2019). Hormetins, or stress sources, are factors that stimulate hormesis. If the stress causes an effect similar to the stimulating effect created by a low dose of a toxin, it is defined as "eustress", namely a beneficial effect, but if it causes irreversible or negative damage, it is defined as "distress", namely harmful stress (Vargas-Hernandez et al., 2017). High doses of ionizing radiation are harmful to plants. However, the concept of high dose differs in plants compared to other organisms. The presence of a cell wall in plants and the totipotency of cells that provide regeneration capability, as well as the presence of a more effective repair system for cellular damage due to the inability of plants to move, result in increased resistance to ionizing radiation (Fornalski et al., 2012). Gamma doses lower than 1 kGy are considered low doses (Iglesias-Andreu et al., 2012), and low-dose gamma application has shown that germination rate and seedling growth in cucumber and okra (Jaipo et al., 2019), germination, fruit number and total yield in tomato (Wiendl et al., 2013), germination index, root and hypocotyl length and photosynthetic pigment content in lettuce (Marcu et al., 2013) are positively affected. Wang et al. (2017) stated that low-dose gamma can affect the expression of genes related to heavy metal transport and abscisic acid metabolism under Pb/Cd stress in barley. Saputro et al. (2019) stated that 25 Gy gamma application positively affected some plant growth parameters under waterlogging stress and caused overexpression of some proteins that are estimated to be 1-aminocyclopropane-1-carboxylate synthase, alcohol-dehydrogenase, and superoxide dismutase. Today, research on radiation hormesis is focused on morphological and photosynthetic changes as well as plant nutrient uptake and yield, whereas studies on biochemical changes are limited.

Common beans (*Phaseolus vulgaris* L.) are an important source of protein, vitamins and minerals as well as an important product for sustainable agriculture. Common beans, which are sensitive to abiotic stress, lose 85% pod yield per plant when they are exposed to a salinity equivalent to 100 mM NaCl (De Pascale et al., 1997). Amino acids, which act as growth factors in plants, are the building blocks of proteins. They are important to stimulate plant growth, act as buffers that help maintain the proper pH in plant cells, are sources of C and energy, contribute to ammonia removal from the cell because they contain both acidic and basic groups, and facilitate the synthesis of different organic compounds such as enzymes (Ali et al., 2019). Amino acid and protein synthesis are high in growing and differentiating cells, and protein and amino acid breakdown increases during aging. Protein and amino acid degradation can also occur under stress conditions (Hildebrandt et al., 2015).

Approximately one-fifth of the arable land in the world is affected by salt stress and every year 1.5 million hectares of land become unsuitable for agriculture (Hasanuzzaman et al., 2014). Global warming has made this stress even more important. Due to tolerance to salt stress is controlled by multiple genes (Zhang et al., 2013), the transfer of these genes in breeding studies is difficult. Different applications are made to overcome this problem. It is necessary to investigate the hormetic effects of gamma rays, which are used in many different industrial areas other than breeding (Aquino, 2012), and the possibilities of use as a pre-treatment should be investigated. The changes caused by this easy and practicable method in the plant biochemical mechanism are not completely known. In vitro studies are important in terms of creating a model before large-scale field studies, as they can get faster results in smaller areas. The use of in vitro culture methods provides many advantages in determining the tolerance of plants not only to salt stress but also to other abiotic and biotic stress conditions. The elimination of the large land need, which is necessary especially in the field conditions, saves time, labor and cost for breeders and researchers. Salt stress tolerance has been successfully carried out in vitro in many economically important plants such as tomato (Zaki and Yokoi, 2016), spinach (Muchate et al., 2019) and potato (Ahmed et al., 2020). This study was aimed to reveal the relationships between low dose gamma rays and changes of amino acid profiles of common beans in non-stress and salt stress conditions depending on the hormetic effect in vitro.

MATERIALS AND METHODS

Plant material and embryo culture

It was used two standard common bean (*Phaseolus vulgaris* L.) cultivars, Öz Ayşe and F16. The source of gamma radiation was cobalt-60 (Ob-Servo Sanguis Co-60, Institute of Isotopes Co., Ltd. [IZOTOP], Budapest, Hungary), with a dose rate 2190 kGy h^{-1}, at the Turkey Atomic Energy Agency. To determine the effect of low-dose gamma irradiation, we irradiated common bean seeds with four different doses (10, 20, 30, 40 Gy). Unirradiated seeds of the cultivars were used as the control group.

The embryo was cultured under in vitro conditions immediately after the seeds were irradiated. The seeds were shaken continuously for 10 min in a 10% sodium hypochlorite solution in a sterile cabinet for surface sterilization. Subsequently, they were placed in 70% ethanol for 5 min and washed three times with distilled sterile water, followed by placing them in distilled sterile water for 12 h. The next day, embryos were taken from seeds and transferred to Murashige and Skooge medium (MS_0) without plant growth regulators containing 30 g L^{-1} sucrose and 7 g L^{-1} agar. Plant growth regulators were not added to any of the media used in the experiment. While only MS_0 medium was used for the control group, 50, 100, 150 and 200 mM NaCl was added to the MS_0 media for salt stress treatments. Cultures were maintained at 24 ± 2 °C and a 16:8 h photoperiod. The experiment was a randomized block trial design with 10 replicates and 10 embryos per replicate. Each Magenta plant culture box (6 × 6 cm) containing 10 embryos was designed as a replicate. Amino acid profiling analyses were performed 21 d after the start of the experiment on leaf samples taken from randomly selected plants.

Extraction of free amino acids

Free amino acids (lysine, Lys; histidine, His; arginine, Arg; cysteine, Cys; serine, Ser; glutamic acid, Glu; threonine, Thr; aspartic acid, Asp; proline, Pro; valine, Val; methionine, Met; tyrosine, Tyr; isoleucine, Ile; leucine, Leu; phenylalanine, Phe) in the ground leaf samples were analyzed using an ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) device according to the method specified by Nimbalkar et al. (2012). Briefly, 0.5 g homogenized sample was extracted with 10 mL water:methanol (80:20) (v/v) solution containing 0.1% (v/v) formic acid. The mixture was vortexed for 5 min and then centrifuged at 4000 rpm for 15 min at 4 °C. The upper phase obtained after centrifugation was passed through a 0.2 µm polytetrafluoroethylene (PTFE) membrane filter and injected into the UHPLC-MS/MS device. Total analysis time was 7 min. Free amino acid profile analysis and determination were performed using a mass spectrometer system (UltiMate 3000 UHPLC, TSQ Fortis, Thermo Scientific, Waltham, Massachusetts, USA). Chromatographic evaluations were made via the Xcalibur software (Thermo Scientific). A Hypersil Gold RP C18 (1.9 µm), 50 × 2.1-mm UHPLC, column was used as analytical column for chromatographic separation.

Statistical analysis

The data obtained at the end of the study were analyzed using the Minitab 17.0 package program (Minitab, State College, Pennsylvania, USA). ANOVA test was used to determine the variations and means were compared by Tukey test. The evaluation and comparison of the confidence intervals of the mean of the treatment were performed with the Interval Plot (Minitab).

RESULTS

Effect of low dose gamma and salt stress on vegetative growth

Shoot length (SL), root length (RL), leaf width (LW), leaf length (LL), shoot fresh weight (SFW), and root fresh weight (RFW) of the two common bean cultivars, together with the statistical analysis results, are presented in Table 1. It was found a significant difference between cultivars in LL, LW, and RFW. Low-dose gamma rays and salt stress, and combinations of low-dose gamma rays and salt stress, had significant effects on all parameters ($p < 0.01$). In the interactions between variation sources, a Cultivar (C) × Low dose (L) interaction in all growth properties, except for SL, and a C × Salt stress (S) interaction for all parameters were significant at the 0.05 and 0.01 levels. The C × L × S triple interaction for all properties was significant at the 0.01 level.

The results of the statistical analysis of the vegetative growth of common bean cultivars treated with different low-dose gamma irradiation are shown in Table 2. Hormetic effect doses differed according to genotypes. Gamma doses had nonsignificant effect on the SL of 'Öz Ayşe'. Further, 10 Gy had a significant effect on RL (98.50 mm), LL (22.11 mm), LW (21.70 mm), SFW (0.3536 g), and RFW (0.5898 g). In 'F16', the stimulating effect of 20 Gy on SL (80.11 mm), RL (114.6 mm), LL (26.34 mm), shoot (0.3480 g), and root (0.5468 g) fresh weight was significant. There was nonsignificant effect of low-dose gamma rays' application on LW in 'F16'. However, the highest value (13.66 mm) was measured at 20 Gy.

Table 3 shows the statistical analysis results of the growth parameters of the embryos regenerated in MS media containing different salt concentrations. Vegetative plantlet growth decreased due to the increase in salt concentration. Especially when the salt concentration was increased to 100 mM, the decline in the growth was obvious. In 'Öz Ayşe', SL (59.22 mm), RL (46.06 mm), and RFW (0.4240 g) obtained at a salt concentration of 50 mM were included in the same statistical group as the control. In addition, SFW (0.4380 g) was higher than in the control (0.3984). Although there was nonsignificant difference among treatments in terms of LW and RFW in 'F16', the highest numerical value was obtained at a salt concentration of 50 mM. Similar to the 'Öz Ayşe', there was nonsignificant difference between the control (38.54 mm) and 50 mM (36.62 mm) for SL in 'F16'. The significantly highest values in RL and SFW were obtained from regenerated embryos at 50 mM. Control plantlets had the highest values in terms of LL (11.90 mm).

Table 1. Variance analysis result with respect to growth parameters of common bean cultivars.

Variation sources	df	SL	RL	LL	LW	SFW	RFW
Cultivar (C)	1	ns	ns	*	**	ns	**
Low-dose (L)	4	*	**	**	*	*	*
Salt stress (S)	4	**	**	**	**	**	**
C × L	4	ns	*	**	*	**	**
C × S	4	*	**	**	**	*	**
C × L × S	24	**	**	**	**	**	**

SL: Shoot length; RL: root length; LL: leaf length; LW: leaf width; SFW: shoot fresh weight; RFW: root fresh weight.
Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).
*, **Significant at $p < 0.05$ and $p < 0.01$, respectively. ns: Nonsignificant.

Table 2. Effects of low-dose gamma on growth parameters in common bean cultivars.

Cultivar	Treatment	SL	RL	LL	LW	SFW	RFW
	Gy	mm				g	
Öz Ayşe	Control	69.73	61.94ab	22.22a	18.68ab	0.3984a	0.5704a
	10	87.65	98.50a	22.11a	21.70a	0.3536a	0.5898a
	20	55.76	94.84a	10.66b	15.74ab	0.2766ab	0.4844ab
	30	73.98	63.74ab	13.05b	21.12a	0.2218b	0.1803bc
	40	51.74	45.82b	8.78b	6.82b	0.1985b	0.1482c
F16		SL	RL	LL	LW	SFW	RFW
	Control	38.54c	19.70b	8.06b	8.52	0.1382b	0.0687b
	10	53.68bc	43.88b	8.35b	8.90	0.2466ab	0.0560b
	20	80.11a	114.60a	26.34a	13.66	0.3480a	0.5468a
	30	62.17ab	44.88b	13.92b	11.78	0.2332ab	0.1264b
	40	40.36bc	32.17b	10.35b	10.19	0.0994b	0.0575b

SL: Shoot length; RL: root length; LL: leaf length; LW: leaf width; SFW: shoot fresh weight; RFW: root fresh weight.
Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

Table 3. Effects of salt stress levels on growth parameters in common bean cultivars.

Cultivar	Treatment	SL	RL	LL	LW	SFW	RFW
	mM	mm				g	
Öz Ayşe	Control	69.73a	61.94ab	22.22a	18.68ab	0.3984a	0.5704a
	50	59.22a	49.06a	11.14b	9.02b	0.4380a	0.4240a
	100	25.08b	26.32b	10.82b	7.90b	0.3050bc	0.0841b
	150	21.86bc	20.48bc	6.78c	6.56b	0.2282c	0.1776b
	200	5.53c	6.82c	3.57d	4.88b	0.1071d	0.0220b
F16		SL	RL	LL	LW	SFW	RFW
	Control	38.54c	19.70b	8.06b	8.52	0.1382b	0.0687b
	50	36.62a	54.48a	11.90a	8.70	0.4736a	0.0869
	100	18.74b	24.68b	8.50ab	6.86	0.2736ab	0.0299
	150	16.46b	13.38b	6.02b	3.48	0.1858b	0.0252
	200	0.00	0.00	0.00	0.00	0.0000	0.0000

SL: Shoot length; RL: root length; LL: leaf length; LW: leaf width; SFW: shoot fresh weight; RFW: root fresh weight.
Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

The comparative analysis of the effects of applications on vegetative parameters in 'Öz Ayşe' is presented in Figure 1. There was a hormetic effect of 30 Gy irradiation on LW (12.72 mm) and SFW (0.5944 g) under 50 mM salt stress. In addition, the hormetic effect of 30 Gy irradiation on SL (42.46 mm), RL (36.88), LW (9.50 mm), shoot (0.3882 g), and RFWs (0.2522 g) was determined at 100 mM salt concentration, where salt stress had a high negative effect on vegetative growth. Low-dose gamma rays applied for any of the parameters at 150 and 200 mM salt stress had no hormetic effect.

The statistical analysis results of the effects of the applications on the vegetative development of the 'F16' are presented as an interval plot (Figure 2). Although there was germination under 200 mM salt stress, no surviving plant could be obtained. Similarly, the embryos of seeds irradiated with 40 Gy did not survive in medium with a salt concentration of 150 mM. In terms of SL (46.16 mm), the embryos of seeds treated with low doses of 40 Gy showed a hormetic effect at a salt concentration of 50 mM. Similarly, it was determined a stimulating effect of 40 Gy on LW (9.06 mm) and RFW (0.2122 g). An irradiation dose of 30 Gy at salt stress of 100 mM had a hormetic effect on all parameters. When a salt concentration of 150 mM was applied, the effect of the 20 Gy irradiation dose was significant in terms of SL (28.30 mm) and RL (14.70 mm). All applications were in the same statistical group in respect to LL. The dose of 10 Gy had a prominent effect on LW (7.08 mm). All applications on SFW gave better results than 150 mM application and were included in a separate group. A hormetic effect of 10 (0.0433 g) and 30 Gy (0.0401 g) on RFW was determined.

Figure 1. Interval plots of the effects of low-dose gamma rays and salt stress on the vegetative growth of common bean 'Öz Ayşe' (mean ± 95% confidence intervals).

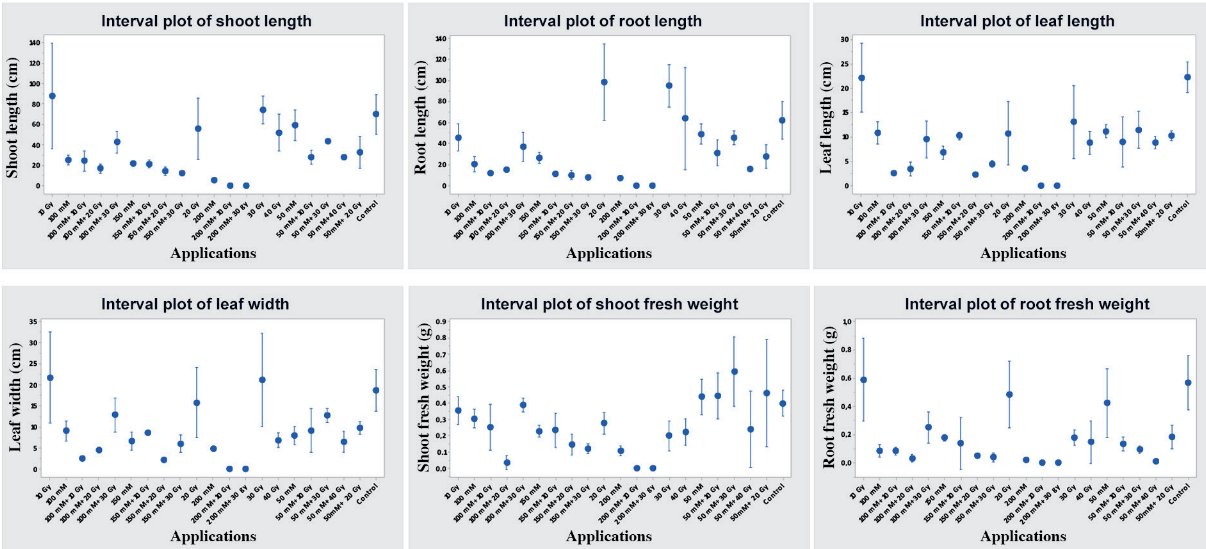
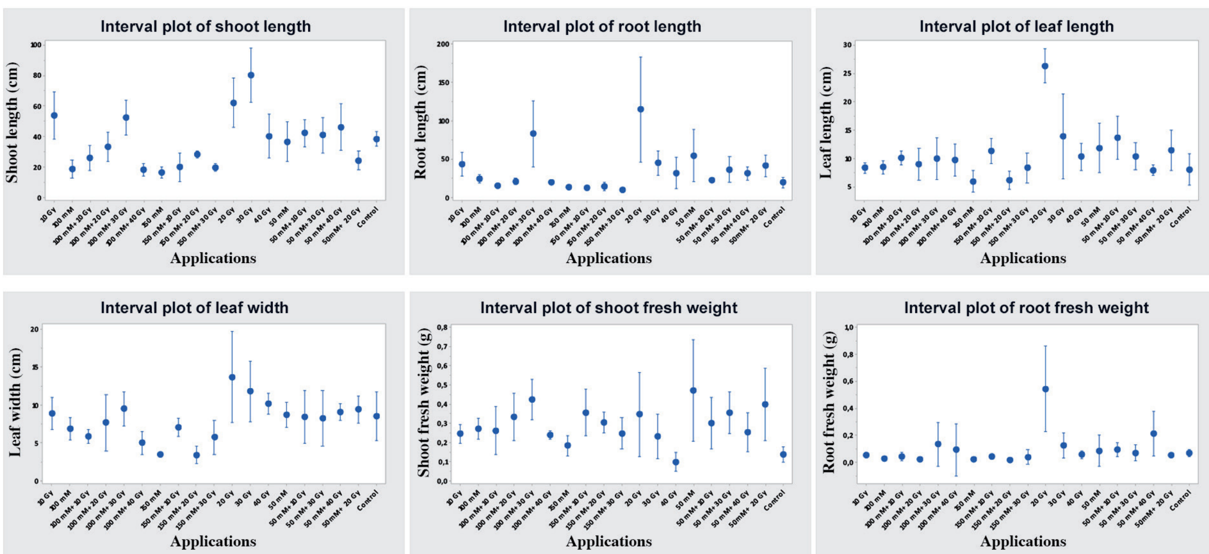


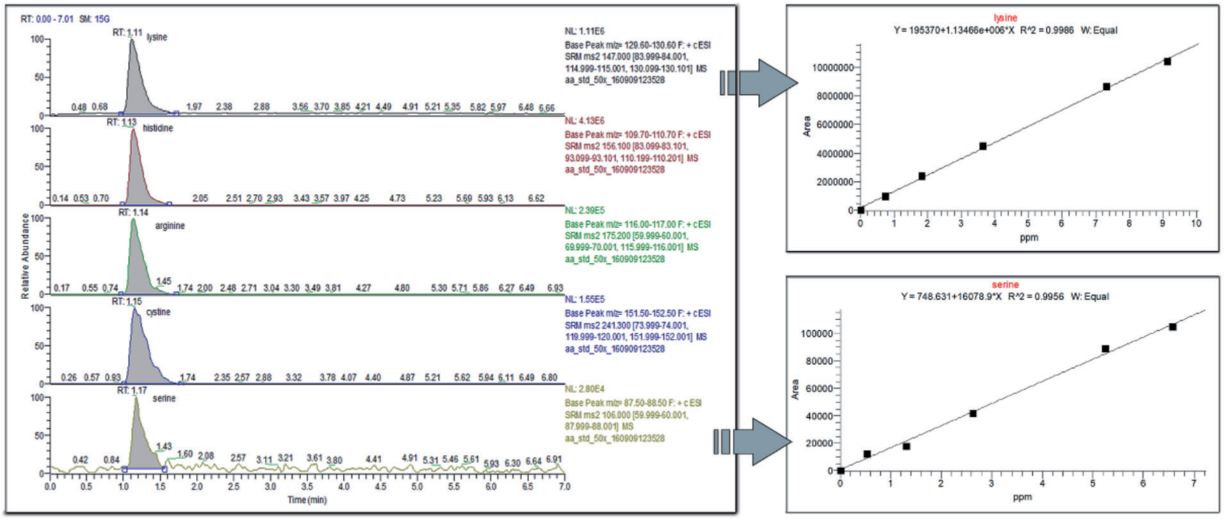
Figure 2. Interval plots of the effects of low-dose gamma and salt stress on the vegetative growth of common bean 'F16' (mean ± 95% confidence intervals).



Free amino acid profiling

Chromatograms and calibration curves of some free amino acids are presented in Figure 3. The correlation coefficients (R^2) of linear regression analysis from calibration curves were between 0.9929 and 0.9999. The limits of detection (LOD) and limits of quantification (LOQ) for sensitivity were evaluated in the ranges of 4.4-7.6 and 14.6-25.5 mg kg^{-1}, respectively. The relative standard deviations (% RSD) of the peak corrected areas obtained by injecting amino acid standard solutions six times in a row were determined; the RSD values corresponding to precision were between 2.3% and 4.1%. The accuracy of the method was determined by adding standard amino acids to the leaf sample whose amino acid analysis was performed and evaluating the recovery. The recovery values varied between 87.2% and 96.3%.

Figure 3. Chromatograms and calibration curves of some free amino acids.



The ANOVA results regarding the amino acid profiles of two common bean cultivars are shown in Table 4. As a source of variation, the cultivars had a significant effect at the level of 0.05 on Thr, Glu, Ala, Pro, Tyr and total free amino acid (TFAA), while on Gln and Val, the effect was significant at 0.01. The effect of low salt stress on Lys, Asp, Ser, Glu, Ala, Gln, Val and TFAA was significant at 0.05, while salt stress had a significant effect on all amino acid contents at 0.01. The interaction Cultivar × Low-dose gamma irradiation was significant at 0.05 for Pro and Leu, while for all other amino acids, it was significant at 0.01. The interaction Variety × Salt was significant at 0.05 only for Met. For the other amino acids, 0.01 was the significance level. The results of triple interaction Cultivar × Low-dose gamma rays × Salt stress revealed that there was a triple interaction at 0.01 for all amino acids.

The amino acid profiles of the common bean cultivars differed according to the gamma ray doses applied (Table 5). For Lys, (43.94 mg kg^{-1}), Cys (14.31 mg kg^{-1}), His (37.04 mg kg^{-1}), Arg (37.46 mg kg^{-1}), Asp (147.55 mg kg^{-1}), Ser (70.08 mg kg^{-1}), Thr (47.25 mg kg^{-1}), Glu (127.29 mg kg^{-1}), Ala (183.25 mg kg^{-1}), Gln (178.82 mg kg^{-1}), Pro (124.32 mg kg^{-1}), Val (69.01 mg kg^{-1}), Ile (69.51 mg kg^{-1}), Leu (57.28 mg kg^{-1}), Phe (88.41 mg kg^{-1}), and TFAA (1307.33 mg kg^{-1}), the levels were highest in the plants obtained from embryos of 40 Gy irradiated 'Öz Ayşe' seeds. Only the levels of Met (8.9 mg kg^{-1}) and Tyr (23.30 mg kg^{-1}) increased at 20 Gy in 'F16', while Met (9.41 mg kg^{-1}) and Tyr (27.29 mg kg^{-1}) were highest at 40 Gy, generally, the amino acid values were increased at 10 Gy. However, Arg (40.70 mg kg^{-1}), Asp (146.26 mg kg^{-1}), Ala (166.24 mg kg^{-1}), Pro (118.86 mg kg^{-1}) and Val (64.02 mg kg^{-1}) reached the highest values at 20 Gy. Comparing the two cultivars, the amino acid values were generally higher for 'F16'.

Table 4. ANOVA result with respect to the amino acid profile of common bean cultivars.

Variation sources	df	Lys	Cys	His	Arg	Asp	Ser	Thr	Glu	Ala
C (Cultivar)	1	ns	ns	ns	ns	ns	ns	*	*	*
L (Low-dose)	4	*	ns	ns	ns	*	*	ns	*	*
S (Salt stress)	4	**	**	**	**	**	**	**	**	**
C × L	4	**	**	**	**	**	**	**	**	**
C × S	4	**	**	**	**	**	**	**	**	**
C × L × S	24	**	**	**	**	**	**	**	**	**
		Gln	Pro	Val	Met	Tyr	Ile	Leu	Phe	TFAA
C	1	**	*	**	ns	*	ns	ns	ns	*
L	4	*	ns	*	ns	ns	ns	ns	ns	*
S	4	**	**	**	**	**	**	**	**	**
C × L	4	**	*	**	**	**	**	*	**	**
C × S	4	**	**	**	*	**	**	**	**	**
C × L × S	24	**	**	**	**	**	**	**	**	**

Ala: Alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine; TFAA: total free amino acid.
*, **Significant at $p < 0.05$ and $p < 0.01$, respectively. ns: Nonsignificant.

Table 5. Effects of low dose gamma rays applications on amino acid profile of common bean cultivars.

Cultivar	Dose	Lys	Cys	His	Arg	Asp	Ser	Thr	Glu	Ala
	Gy					mg kg^{-1}				
Öz Ayşe	Cnt	32.63b	12.05a	29.44c	33.81b	111.39c	49.25c	35.76c	93.08c	126.56c
	10	41.54a	13.80a	33.14b	36.78a	132.28b	62.70b	43.99b	118.51b	161.13b
	20	30.43bc	6.48b	21.34d	24.86c	103.69d	47.70c	21.24d	82.72d	110.13d
	30	28.22c	6.09b	20.86d	24.30c	99.56e	44.24d	20.04d	76.72e	102.14e
	40	43.94a	14.31a	37.04a	37.46a	147.55a	70.08a	47.25a	127.293a	183.25a
		Gln	Pro	Val	Met	Tyr	Ile	Leu	Phe	TFAA
	Cnt	133.06c	90.67c	49.61c	3.85c	4.33b	50.69c	42.62c	62.25c	961.15c
	10	169.41b	115.44b	63.16b	4.90bc	5.51b	64.54b	54.27b	79.26b	1200.46b
	20	125.11d	75.23d	49.03c	8.09a	23.30a	40.33d	37.07d	55.43d	862.25d
	30	116.03e	69.77e	45.47d	7.50ab	21.61a	37.40e	34.38e	51.40e	805.81e
	40	178.82a	124.32a	69.01a	5.57abc	6.16b	69.51a	57.28a	88.41a	1307.33a
F16		Lys	Cys	His	Arg	Asp	Ser	Thr	Glu	Ala
	Cnt	48.95b	13.80ab	38.71a	34.01c	142.82b	73.80b	42.70bc	122.18b	159.66b
	10	51.76a	15.86a	40.83a	36.50b	144.10ab	76.36a	46.33a	126.03a	167.76a
	20	41.97c	13.69ab	35.29b	40.70a	146.26a	61.22c	43.69ab	119.52c	166.24a
	30	35.81d	12.32b	31.31c	32.83c	118.26d	55.04d	39.40c	98.69d	134.01c
	40	35.11d	7.73c	24.52d	28.61d	121.82c	53.31d	23.99d	96.71d	124.39d
		Gln	Pro	Val	Met	Tyr	Ile	Leu	Phe	TFAA
	Cnt	179.41a	105.92c	61.67a	5.87b	6.50bc	77.23a	63.88a	90.18b	1267.36b
	10	183.00a	112.10b	62.93a	6.06b	6.93b	83.13a	66.46a	94.86a	1321.07a
	20	162.17b	118.86a	64.02a	4.97b	5.78bc	62.95b	54.02b	75.87c	1217.29c
	30	138.75d	91.05d	50.72c	4.31b	4.51c	50.90c	45.14c	68.32d	1009.72d
	40	144.17c	88.39e	57.50b	9.31a	27.29a	46.41c	44.16c	62.66e	997.90e

Cnt: Control; Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine; TFAA: total free amino acid. Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

The responses of the cultivars to different salt stress levels in terms of amino acid amounts differed (Table 6). In 'Öz Ayşe', the highest values were generally detected in plants obtained from embryos cultured at a salt concentration of 100 mM, where the physiological effects of salt stress could be observed. In contrast the levels of Met (8.84 mg kg^{-1}) and Tyr (25.06 mg kg^{-1}) increased at 150 mM. A more complex situation was observed for 'F16'. While the highest amounts of Cys (11.41 mg kg^{-1}), His (30.52 mg kg^{-1}), Arg (34.53 mg kg^{-1}), Thr (37.02 mg kg^{-1}), Glu (96.37 mg kg^{-1}), Pro (96.06 mg kg^{-1}), Ile (48.89 mg kg^{-1}), and Leu (45.08 mg kg^{-1}) were detected at 100 mM; Lys (36.90 mg kg^{-1}), Asp (123.12 mg kg^{-1}), Ser (56.55 mg kg^{-1}), Ala (128.36 mg kg^{-1}), Gln (143.30 mg kg^{-1}), Val (56.27 mg kg^{-1}), Met (9.10 mg kg^{-1}), Tyr (27.77 mg kg^{-1}), Phe (67.22 mg kg^{-1}), and TFAA (999.00 mg kg^{-1}) reached the highest values at 150 mM. Unlike for low gamma rays doses, when each stress level was examined separately, the amino acid amounts of 'Oz Ayşe' were generally higher.

The interval plot in Figure 4 allows the comparison of all applications and combinations of these applications. We observed differences in the amino acid values of 'Öz Ayşe' depending on low-dose gamma rays applications and salt stress. Generally, amino acid accumulation occurred at 30 Gy at 50 and 100 mM NaCl. It cannot be said that a single irradiation dose stands out at 150 mM.

Low-dose gamma irradiation and salt stress applications caused differences in the plant amino acid profile in 'F16' (Figure 5). Significantly highest values were generally obtained for control and 30 Gy gamma irradiation. In general, amino acid accumulation occurred at 40 Gy at 50 mM and 30 Gy at 100 mM. Similar to 'Öz Ayşe' at 150 mM, it was determined that there was no relationship between the increase in amino acid amount and gamma rays dose. On the other hand, there was nonsignificant difference in Thr amounts among the applications ($p < 0.05$).

Table 6. Effects of salt stress levels on amino acid profile of common bean cultivars.

Cultivar	NaCl	Lys	Cystine	His	Arg	Asp	Ser	Thr	Glu	Ala
	mM					mg kg^{-1}				
Öz Ayşe	Cnt	32.63c	12.05b	29.44c	33.81b	111.39c	49.25c	35.76c	93.08c	126.56c
	50	36.32b	12.28b	32.22b	38.37a	126.38b	53.89b	39.97b	96.53b	135.93b
	100	45.46a	15.13a	37.69a	38.86a	142.66a	65.02a	48.06a	129.90a	173.29a
	150	34.61bc	7.09c	24.21d	28.21c	111.66c	49.50c	22.82e	90.67c	118.77d
	200	28.97d	9.98b	25.31d	24.72d	125.72d	42.28d	32.36d	84.26d	112.52e
		Gln	Pro	Val	Met	Tyr	Ile	Leu	Phe	TFAA
	Cnt	133.06d	90.67c	49.61c	3.85b	4.33b	50.69c	42.62b	62.25c	961.15c
	50	148.42b	101.14b	55.23b	4.15b	4.41b	55.39b	44.99b	65.71b	1051.41b
	100	181.96a	126.76a	64.33a	5.37b	6.14b	69.41a	58.37a	86.74a	1295.24a
	150	139.83c	82.47d	55.63b	8.84a	25.06a	44.14d	37.76c	62.89c	944.22c
	200	120.62e	77.92e	44.98d	3.49b	3.62b	44.30d	36.39c	55.26d	889.18c
F16		Lys	Cys	His	Arg	Asp	Ser	Thr	Glu	Ala
	Cnt	48.95a	13.80a	38.71a	34.53a	142.82a	73.80a	42.70a	122.18a	159.66a
	50	28.56d	9.97bc	25.27c	27.14b	90.44d	43.03d	30.12c	82.93c	110.41d
	100	33.16c	11.41ab	30.52b	34.53a	111.72c	50.29c	37.02b	96.37b	122.07c
	150	36.90b	7.56c	24.93c	27.98b	123.12b	56.55b	23.90d	94.94b	128.36b
	200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Gln	Pro	Val	Met	Tyr	Ile	Leu	Phe	TFAA
	Cnt	179.41a	105.92a	61.67a	5.87b	6.50b	77.23a	63.88a	90.18a	1267.36a
	50	114.40d	77.84d	41.74d	3.29b	3.78c	44.99c	35.24c	51.47d	820.71d
	100	138.32c	94.06b	50.66c	4.06b	4.41bc	48.89b	45.08b	64.58c	977.25c
	150	143.30b	83.15c	56.27b	9.10a	27.77a	44.57c	43.32b	67.22b	999.00b
	200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Cnt: Control; Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine; TFAA: total free amino acid. Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

DISCUSSION

The hormetic effect of low-dose gamma rays was determined based on the comparison of vegetative growth and amino acid profiles of irradiated and non-irradiated common bean seeds under salt stress conditions. Low-dose gamma irradiation and salt stress had significant effects on vegetative growth. The stimulating effect of gamma rays is based on accelerating cell division, positively affecting vegetative growth (Marcu et al., 2013). Root development is an important parameter in salt stress. In 'Öz Ayşe', 10 Gy increased RL. The most effective irradiation dose in 'F16' was 20 Gy; it dramatically increased RL and, considering its effect on RFW, plays a role not only in the elongation of roots but also in the production of organic matter. In a study conducted on cowpea, low-dose gamma rays application increased mitotic activity at the root tips in M_1 and M_2 plants (Badr et al., 2014). Similarly, low-dose gamma rays application in onion causes an increase in the proportion of dividing cells (Kumar et al., 2011). In addition, 10 Gy gamma irradiation positively affected SL, RFW, SFW and leaf development in 'Öz Ayşe'. Salt stress adversely affected plant growth as expected. In both cultivars, this negative effect was clearly observed at 100 mM. The counteractive effect of low-dose gamma rays against this negative influence was determined at 30 Gy at 50 and 100 mM in 'Öz Ayşe'. In 'F16', 30 Gy for 100 mM salt stress had hormetic effects. The results obtained were consistent with the findings of studies performed to demonstrate the hormetic effect of low-dose gamma irradiation on apricot (El-Sabagh et al., 2011), lettuce (Marcu et al., 2013), and *Arabidopsis* (Qi et al., 2014). Zaka et al. (2004) drew attention to point mutations that occur in apical meristem cells caused by low-gamma irradiation at an early stage of plant development. The authors state that the repair mechanism is triggered at high gamma doses, but at low doses, it is transferred without induction of the repair mechanism, and accumulation occurs during successive cell divisions. Low-dose gamma irradiation accelerates cell division, whereas the repair mechanism is not induced; this explains the increase in plant growth. Changes caused by gamma rays in chromosomes vary depending on the dose and variety.

Figure 4. Interval plot of the effect of NaCl and low-dose gamma ray applications on the amino acid profile of 'Öz Ayşe' cultivar (mean ± 95% confidence intervals).

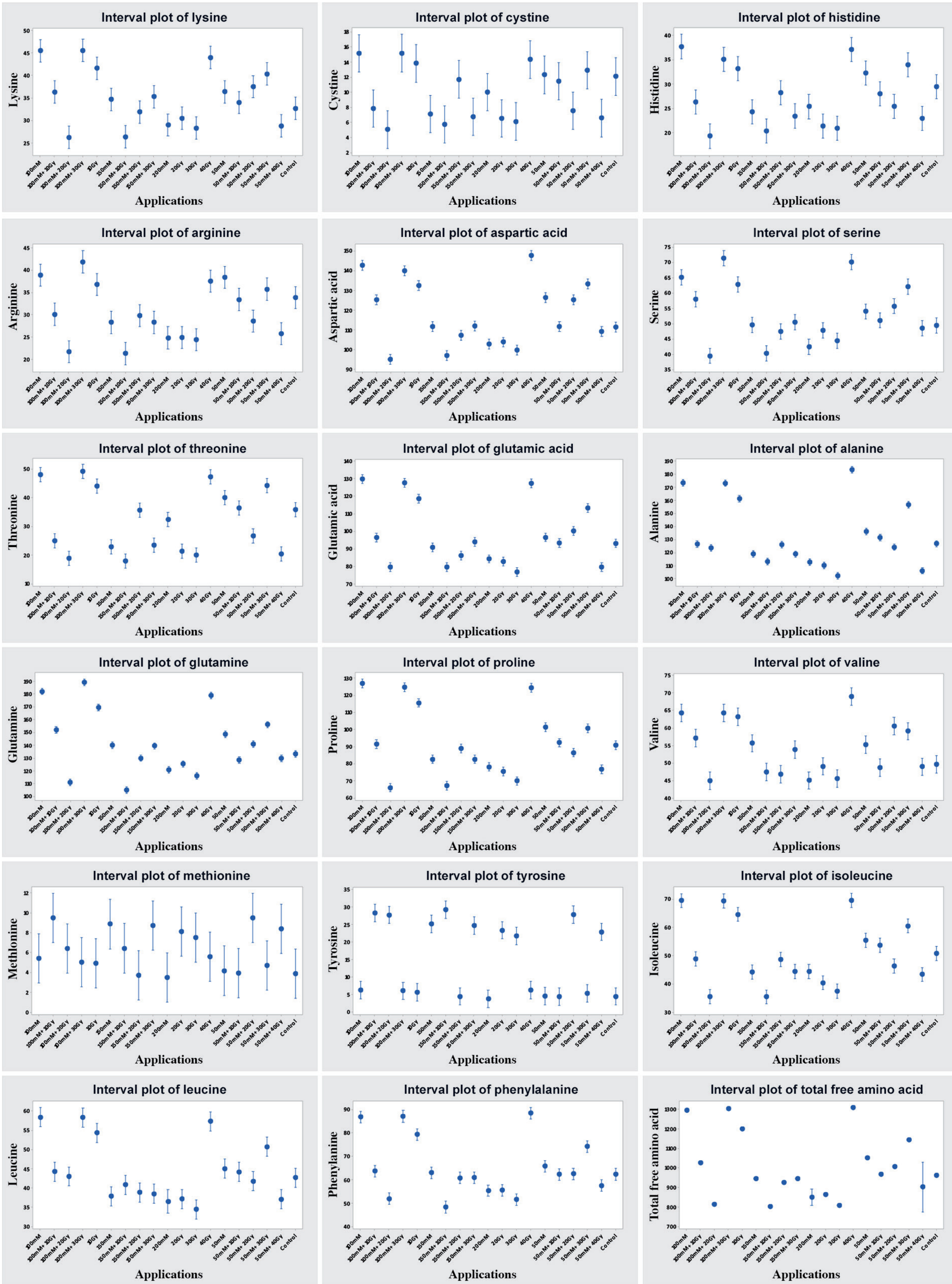
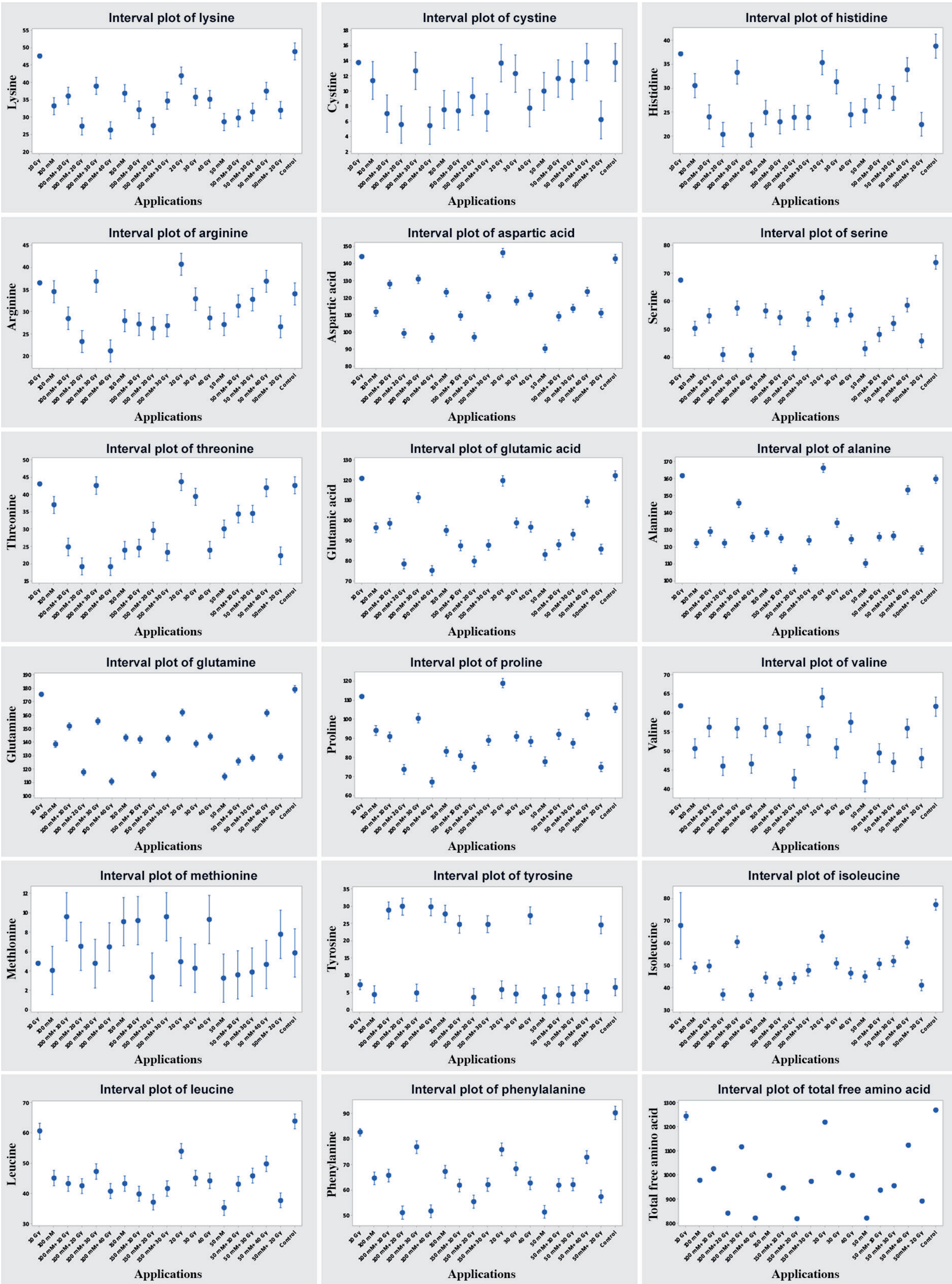


Figure 5. Interval plot of the effect of NaCl and low-dose gamma ray applications on the amino acid profile of common bean ‘F16’ (mean ± 95% confidence intervals).



Although high gamma doses in cowpea increase chromosomal abnormalities such as anaphase delays, anaphase bridging, chromosome clustering, chromosome breaks (Dhanavel et al., 2012), low-dose gamma applications in some cowpea varieties have led to more chromosomal abnormalities (Badr et al., 2014). Chromosomal abnormalities do not always cause negative consequences. It is also reported that there is a positive correlation between chromosomal abnormalities and the increase in antioxidant enzyme activity that activates the defense mechanisms of cells (Datta et al., 2011).

Amino acids, one of the important components of plant metabolism, play various roles in growth, development and defense processes (Ali et al., 2019). Changes in the amounts of amino acids, modifying plant metabolism under stress conditions, can determine defense mechanisms in plants and help in understanding tolerance levels. From this viewpoint, the effects of gamma ray and salt stress on the amino acid profile differed between cultivars. When only low-dose gamma irradiation was applied without salt stress, in general, 40 Gy in 'Öz Ayşe' and 10 Gy in 'F16', this caused an increase in amino acid amounts. However, the increase in plant growth at some low-dose gamma rays levels is thought to be related to the stimulating effect of the gamma rays rather than the stress effect. The results for salt stress provide more explanatory information for amino acid changes. The levels of the amino acids that decreased with the application of salt stress increased with increasing stress levels. In studies conducted in soybean (Farhangi-Abriz and Ghassemi-Golezani, 2016), wheat (Aly et al., 2019a), moringa (Hussen et al., 2013), the amount of amino acids increased under salt stress, which was associated with tolerance to salt stress. However, the increase in amino acids differs according to the species and genotype. For example, Asp, Thr, Ser, Ala, Val, Gly, Ile and His were increased in moringa depending on the concentration, and in this study, there was a significant increase in the amounts of Glu, Ala, Gln, Pro, Tyr in 'Öz Ayşe' and in Tyr in 'F16'. Cavusoglu (2019) reported that Tyr, when applied externally, mitigated the negative effects of salt stress on physiological and cytological parameters. The fact that more Tyr was synthesized under severe stress may be related to the fact that Tyr acts as the precursor of several specific metabolites that perform a variety of physiological functions such as defense mechanisms, structural support, electron carrying and antioxidant activity. In this study, in both common bean cultivars, 150 mM reached the highest value.

The fact that Ala is one of the amino acids associated with stress response (Galili, 2011) explains the increase in its amount. In wheat, Cys, Arg and Met constitute 55% of the total free amino acids. However, Xie et al. (2020) reported that in *Phragmites australis*, 19 amino acids (Val, Ile, Pro, His...) accumulate depending on the salt stress period and the prolongation of the process causes an increase in the amino acid concentration. Lysine is an important signaling amino acid that regulates plant growth and responses to the environment. It is also synthesized in higher plants in a route that begins with aspartate, leading to the formation of Thr, Met and Ile (Ali et al., 2019). In this respect, when the amounts of Lys, Glu, Thr, Met and Ile were examined, they increased at 100 mM in 'Öz Ayşe', and this increase in Met continued at 150 mM. We assume that the lack of such an increase in 'F16' is due to the sensitivity of the cultivar. In addition, the fact that the plants could not be obtained at a salt stress of 200 mM was another evidence of the sensitivity of 'F16'. Simple organic molecules, such as free amino acids, are involved in the regulation of plant osmosis in saline soil conditions (Aly et al., 2019b). Consistent with this study, studies in different plants show that amino acid synthesis is related to the plant's stage of development, intensity, and duration of the stress (Yang et al., 2015; Farhangi-Abriz and Ghassemi-Golezani, 2016; Xie et al., 2020). The decrease of Pro after 100 mM salt application in 'Öz Ayşe' was an indication of this situation.

The amino acid contents of plants grown at salt concentrations of 50 and 100 mM were generally highest in seeds irradiated with 30 Gy in 'Öz Ayşe'. The plant growth values also support this result. It is not possible to mention a single gamma rays dose for the stimulative effect of gamma irradiation at 150 mM. Since common beans are sensitive to stress conditions, gamma rays did not have a hormetic effect for highly severe stress, but low-dose gamma rays affected the amino acid profile positively under moderate stress conditions, which was also reflected in plant growth. In 'F16', 40 Gy at a salt concentration of 50 mM had an obvious hormetic effect. In addition, the stimulating effect of 30 Gy at 100 mM is generally reflected in the accumulation of all amino acids. Branched-chain amino acids (Leu, Ile and Val) accumulate during stress due to protein breakdown and can act as respiratory substrates. In *Arabidopsis*, a model plant, branched amino acids accumulate in response to some stress conditions such as salt, drought and herbicide application, and this accumulation is more significant than that of Pro, which has been the subject of many studies (Huang and Jander, 2017). Such an accumulation occurred for 30 Gy in 'Öz Ayşe' and 'F16', and the fact that these applications also promote plant growth suggests that amino acid accumulation may be due to the stimulation of amino acid synthesis rather than protein degradation. The regulatory role of amino acid metabolism in the response of plants to stress conditions includes the suppression of genes associated with amino acid biosynthesis and the stimulation of genes associated with amino acid catabolism in stress-induced transcriptome changes (Galili, 2011). The increase in vegetative plant growth in this study can be explained by the activation of genes that stimulate amino acid biosynthesis with a low-dose gamma rays effect.

The stability of protein synthesis in common beans and the synthesis of certain polypeptides continued under salt stress with low-dose gamma rays application (Beltagi et al., 2006). In accordance with this study, the stimulating effect of low-dose gamma rays administration at moderate salt stress was demonstrated. Similar results were obtained in a study conducted on wheat, where salt stress and gamma rays caused an increase in the amino acid content, and the authors reported changes in the protein profile depending on the irradiation dose and the salinity level (Aly et al., 2019a). In this study, since there was no external support, internal synthesis increased as a result of the stimulation by gamma rays, and tolerance to salt was achieved by stimulating plant growth. These increases are due to changes in the metabolism of free amino acids that act as osmoprotectants or to protein turnover under stress conditions. For example, in a study on *Arabidopsis* the free amino acid profile under water stress, as in this study, depended on the type and severity of the stress (Hildebrandt, 2018; Yobi et al., 2020). In another study, the amino acid content of beans also increased under moderate drought stress, suggesting that the amino acid level can be used as a biochemical marker in breeding studies (Andrade et al., 2016). The accumulation of amino acids indicates plant tolerance, as the accumulation of specific amino acids such as Pro, as well as secondary metabolites derived from amino acid metabolism, are associated with increased tolerance to adverse environmental conditions.

CONCLUSIONS

In this study, low-dose gamma rays stimulated vegetative growth in common beans. Amino acid accumulation in non-irradiated plants was at 100 mM NaCl in 'Öz Ayşe'. In 'F16', the control was the group with the highest amount of amino acids. This is thought to be due to the fact that 'F16' is very sensitive to salt stress. When salt stress was applied, amino acid accumulation occurred at certain gamma rays doses. Consistent with the vegetative development of plants under salt stress conditions, low-dose gamma rays have a hormetic effect at moderate salt stress, but not under severe salt stress.

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