

## The effects of methanol and amino acid glycine betaine on qualitative characteristics and yield of sugar beet (*Beta vulgaris* L.) cultivars

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### Abstract

In order to investigate the effects of methanol and glycine betaine application on quality traits and yield of different fodder beet cultivars, the experiment was performed as a combined split-factorial design based on randomized complete block design with 3 replications in two years in Isfahan, Iran. The concentrations of methanol in 3 levels (control, 15 and 30% v/v methanol) were considered as the first treatment, the concentrations of glycine betaine in 2 levels (control and 4 g per liter) as the second treatment and different cultivars ('Sentinel', 'Drafter', 'Rivolta', 'Elanta', 'Rasta', and 'Qualita') were considered as the factorial. Foliar spraying was performed at three intervals every two weeks. The results showed that the methanol spraying affected on root yield, sugar, potassium and sodium content, catalase enzyme activity, superoxide dismutase, rubisco, and malondialdehyde significantly. Glycine betaine foliar application showed significant differences in root yield, sugar, potassium and sodium content, enzyme catalase activity, superoxide dismutase, rubisco, and malondialdehyde. Based on the results, the utilization of methanol and glycine betaine caused quality improvement of the sugar beet under similar conditions of the present experiment.

**Keywords:** catalase; malondialdehyde; nitrate reductase; rubisco; superoxide dismutase

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### Introduction

Most parts of the world are exposed to drought, and water scarcity is one of the critical issues leading to reduced crop yields. Beet cultivars have shown significant differences in terms of yield under different environmental conditions, which indicates their adaptability to environmental stresses (Albayrak and Camas, 2007). Increasing the concentration of carbon dioxide due to reduced light respiration and counteracting the destructive effects of environmental stress can improve plant performance by preserving or increasing dry matter. One way to increase the concentration of carbon dioxide and reduce the destructive effects of drought stress on plants is to apply the alcoholic compounds and glycine betaine (GB) amino acid. Methanol is the simplest compound produced in the plant (Felix *et al.*, 2019). Methanol produces carbon dioxide in the leaves

and escalates photosynthesis, so it can be used as a carbon source (Saneienejad *et al.*, 2019). Methanol also controls the growth and development of plants by affecting the intracellular transport of macromolecules (Dorokhov *et al.*, 2018). It has been indicated that when the cotton treated with exogenous methanol, the Rubisco activities increased by above 60%, and also the net photosynthesis rate and intercellular CO<sub>2</sub> concentration were increased. Many studies have shown the role of methanol as a biochemical by-product as a signal molecule in plant-plant communication, but studies in the last decade have revealed that it has participated in metabolic biochemical processes during growth and development. Methanol emissions escalate in response to mechanical wounding or other stresses due to damage of the cell wall, which is the main source of methanol production. The insoluble compounds of electrical chargeless such as alcohols, aldehydes, and sugars easily penetrate and pass-through protoplasmic membranes. Also, methanol passes through by diffusion from the membrane of the plant cells (Reinhold and Kaplan, 1984). Under drought stress condition, methanol spraying can prevent their biomass reduction (Dorokhov *et al.*, 2018). However, methanol spraying has an negative effect on plants that do not have moisture restrictions (Nadali *et al.*, 2010; Rehman and Khalil, 2018).

The toxicity of methanol solutions may be related to the ability to oxidize it. Methanol in some plants could be oxidized, immediately, to CO<sub>2</sub>, formaldehyde, and formic acid (Fall and Benson, 1996). The intermediate formaldehyde represents a reactive electrophilic species with high toxicity that is rapidly detoxified by a pathway involving three key enzymes: (1) NAD dependent formaldehyde dehydrogenase (FALD), (2) thioesterase S-formylglutathione (FGH), and (3) NAD-dependent formate dehydrogenase (FDH), which oxidizes the formate to CO<sub>2</sub> (Haslam *et al.*, 2002; Kordic *et al.*, 2002; Achkor *et al.*, 2003). According to Demmers-Derks *et al.* (1996), increasing dioxide carbon content will not essentially result in increased sugar content in plants, because there is a negative correlation between sugar content and root yield. Excessive synthesis of methanol has been observed to lead to the shortening of tobacco (Hasunuma *et al.*, 2004; Sheshukova *et al.*, 2017). Therefore, methanol seems to be essential for balance in plant growth and development. Methanol application on C<sub>3</sub> plants can compensate for part of the photosynthetic carbon losses, so increases the efficiency of photosynthesis (McGiffen and Manthey, 1996; Behrouzfar and Yarnia, 2016; Rehman and Khalil, 2018). Methanol is oxidized in the form of aldehyde and carbon dioxide in the plant and is synthesized as amino acids (serine and methionine) and carbohydrates in various tissues of C<sub>3</sub> plants. Methanol-treated plants can increase their net assimilation rate (Nonomura and Benson, 1992). Exogenous methanol accelerates the production of sugars and amino acids and increases the production of dry matter, leaf photosynthesis, grain yield, and also reduces the water use efficiency on the plant (Valizadeh-Kamran *et al.*, 2019). Increasing the crop growth rate after methanol spraying is due to increasing the concentration of carbon dioxide in the leaves and using methanol as a direct source for the synthesis of serine amino acids besides reducing carbon loss through light respiration (Dorokhov *et al.*, 2018). Glycine betaine plays an important role in regulating cellular osmosis, maintaining organs (mitochondria, chloroplasts), and water efficiency in plants under water deficit (Ashraf and Foolad, 2007; Kurepin *et al.*, 2015). The exogenous glycine betaine has been proven to improve growth traits and plant performance under drought stress (Khan *et al.*, 2015; Joshi *et al.*, 2016).

Nawaz and Wang (2020) report drought stress decreased photosynthetic pigments and increased reactive oxygen species, lipid peroxidation, osmolytes, and antioxidants in the plant. Nonetheless, exogenous GB alone improved drought tolerance. The maximum decrease in malondialdehyde, and increase in soluble sugars, chlorophyll contents, and superoxide dismutase, catalase, peroxidase was recorded when GB was applied alone under drought. In many plants, the accumulation of GB is lower than sufficient to modify the adverse effects of dehydration caused by various environmental stresses. Exogenous application of GB could help diminish the adverse effects of environmental stresses (Mäkelä, 2004; Ashraf and Foolad, 2007). Sprayed GB is immediately absorbed by plant tissues and is readily translocated to the roots, meristems, and leaves (Mäkelä *et al.*, 2000). Because GB is metabolically quite inert in plants, it remains in the plant cytosol and chloroplasts for several weeks (Preedy, 2015). When applied to the leaves of plants, glycine betaine is taken up by leaf tissues and roots (Park *et al.*, 2006). The most of the glycine betaine that is taken up by the leaves is localized in the

cytosol and only a small fraction of the cytosolic glycine betaine is translocated to chloroplasts (Park *et al.*, 2006). Glycine betaine was translocated to actively growing and expanding parts of plants, the long-distance translocation of glycine betaine being mediated by the phloem (Mäkelä *et al.*, 1996).

All forms of abiotic stress cause an oxidation reaction in plant cells. However, the presence of glycine betaine significantly reduced the production of  $H_2O_2$  and efflux of  $K^+$  ions dose-dependent on against abiotic stress (Cuin and Shabala, 2007; Chen and Murata, 2008). Glycine betaine mitigates the damaging effects of oxidative stress by activating or stabilizing reactive oxygen species (ROS)-scavenging enzymes and repressing the production of reactive oxygen species (Park *et al.*, 2006). The accumulation of glycine betaine in chloroplasts and cytosol is effective in tolerating plants to abiotic stress (Wiśniewska *et al.*, 2019). Glycine betaine counteracted the inhibitory effect of drought stress by the repair of photosystem II (PSII) (Ohnishi and Murata, 2006). Glycine betaine stabilizes and strengthens structures, increases enzymatic activity, and cell wall stability in the face of the damaging effects of stress is one of its activities. Therefore, the effect of methanol and glycine betaine as reducing agents of drought stress damage on sugar beet was investigated. Koukourikou-Petridou and Koukounaras (2002) report methanol with glycine treatment increased root length and weight, and chlorophyll content. when used methanol alone, lead to achieving similar results as the control.

## Materials and Methods

### *Study context*

This experiment was performed as a combined split-factorial design based on randomized complete block design with 3 replications in two years (2018 and 2019) in, Isfahan, Iran (32°43'29.8"N 51°50'09.8"E with 1543 meters above sea level).

### *Sampling design and biological material*

The concentrations of methanol in 3 levels (control, 15 and 30% v/v methanol) as the first treatment (A), and the concentrations of glycine betaine in 2 levels (control and 4 grams per liter (called 4 g)) as the second treatment (B) and 6 cultivars ('Sentinel', 'Drafter', 'Rivolta', 'Elanta', 'Rasta', and 'Qualita') were considered as the first factor. Soil texture was silty clay loamy with pH 7.5. At the harvest stage, random sampling of farms was done without margin effects.

### *Qualitative analysis*

The plants were dried at 80 °C to constant weight. The content of  $Na^+$  and  $K^+$  were determined using the flame spectrophotometer (FP640, Shanghai Precision & Scientific Instrument) (Pi *et al.*, 2016). The activity of nitrate reductase in leaves was investigated using Nicotinamide adenine dinucleotide (NADH) as a hydrogen donor by colorimetry (Zbiec *et al.*, 2003). The activity of catalase enzyme measured catalase (CAT) activity was determined by measuring the decomposition rate of  $H_2O_2$  in 60 s with spectrophotometer at 250 nm by Darwesh *et al.* (2018), CAT enzymatic activity was calculated using the system reported by Aebi (1984). For measurement of superoxide dismutase (SOD), 1 mL of reaction mixture was prepared in 50 mM K-P buffer (pH 7.8) containing 2  $\mu$ M riboflavin, 75  $\mu$ M nitroterazolium blue (NBT), 100  $\mu$ M EDTA, 13 mM DL-methionine and 60  $\mu$ L of enzyme extract and the absorbance was taken at 560 nm (Stewart and Bewley, 1980). Lipid peroxidation was determined by measuring the content of malondialdehyde (MDA) in leaves (Yang *et al.*, 2015).

### *Statistical procedures*

Analysis of variance was performed as a combined split-split factor based on randomized complete block design with 3 replications in two experimental years by SAS 9.4 software and Duncan's multiple range tests

were used for means comparison. The results of soil at experimental station and the average annual temperature of 2018 and 2019 during the study shown in Table 1, and Table 2, respectively.

**Table 1.** The analysis of pH, texture, organic carbon, nitrogen, phosphor and potassium of soil of study

pH	Texture	EC dS.m-1	O.C (%)	N (%)	P <sub>2</sub> O <sub>5</sub> (ppm)	K <sub>2</sub> O (ppm)
7.5	SCL	1.01	0.09	9	17.7	650

**Table 2.** The average annual temperature during the years 2018 and 2019 of study

	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19
High Temp	33.8	23	17.2	17.2	22.6	23.6	29	36.2
Low Temp	8	-2.8	-5.4	-5.6	-8.2	-1.2	4.4	9.6
Aver Temp	18.8	8.9	6.2	4.5	7.5	10.4	15.6	23.2
	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20	Mar-20	Apr-20	May-20
High Temp	33.8	23	16.6	13	22.6	23.6	29	36.2
Low Temp	8.6	-2.8	-3.4	-5.6	-8.2	-1.2	6	9.6
Ave Temp	19	7.9	5.9	3.4	8.3	11.2	16.2	23.7

## Results

### *Root yield*

The effect of year on root yield was significant at the 1% probability (Table 3). The highest root yield was observed in year 2 and the lowest root yield was observed in year 1 (Table 4). Increasing the concentrations of glycine betaine by maintaining photosynthetic capacity, and the membrane structure improved plant performance (Ma *et al.*, 2007; Cha-um *et al.*, 2019). Similarly, Afshar *et al.* (2008) reported that glycine betaine significantly increased corn yield. The effect of methanol spraying on root yield was significant at the 1% probability level (Table 3). The highest root yield was observed in methanol treatment of 30% v/v and the lowest root yield was observed in non-methanol application treatment (Table 4). Dorokhov *et al.* (2018) has shown that methanol-treated plants can increase their net photosynthesis and improve performance. It was concluded that application of the highest level of methanol spraying, showed 170% increase in the amount of the amino acid glycine betaine under drought stress condition. Drought stress increases the proline and glycine betaine amino acids in the leaves and increased with the exogenous application of glycine betaine. Also, Iqbal, Ashraf (2008) reported that glycine betaine led to an increase in relative water content (RWC) of sunflower leaves under water stress. The effect of cultivar on root yield was significant at the 1% probability (Table 3). The highest root yield was observed in cultivar treatment 3 and the lowest root yield was observed in 'Rasta' (Table 4). The effect of foliar application and root yield was significant at the five percent probability (Table 3). The highest root yield was achieved in year 2 with methanol 15% and glycine betaine 4 g, and the lowest root yield was observed in year1 along with the non-application of methanol and glycine betaine (Table 4). Kurepin *et al.* (2019) showed that glycine betaine foliar application treatments increased sugar beet root yield compared to the control treatment. The increase in growth and yield of plants was due to the use of anti-stress solutions due to their effectiveness as an inhibitor of light respiration (Zbiec *et al.*, 2003; Stepanov *et al.*, 2020).

**Table 3.** Analysis of variance for yield, sugar, potassium, sodium, CAT, SOD, nitrate reductase, rubisco, MDA

S.O.V	DF	Yield	Sugar	Potassium	Sodium	CAT	SOD	Nitrate reductase	RUBISCO	MDA
Year	1	37897.79**	2084425.09**	64565.49**	13422.84**	23.89**	196.86**	37.45*	12046.8**	18899.66**
Rep (Year)	4	274.9**	39542.03*	114.95*	13.42*	0.11	0.93*	2.52	86.04*	64.91
A	2	3815.72**	552236.82**	1205.32**	126.16**	2.11**	19.15**	7.27	1129.46**	2039.46**
Year*A	2	378.39*	58666.62*	331.83**	56.22**	0.47**	2.41**	10.24	132.89*	459.56**
Rep* Year*A	8	66.48	5939	18.81	6.9	0.07	0.34	3.77	22.62	31.4
B	1	5081.26**	626817.64**	1370.35**	151.22**	3.56**	20.81**	2.65	1612.32**	1716.54**
A*B	2	399.7*	46372.51*	88.26	16.64*	0.16*	1.66*	0.73	112.75*	127.43*
Year*A*B	3	319.27*	33186.76*	223.84**	36.66**	0.27*	0.72	14.45*	111.25*	21
Rep* Year*A*B	12	69.38	9487.34	43.34	5.2	0.07	0.38	4.71	27.18	33.77
C	5	1528.86**	204233.34**	572.88**	63.73**	0.79**	6.93**	13.12*	464.11**	635.3**
Year*C	5	111.14	12957.42	111.7*	18.98**	0.13*	0.55	4.78	35.97	74.55*
A*C	10	168.4*	25040.29*	71.68*	10.92*	0.09	0.87*	1.74	53.18*	93.07**
B*C	5	102.07	15196.35	50.03	4.85	0.05	0.58	2.53	30.27	56.43
A*B*C	10	86.32	10650.56	43.61	4.1	0.04	0.44	1.63	24.53	39.64
Year*A*B*C	25	134.32*	18881.84*	56.33*	7.31*	0.1*	0.7*	4.39	42.48*	64.95*
Error	120	67.49	10101.9	29.45	3.56	0.05	0.32	4.04	21.28	28.19

\* Significant at 0.05 significance, \*\*significant at 0.01. A: Methanol concentration; B: Glycine betaine concentration; C: Cultivar

**Table 4.** Mean comparison of year, methanol and glycine betaine foliar application on yield, sugar, potassium, sodium, CAT, SOD, nitrate reductase, rubisco, MDA

Year	Yield (t.ha <sup>-1</sup> )	Sugar (mg.g <sup>-1</sup> DW)	Potassium (mg.g <sup>-1</sup> DW)	Sodium (mg.g <sup>-1</sup> DW)	CAT (u.mg <sup>-1</sup> pr <sup>-1</sup> )	SOD (u.mg <sup>-1</sup> pr <sup>-1</sup> )	Nitrate reductase (μmol.NO <sub>2</sub> .g <sup>-1</sup> h <sup>-1</sup> )	RUBISCO (μmol.m <sup>-2</sup> s <sup>-1</sup> )	MDA (nm.mg <sup>-1</sup> pr <sup>-1</sup> )
Year 1	47.01b	595.27b	20.94b	5.23b	1.58b	5.65b	9.11b	26.09b	65.29b
Year 2	73.51a	791.74a	55.52a	21a	2.25a	7.56a	9.95a	41.02a	84a
Methanol concentration (A)									
0 (Met 0)	51.85b	592.64b	33.71c	11.74c	1.72b	6.01b	9.26a	28.98b	80.78a
15 (Met 15)	64.46a	737.61a	41.69a	14.39a	2.02a	6.89a	9.46a	35.88a	71.77b
30 (Met 30)	64.47a	750.26a	39.29b	13.21b	2.01a	6.91a	9.88a	35.8a	71.37b
Glycine betaine (B)									
0 (Gly 0)	55.41b	639.63b	35.71b	12.28b	1.79b	6.29b	9.42a	30.82b	71.82b
4 (Gly 4)	65.11a	747.37a	40.75a	13.95a	2.04a	6.91a	9.64a	36.29a	77.46a
Cultivar (C)									
Sentinel	60.97c	697.97c	38.49b	13.29b	1.91b	6.64b	9.65a	33.83b	74.2b
Drafter	58.02c	662.74c	37.17b	12.76b	1.89b	6.44b	9.39a	32.38b	76.71b
Rivolta	68.97a	795.09a	43.33a	14.72a	2.1a	7.17a	9.78a	38.35a	69.51c
Elanta	58.47c	683.86c	37.34b	12.79b	1.85b	6.5b	10.26a	32.42b	74.75b
Rasta	50.04d	574.56d	31.74c	10.92c	1.69c	5.91c	8.44b	27.99c	81.42a
Qualita	65.09b	746.79b	41.29a	14.2a	2.05a	6.95a	9.67a	36.35a	71.27c

Common letters within each column do not differ significantly

### Potassium

The effect of location on potassium levels was significant at the 1% probability (Table 3). The highest amount of potassium was observed in year 2 treatment and the lowest amount of potassium was obtained in year 1 treatment (Table 4). Studies by Amin *et al.* (2013) showed that drought adversely affects the quality of sugar beet by increasing impurities such as alpha-amino, nitrogen, sodium, potassium, and reducing the amount of sugar that can be extracted. The effect of methanol foliar application of potassium levels was significant at the 1% probability level (Table 3). The highest amount of potassium was observed in the treatment of 15% v/v methanol and the lowest amount of potassium was observed in the treatment of non-application of methanol (Table 4). Sucrose has the ability to replace K<sup>+</sup> and Na<sup>+</sup> in vacuoles (Cha-um *et al.*, 2019) and during growth, the concentrations of sucrose and K<sup>+</sup> are inversely related (Hoffmann *et al.*, 2018). Under condition of drought stress, the stomata are closed and the stomatal conductance in the leaves and the penetration of CO<sub>2</sub> and the production of carbohydrates is limited (Hsiao, 2000). However, the results of the experiments of Nadali *et al.* (2010) on sugar beet indicated that sodium, potassium, and nitrogen were not affected by methanol levels, which was inconsistent with the results of this experiment. The effect of glycine betaine foliar application concentration on potassium levels at the 1% probability level was significant (Table 3). The highest amount of potassium in glycine betaine treatment was 4 g, and the lowest amount of potassium was observed in the control

treatment (Table 4). Mäck and Hoffmann (2006) showed that glycine betaine, along with potassium, play a major role in the osmotic activity of sugar beet. The effect of cultivar on potassium levels was significant at the 1% probability level (Table 3). The highest amount of potassium was observed in the treatment of 'Rivolta' and the lowest amount of potassium was observed in the treatment of 'Rasta' (Table 4). Hadir *et al.* (2021) reported that a significant difference between the six cultivars evaluated in terms of root potassium. The effect of foliar application and potassium content was significant at the 1% probability level (Table 3). The highest potassium was observed in the year 2 treatment with 15% methanol and 4 g of glycine betaine and the lowest potassium was observed in the year 1 treatment along with non-application of methanol and glycine betaine (Table 5).

**Table 5.** Mean comparison of interaction between year, methanol and glycine betaine foliar application on yield, sugar, potassium, sodium, CAT, SOD, nitrate reductase, rubisco

Treatment	Yield (t.ha <sup>-1</sup> )	Sugar (mg.g <sup>-1</sup> DW)	Potassium (mg.g <sup>-1</sup> DW)	Sodium (mg.g <sup>-1</sup> DW)	CAT (u.mg <sup>-1</sup> pr <sup>-1</sup> )	SOD (u.mg <sup>-1</sup> pr <sup>-1</sup> )	Nitrate reductase ( $\mu$ mol.NO <sub>2</sub> .g <sup>-1</sup> h <sup>-1</sup> )	RUBISCO ( $\mu$ mol.m <sup>-2</sup> s <sup>-1</sup> )	MDA (nm.mg <sup>-1</sup> pr <sup>-1</sup> )
Year1 Met0 Gly0	36.1g	459.38f	16.19g	4.1g	1.33g	4.81f	8c	20.15g	93.73a
Year1 Met0 Gly4	38.67g	489.97f	17.69g	4.39fg	1.4g	5.02f	8.94bc	21.39g	91.47a
Year1 Met15 Gly0	47.59f	598.18e	21.33f	5.27efg	1.56f	5.7e	9.25abc	26.33f	83.21b
Year1 Met15 Gly4	52f	654.58de	22.75ef	5.71e	1.67f	6.04e	8.81bc	28.85f	79.29c
Year1 Met30 Gly0	48.83f	626.08de	21.82f	5.45ef	1.65f	5.79e	9.31abc	27.19f	82bc
Year1 Met30 Gly4	58.9de	743.42b	25.84e	6.45e	1.89e	6.54d	10.38a	32.6e	74.28d
Year2 Met0 Gly0	62.68de	673.31cd	48.35d	18.34d	1.95e	6.88cd	10.33a	34.96de	70.89de
Year2 Met0 Gly4	69.97bc	747.9b	52.6c	20.14bc	2.19c	7.32b	9.75ab	39.43bc	67.05f
Year2 Met15 Gly0	71.51b	758.91b	54.74bc	20.72bc	2.19cd	7.47b	9.23abc	39.74b	65.82f
Year2 Met15 Gly4	86.74a	938.78a	67.92a	25.84a	2.64a	8.36a	10.55a	48.61a	58.76g
Year2 Met30 Gly0	65.75cd	721.93bc	51.81cd	19.77c	2.04de	7.11bc	10.4a	36.56cd	69.11ef
Year2 Met30 Gly4	84.38a	909.59a	57.66b	21.16b	2.47b	8.21a	9.42ab	46.83a	60.1g

Common letters within each column do not differ significantly

### Sodium

The effect of location on sodium levels was significant at the 1% probability level (Table 3). The highest amount of sodium was observed in year 2 treatment and the lowest amount of sodium was observed in year 1 treatment (Table 4). The effect of methanol foliar application of sodium content was significant at the 1% probability level (Table 3). The highest amount of sodium was observed in the treatment of 15% v/v methanol and the lowest amount of sodium was observed in the treatment of non-consumption of methanol (Table 4). The effect of glycine betaine foliar application concentration on sodium content was significant at the 1% probability level (Table 3). The highest amount of sodium in glycine betaine treatment was 4 g and the lowest amount of sodium was observed in the control treatment (Table 4). Glycine betaine by regulating the ratio of sodium: Potassium is involved in the plant's tolerance to salt. Reza *et al.* (2006) noted that sodium accumulation was significantly increased in aerial parts and wheat roots due to salinity stress, and the use of glycine betaine increased sodium accumulation with increasing potassium accumulation. The effect of cultivar on the sodium level at the probability level was one percent significant (Table 3). The highest amount of sodium was observed in the treatment of 'Rivolta' and the lowest amount of sodium was observed in the treatment of 'Rasta' (Table 4). The effect of foliar application and the amount of sodium on the probability level was one percent significant (Table 3).

### Catalase activity

The effect of location on catalase enzyme activity (CAT) was significant at the 1% probability level (Table 3). The highest activity of catalase enzyme was observed in year 2 treatment and the lowest activity of catalase enzyme was observed in year 1 treatment (Table 4). Branch (2009) reported that plants under drought stress have a significant increase in SOD and CAT activity in canola leaves. The different activities of antioxidant enzymes in different genotypes can be related to different genetic behaviours to tolerate drought stress conditions. The effect of methanol spraying on catalase enzyme activity was significant at the 1% probability level (Table 3). The highest activity of catalase enzyme in the treatment of methanol was observed

in 15% v/v and the lowest activity of catalase enzyme in the treatment of non-methanol application was observed (Table 4). It has been reported an increase of nearly 10 times in the production of catalase enzyme was observed on plants under drought stress conditions. The enzyme catalase protects cells against hydrogen peroxide and plays an important role in resistance to oxidative stress. Methanol through the absorption of iron, which is a prosthetic group of hemoproteins such as catalase, peroxidase, and superoxide dismutase, could be involved in the destruction of active oxygen species in plants (Blokhina *et al.*, 2003; Keles and Öncel, 2004).

The effect of glycine betaine foliar application concentration on catalase activity was significant at the 1% probability level (Table 3). The highest catalase activity was observed in 4 g glycine betaine treatment and the lowest catalase activity was observed in the control treatment (Table 4). Cruz *et al.* (2013) study mentioned that the CAT activities in well-watered plants were not influenced by the glycine betaine exogenous application, but in water-stressed plants, the CAT activity significantly increased. The effect of cultivar on catalase activity was significant at the 1% probability level (Table 3). The highest catalase activity was observed in the treatment of 'Rivolta' and the lowest catalase activity was observed in the treatment of 'Rasta' (Table 4). Prajapat *et al.* (2018) stated the activity of CAT increased in drought-tolerant cultivars of maize. Besides, Fu and Huang (2001) reported that the ability for adaptation to drought stress depended on the maintenance of or increases in the capability to detoxify superoxide radical by antioxidant enzymes. The effect of foliar application and catalase activity at the level of probability was significant at five percent (Table 3). The highest activity of catalase enzyme was observed in the treatment of year 2 with methanol 15 and glycine betaine 4 g, and the lowest activity of catalase enzyme was observed in the treatment of year 1 with non-consumption of methanol and glycine betaine (Table 5). Plants treated with glycine betaine showed an increase in glycine betaine-treated plants exhibited increased levels of photosystem II (PSII) activity compared with control plants. Glycine betaine-treated plants had significantly greater catalase activity. This result suggests that glycine betaine may enhance the induction of antioxidant mechanisms under abiotic stress conditions.

#### *Superoxide dismutase activity*

The effect of location on the level of superoxide dismutase activity (SOD) was significant at the 1% probability level (Table 3). The highest superoxide dismutase activity was observed in year 2 treatment and the lowest superoxide dismutase activity was observed in year 1 treatment (Table 4). It has been reported that in the absence of stress, the application of methanol did not have a significant effect on the SOD in the leaves. However, under stress conditions, methanol levels significantly reduced the activity of this enzyme compared to the non-methanol application under stress conditions. The effect of methanol spraying on the level of SOD was significant at the 1% probability level (Table 3). The highest SOD in methanol treatment was observed to be 30% v/v, and the lowest SOD was observed in the treatment of the non-methanol application (Table 4). Application of methanol in different climatic conditions due to the fact that it provides more carbon dioxide to the leaves of the plant, the plant is less exposed to adverse environmental conditions and as a result, the production of oxygen free radicals is reduced. Due to the reduction in free radicals, the plant needs less antioxidant enzymes. However, Romandini *et al.* (1994) believed that the presence of methanol did not affect the enzymatic levels, while the absence of glucose gave higher SOD levels. It has been reported that the highest amount of superoxide dismutase enzyme was observed in soybean with 6 units per mg of protein under conditions of extreme moisture stress without methanol spraying and increasing the intensity of moisture stress increased the production of this enzyme. The effect of glycine betaine foliar application concentration on the superoxide dismutase activity was significant at the 1% probability level (Table 3). The highest superoxide dismutase activity was observed in 4 g glycine betaine treatment and the lowest superoxide dismutase activity was observed in the control treatment (Table 4). The effect of glycine betaine foliar application concentration on the superoxide dismutase activity at a probability level of one percent was significant (Table 3). The highest superoxide dismutase activity in glycine betaine treatment was 4 g, and the lowest superoxide dismutase activity was observed in the control treatment (Table 4). The effect of cultivar on the superoxide dismutase activity was significant at the 1% probability level (Table 3). The highest and lowest

superoxide dismutase activity was observed in the treatment of 'Rivolta' and treatment of 'Rasta', respectively (Table 4). The reason for the difference in the response of cultivars seems to be related to their genetic differences and the process of their different growth. Superoxide dismutase is an antioxidant enzyme that catalyzes active superoxide anions and converts them to oxygen and low-activity types of hydrogen peroxide (Kumar *et al.*, 2020). According to the results, cultivars that had better yield had higher superoxide dismutase activity under stress conditions. The effect of the year and foliar application and cultivar on the superoxide dismutase activity was not significant (Table 3).

#### *Nitrate reductase*

The effect of location on the nitrate reductase activity was significant at the five percent probability level (Table 3). The highest activity of the reductase nitrate enzyme was observed in year 2 treatment and the lowest activity of the reductase nitrate enzyme was observed in year 1 treatment (Table 4). The effect of methanol spraying on nitrate reductase activity was not significant (Table 3). According to previous study (Zbieć *et al.*, 2003), increasing the amount of nitrate reductase during methanol application showed a significant difference compared to the control, also methanol increased CGR and nitrate reductase activity on rapeseed. The effect of glycine betaine foliar application and also the effect of cultivar on the nitrate reductase activity was not significant (Table 3). The effect of foliar application and cultivar on the nitrate reductase activity was significant at the 5% probability level (Table 3). The highest nitrate reductase activity was observed in year 2 treatment with methanol 15% and glycine betaine 4 g and the lowest nitrate reductase activity was observed in year 1 treatment along with the non-application of methanol and glycine betaine (Table 3).

#### *Rubisco activity*

The effect of location on rubisco activity was significant at the 1% probability level (Table 3). The highest rubisco activity in Year 2 treatment and the lowest rubisco activity in year 1 treatment was achieved (Table 4). In the year 1 region, due to higher temperatures and unfavourable environmental conditions the sugar beet plant, which is a C3 plant, had more light respiration (Table 3). With increasing light respiratory, the rubisco showed a lower level of activity. Cruz *et al.* (2013) showed that dehydration significantly reduced the stomatal conductivity and the ratio of CO<sub>2</sub> concentration. In stress-tolerant plants, the rate of net assimilation rate decreased with the absence of CO<sub>2</sub>, which reduced the activity of carboxylase, rubisco, and stimulates plant respiration. The effect of methanol spraying on rubisco activity was significant at the 1% probability level (Table 3). The highest rubisco activity in methanol treatment was 15% v/v and the lowest rubisco activity was observed in the methanol non-application treatment (Table 4). Crafts-Brandner and Law (2000) stated that rubisco activity and photosynthesis declined under thermal stress conditions. They reported that increased in CO<sub>2</sub> reduced rubisco activity on the leaves. Therefore, by increasing the CO<sub>2</sub> concentration during methanol application, it can be shown that methanol could increase rubisco activity. The effect of glycine betaine foliar application on rubisco activity was significant at the 1% probability level (Table 3). The highest rubisco activity was observed in glycine betaine 4 g treatment, and the lowest rubisco activity was observed in the control treatment (Table 4). glycine betaine application protected chloroplast ultrastructure and prevented the decrease of chlorophyll and rubisco activity under salt- and drought-stress conditions (Mäkelä *et al.*, 2000). The effect of cultivar on rubisco activity was significant at the 1% probability level (Table 3). The highest rubisco activity was observed in 'Rivolta' and the lowest rubisco activity was observed in 'Rasta' (Table 4). Studies have shown that there is more inhibition of the rubisco activity in the heat-sensitive cultivar than in the tolerant cultivars (Bose and Ghosh, 1995). The effect of the Year and foliar application and cultivar on rubisco activity was significant at the probability level of five percent (Table 3). The highest rate of rubisco activity was observed in year 2 treatment with 15 mg of methanol and 4 g of glycine betaine, and the lowest level of rubisco activity in year 1 treatment was observed with non-methanol and glycine betaine application (Table 5).



*Malondialdehyde concentration*

The effect of location on malondialdehyde concentration (MDA) was significant at the 1% probability level (Table 3). The highest malondialdehyde concentration was observed in year 2 treatment and the lowest malondialdehyde concentration was observed in year 1 treatment (Table 4). Malondialdehyde is considered a good indicator of membrane lipid peroxidation (Grotto *et al.*, 2009). Drought stress increases with the content of oxygen free radicals, which increases the content of malondialdehyde to product the membrane peroxidation. An examination of the MDA content under a hydroponic cultivation system showed that the MDA malondialdehyde concentration in the roots of the two genotypes increased due to increased salinity (Chen *et al.*, 2011). The effect of methanol foliar application on malondialdehyde concentration was significant at the 1% probability level (Table 3). The highest and lowest malondialdehyde concentration was observed in methanol non-application treatment and methanol treatment 30% v/v (Table 4). It has been stated that the highest malondialdehyde concentration was observed in conditions of non-methanol application and its value decreases significantly with increasing percentage of methanol. The effect of glycine betaine foliar application on malondialdehyde concentration was significant at the 1% probability level (Table 3). The highest malondialdehyde concentration in glycine betaine treatment was observed at 4 g, and the lowest malondialdehyde concentration was observed in the control treatment (Table 4). It has been reported that, glycine betaine foliar application in sorghum and canola caused the path of amino acid synthesis changed to proline production, so the amount of proline increased with glycine betaine foliar application in the plant (Kadkhodaei *et al.*, 2016). The effect of cultivar on malondialdehyde concentration was significant at the 1% probability level (Table 3). The highest malondialdehyde concentration was observed in the treatment of Rasta and the lowest malondialdehyde concentration was observed in the treatment of 'Rivolta' (Table 4). The effect of foliar application and cultivar on malondialdehyde concentration was not significant (Table 3). The shoot GB concentrations were closely correlated with leaf water potential. Salt-induced GB accumulation in the shoot could also contribute to cytoplasmic osmoregulation in beet leaves, and so play an important role in their salt tolerance. However, the contribution of GB to achieve osmotic balance in roots varied among genotypes. The response of sugar beet, and differed with regard to the accumulation of solutes in the storage root after drought stress (Hoffmann, 2014).

**Conclusions**

The effect of year was significant on yield, sugar, potassium, sodium, CAT, SOD, nitrate reductase, RUBISCO, and MDA. The effect of methanol spraying on root yield was significant the highest yield was observed in methanol treatment of 30% v/v and the lowest root yield was observed in non-methanol application treatment. The maximum amount of potassium was observed in the treatment of 15% v/v methanol, and the lowest amount of potassium was related to the treatment of non-application of methanol. The highest amount of potassium was observed in the treatment of 'Rivolta' and the lowest amount of potassium was observed in the treatment of 'Rasta'. Application of 15% methanol and 4 g of glycine betaine had obtained the highest potassium content and the minimum one was observed in the year 1 treatment along with non-application of methanol and glycine betaine. The highest amount of sodium was observed in the treatment of 'Rivolta', and the lowest amount of sodium was related to treatment of 'Rasta'. The impact of methanol spraying and glycine betaine foliar application on catalase enzyme activity was meaningful at the 1% probability level. The highest activity of catalase enzyme was observed in the treatment of year 2 with methanol 15 and glycine betaine 4 g, and the lowest activity of catalase enzyme was observed in the treatment of year 1 with non-consumption of methanol and glycine betaine. The effect of the year and foliar application as well as cultivar on the superoxide dismutase was not meaningful. The maximum nitrate reductase activity was observed in year 2 treatment along with methanol 15% and glycine betaine 4 g and the lowest nitrate reductase activity was related to year 1 treatment along with the non-application of glycine betaine and methanol. The

highest malondialdehyde concentration was related to the 'Rasta' and the lowest one was observed in the treatment of 'Rivolta'. The impact of foliar application and cultivar on malondialdehyde concentration was not significant. Based on the results, the application methanol 15% v/v with glycine betaine 4 g per liter are recommended to improve the quality of the sugar beet under similar conditions of the present experiment.

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All authors read and approved the final manuscript.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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