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Evaluation Effect of Salicylic Acid and Glycine Betaine on Quantitative Properties of Alfalfa (*Medicago sativa* L.) under Drought Stress Conditions

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Fertilizer management plays an important role for obtaining satisfactory yields and to increase crop productivity. One of limitation factor in agricultural plants production in dry areas in the water tension at growth step negative effect of water tension on corn growth depends on the time of tension occurrence, the intensity, plant growth and genotype step.

OBJECTIVES: This study was done to assess effect of amino acids on alfalfa crop production under water stress condition.

METHODS: Current research was conducted according split plot based on randomized complete blocks with three replications in Karaj Research Farm. The main factor included water stress (S_1: full irrigation, S_2: Cut off irrigation from the beginning of flowering until the end of the growing season, S_3: Cut off irrigation from 50% of flowering until the end of the growing season) and Foliar application of amino acid (A_1: without foliar spraying, A_2: 0.5 mM salicylic acid, A_3: 1 mM salicylic acid, A_4: 10 mM glycine, A_5: 50 mM glycine) belonged to sub plots.

RESULT: The difference between the mean of the traits in the treatments of using the reducing agent and not using these substances were considered significant. The results showed the adverse effect of drought stress on all studied traits and the reduction of its destructive effects by stress reducing agents. In determining the yield of wet and dry forage, the simultaneous use of full irrigation and glycine foliar application was the best treatment.

CONCLUSION: According to the results, the use of 10 mM glycine betaine is recommended. In both stress treatments (cessation of irrigation from the beginning of flowering and cessation of irrigation from 50% of flowering) when stress relieving treatments were used, the effect of stress was significantly reduced as in most traits.

KEYWORDS: *Amino acid, Foliar application, Forage, Irrigation, Yield.*

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1. BACKGROUND

The world population is increasing day by day, while the expansion and development of agricultural lands are very small due to the lack of fertile land. Therefore, one of the important goals to coordinate with the increase in world population is to increase the yield of crops (Maleki *et al*., 2011). Drought and water shortage are considered an objective reality. In the past, water crisis was not as significant as today, since the population was less, but with the population increase by about six times and the need for more food during the last 100 years, the incidence of this crisis has become more evident than the past (Chimenti *et al*., 2002). Water scarcity and drought stress are important factors in reducing yields and challenging food security, especially in arid and semi-arid regions. Drought stress disrupts many morphological features and physiological processes associated with plant growth (Maleki and Fathi, 2019; Ezati *et al*., 2020). It is very necessary to study the effect of environmental stresses and their role in predicting and evaluating the growth and yield of agricultural products (Maghsoudi *et al*., 2020). Crops grown in saline soils may be dehydrated due to their osmotic properties, resulting in reduced growth rate (Yohanns and Abraha, 2013). Alfalfa is one of the most important forage plants in Iran and is very important in animal nutrition and increase of animal products. This plant has a high water requirement and its extensive and deep root system is one of the effective factors in combating drought. Water shortage is one of the non-living factors that negatively affect the growth and yield of crops (Chinnusamy and *et al*., 2004). Drought stress reduces leaf size, stem expansion, root propagation, water use efficiency and relative water content (Farooq *et al*., 2008). One of the strategies used to maintain and expand agricultural production in water-scarce areas is the use of stress-reducing materials. For example, salicylic acid is one of the most important messenger molecules and causes the plant to react to environmental stresses. Salicylic acid, like a non-enzymatic antioxidant, plays an important role in regulating physiological processes in plants (Misra and Saxena, 2009). In the case of plant stresses, salicylic acid is considered as a signal molecule. This signal molecule increases defense compounds such as proline and betaine glycine. Increased growth due to salicylic acid application has also been reported in wheat, soybeans (Azarfard, 2008) and corn (Garcia *et al*., 2002). Salicylic acid can also increase the photosynthesis of the whole plant in plants exposed to drought stress (Larqué, 1979). Glycine betaine is highly synthesized in response to a variety of environmental stresses in many plant species. Glycine betaine is also increased in strains in plants such as spinach, barley, wheat and sorghum (Yang *et al*., 2003). Application of compatible solutions such as propylene and glycine betaine to plants, before stress, at the same time as stress and after stress, has increased the growth and yield of crops (Ashraf and Foolad, 2007).

2. OBJECTIVES

Current study was done to assess effect of amino acids on alfalfa crop production under water stress condition.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

The research was conducted according of split plots experiment based on completely randomized blocks design with three replications in the research farm of Karaj. The geographical location of Karaj included 35° 48' N latitude and 50° 57' E longitude, with an altitude of 1321 meters above sea level, an average annual rainfall of 239.5 mm and an average minimum annual temperature of 8.7°C and a maximum annual temperature of 21.1 °C. In this experiment, two factors including irrigation and foliar application of stress relievers were investigated. Experimental factors each have different levels whose values are as follows. The main plots included different levels of irrigation and the sub-plots included foliar application of stress reducing materials. Each subplot consisting of 25 planting lines with a length of 6 m with row spacing of 50 cm was considered. All of treatments were mentioned in table 1.

Table 1. Treatments, their values and their Symbol

No.	Treatments		Symbol
1	Full irrigation	No foliar spraying	Control
2		Salicylic acid 0.5 mM	Co Ir+As0.5
3		Salicylic acid 1 mM	Co Ir+As1
4		Glycine 10 mM	Co Ir+Gl10
5		Glycine 50 mM	Co Ir+Gl50
6	Cut off irrigation from the beginning of flowering until the end of the growing season	No foliar spraying	Cu Ir Fl0%+No Sp
7		Salicylic acid 0.5 mM	Cu Ir Fl0% +As0.5
8		Salicylic acid 1 mM	Cu Ir Fl0%+As1
9		Glycine 10 mM	Cu Ir Fl0%+Gl10
10		Glycine 50 mM	Cu Ir Fl0%+Gl50
11	Cut off irrigation from 50% of flowering until the end of the growing season	No foliar spraying	Cu Ir Fl50%+ No Sp
12		Salicylic acid 0.5 mM	Cu Ir Fl50%+As0.5
13		Salicylic acid 1 mM	Cu Ir Fl50%+As1
14		Glycine 10 mM	Cu Ir Fl50%+Gl10
15		Glycine 50 mM	Cu Ir Fl50%+Gl50

3.2. *Farm Management*

Salicylic acid and glycine were prepared from the German company MERCK and prepared based on the expressed concentrations. Foliar spraying of plants started from the time of flowering and was done every two weeks. Foliar spraying was continued until all leaf surfaces were wet. By covering the soil surface, salicylic acid and glycine solution was prevented from spilling on the soil surface. Irrigation of the field, according to soil moisture and rainfall status, was started after planting and once every 8 days in a sufficient amount and in the form of a plot. Irrigation was performed after the completion of vegetative growth period and at the beginning of flowering, based on experimental treatments.

3.3. Measured Traits

The studied traits included wet forage yield, dry forage yield, and number of stems per plant and internode distance. Generally plants were harvested in three turns. The first harvest was when the plant approached the height of approximately 20 cm. Next harvests were done when the plant reached the height of 20 cm. In each harvest, the aerial parts of the plant were taken from 2 cm height from the soil surface. After getting prepared, the plant samples were moved to paper envelopes and they were dried and weighted in a ventilating oven for 72 hours at 65 ºC.

3.4. Statistical Analysis

The data were analyzed by SAS software (Ver.9). Mean comparisons were compared by Duncan test (at 5% probability level) and graphs were drawn by Excel software (Ver.2015).

4. RESULT AND DISCUSSION

4.1. *Total dry matter* (*TDM*)

After harvesting and collecting data, the studied traits were analyzed. The comparison table of the average effects of irrigation and stress relievers, wet forage yield and dry forage yield is as follows.

4.2. Wet forage yield

According to the results, the main effects of irrigation and stress relievers as well as their interactions on wetland yield were quite significant. Paay gozar *et al.* (2009) Reported significant effects of stress, foliar application and their interactions were significant. The interaction of irrigation treatments and stress reducing agents on wet forage yield (Fig. 1) showed that treatments "Co Ir+As1" and "Co Ir+Gl50" have the highest wet forage yield.

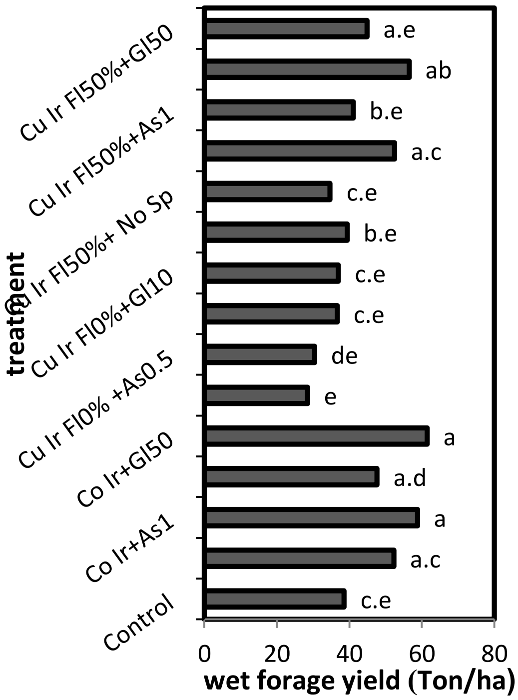

Fig. 1. Comparison of the average effect of irrigation and foliar application of stress reducing agents on wet forage yield using Duncan test at 5% probability level.

Treatment "Cu Ir Fl0%+No Sp" has the lowest forage yield. The effect of salicylic acid on increasing forage yield has been reported by (Misra, and Saxena. 2009). According to the regression results, the highest amount of fresh forage is obtained in the consumption of 2 mM salicylic acid and more consumption, will not have any effect. Consumption of 1 mM to 2 mM salicylic acid is not associated with an increasing slope. As forage yield does not increase as expected, consumption of more than 1 mM salicylic acid is not recommended (Fig. 2).

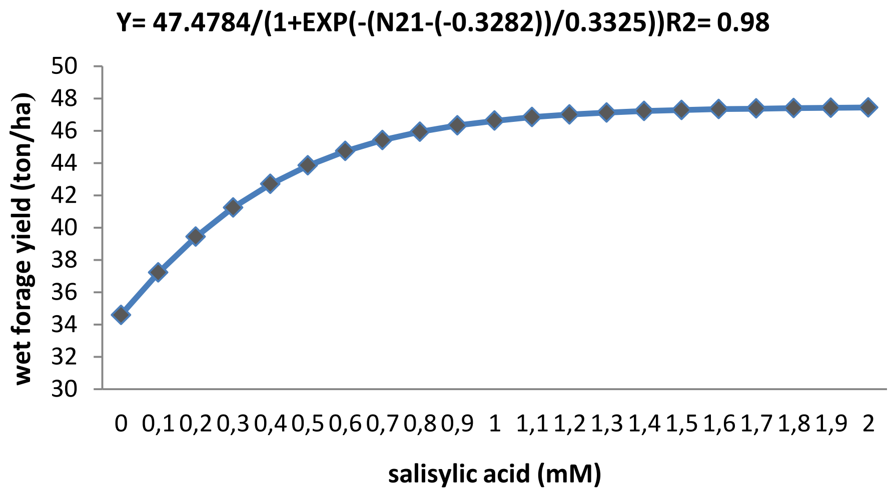

Fig. 2. Regression model of wet forage yield with changes in salicylic acid intake

In the case of glycine betaine, the highest value predicted by the regression model is 20 mM. As a result, consumption of more than 20 mM is not recommended (Fig. 3).

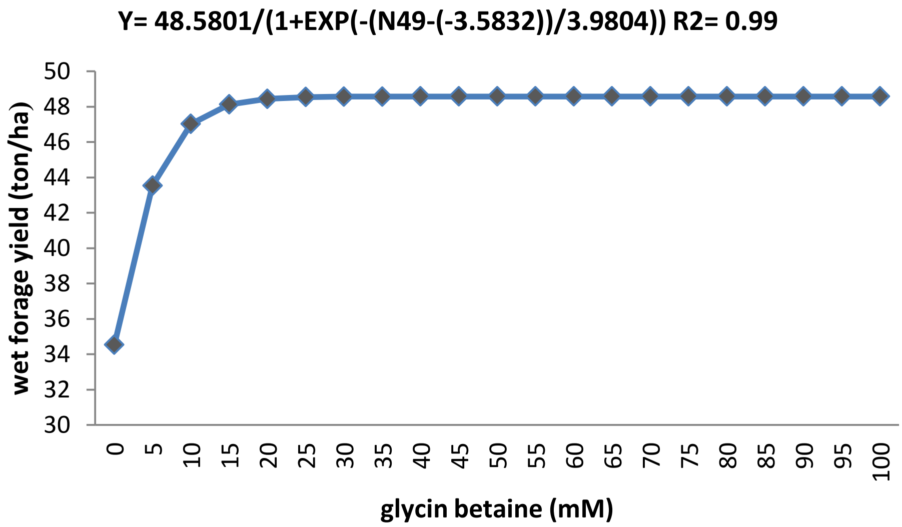

Fig. 3. Regression model of wet forage yield with changes in glycine betaine intake

4.3. Dry forage yield

The results of comparing the mean of interactions between irrigation treatments and stress relievers on dry forage yield are given in Fig. 4. As shown in the diagram, Co Ir + Gl10 treatment with dry forage yield of 17.35 ($t.ha^{-1}$) and Co Ir + Gl50 treatment with dry forage yield of 17.81 ($t.ha^{-1}$) had the highest yield. Cu Ir Fl0% + No Sp treatment with dry forage yield of 6.02 ($t.ha^{-1}$) has the lowest yield. Miri and Zamani Moghaddam, in their study of forage maize, stated that drought stress reduces dry matter accumulation by shrinking leaves and reducing light absorption for photosynthesis. Foliar application at the beginning of reproductive growth, increases cell turgenesis and improves water relations and increases the transfer of photosynthetic material in the plant (Miri and Zamani Moghaddam, 2014). The lowest dry forage yield was obtained due to the interaction of irrigation interruption at the beginning of flowering stage and no use of stress reducing agents. Given the high sensitivity of plants in the reproductive stage (Ashraf and Foolad, 2007) and the explanations given about the wet forage yield, this result is logical.

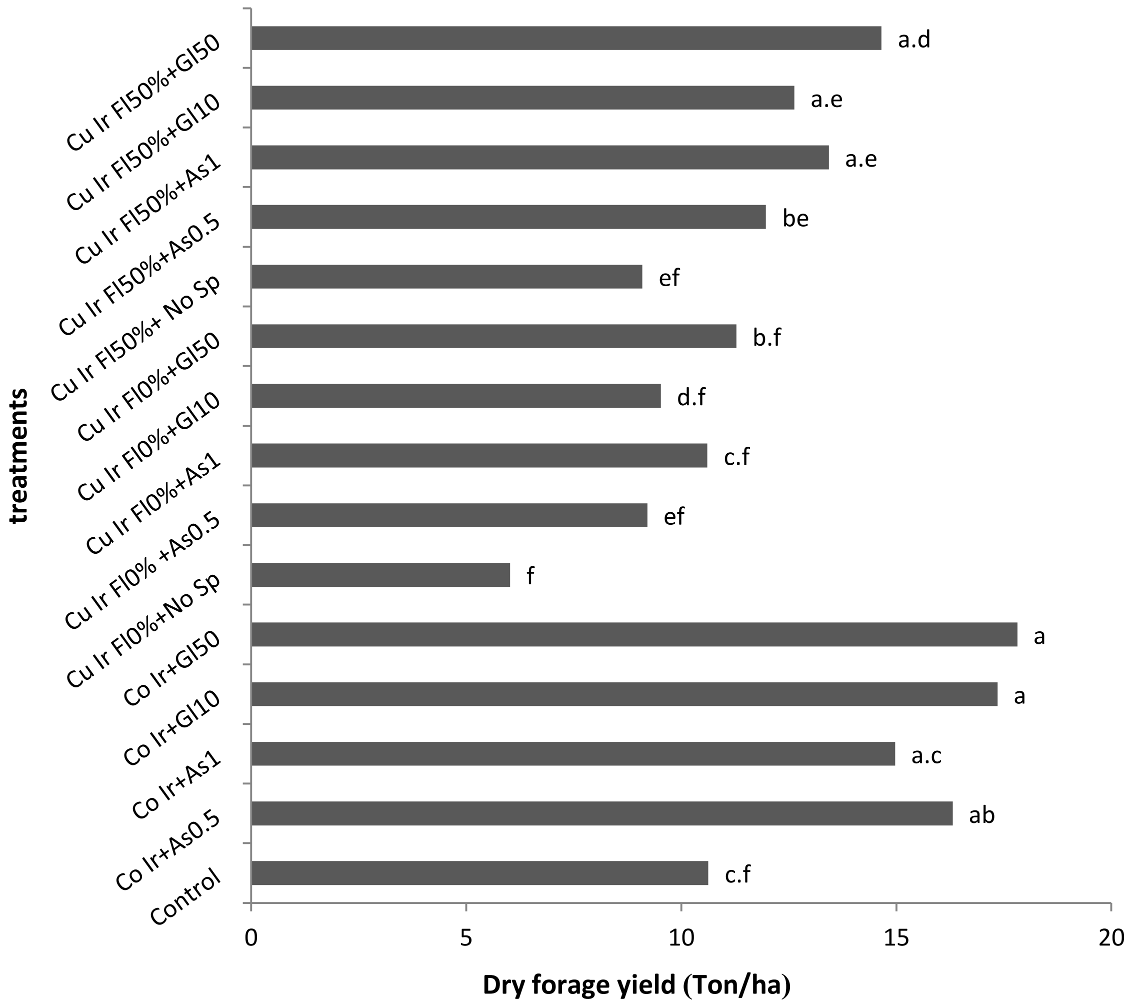

Fig. 4. Comparison of the average effect of irrigation and foliar application of stress reducing agents on dry forage yield using Duncan test at 5% probability level.

4.4. Number of stems per plant

The results showed that the effects of irrigation and stress reducing agents on the number of stems per plant were significant, but their interaction on this trait was not significant. In the research of Mir Ansari et al., The significance of the main effects and the interaction effect of foliar application on the number of stems has been mentioned, which is consistent with the results obtained in this study (Mir Ansari et al 2014). The results of table 2 showed that the complete irrigation treatment with 32.20

stems per plant has the highest value and is in group (a). Irrigation cut-off treatment from the beginning of flowering with 21.02 stems per plant has the lowest value and was placed in group (c). The results of table 3 showed that Gl50 treatment increased the number of stems per plant (31.72) compared to non foliar application conditions (22.01). This difference was very significant. In other words, Gl50 treatment increased the number of stems per plant by 44.11% compared to non-foliar treatment. The reason for increasing the number of stems is to improve the photosynthetic conditions of the plant and better absorption of water and nutrients. The high correlation between the number of stems per plant and wet forage yield and dry forage yield confirms the obtained results.

Table 2. Comparison of average irrigation effect

Treatment	Wet forage yield ($t.ha^{-1}$)	Dry forage yield ($t.ha^{-1}$)	Number of stems per plant
Full irrigation	51.74a	15.41a	32.20a
Stop watering from the beginning of flowering	34.37c	9.32c	21.02c
Cut off irrigation from 50% of flowering	45.92b	12.35b	27.85b

*Means with similar letters in each column are not significantly differentt by Duncan's test at 5% probability level.

Table 3. Comparison of the average effect of stress relievers

Treatment	Wet forage yield ($t.ha^{-1}$)	Dry forage yield ($t.ha^{-1}$)	Number of stems per plant
No foliar spraying	34.54b	8.58b	22.01b
Salicylic acid 0.5 mM	44.41a	12.49a	25.93ab
Salicylic acid 1 mM	45.50a	13.01a	27.36ab
Glycine 10 mM	47.03a	13.16a	28.10ab
Glycine 50 mM	48.58a	14.58a	31.72a

*Means with similar letters in each column are not significantly differentt by Duncan's test at 5% probability level.

4.5. The distance between the nodes

The main effects of irrigation and stress relievers as well as their interactions on distance between nodes were very significant. Results of comparing the mean interaction of irrigation treatments and stress reducing agents on mentioned trait in Fig. "5" showed that Co Ir + Gl50 treatment increased the distance between nodes (7.94 cm) compared to Cu Ir Fl0% + No Sp (3.97 cm). This difference was very significant. Zabet *et al*. (2003) stated that the reason for the reduction in length between nodes in mung bean stems during drought stress was a decrease in cell division. Glycine betaine increases the distance between nodes and ultimately increases plant height due to increased stomata conductance, increased photosynthesis, cell turgenesis and cell enlargement (Ma *et al*., 2007). A concentration of 50 mM glycine had a greater effect on distance between nodes than a concentration of 10 mM. So, increasing concentration of substance has probably increased its positive effects by increasing internal concentration of substance in the plant.

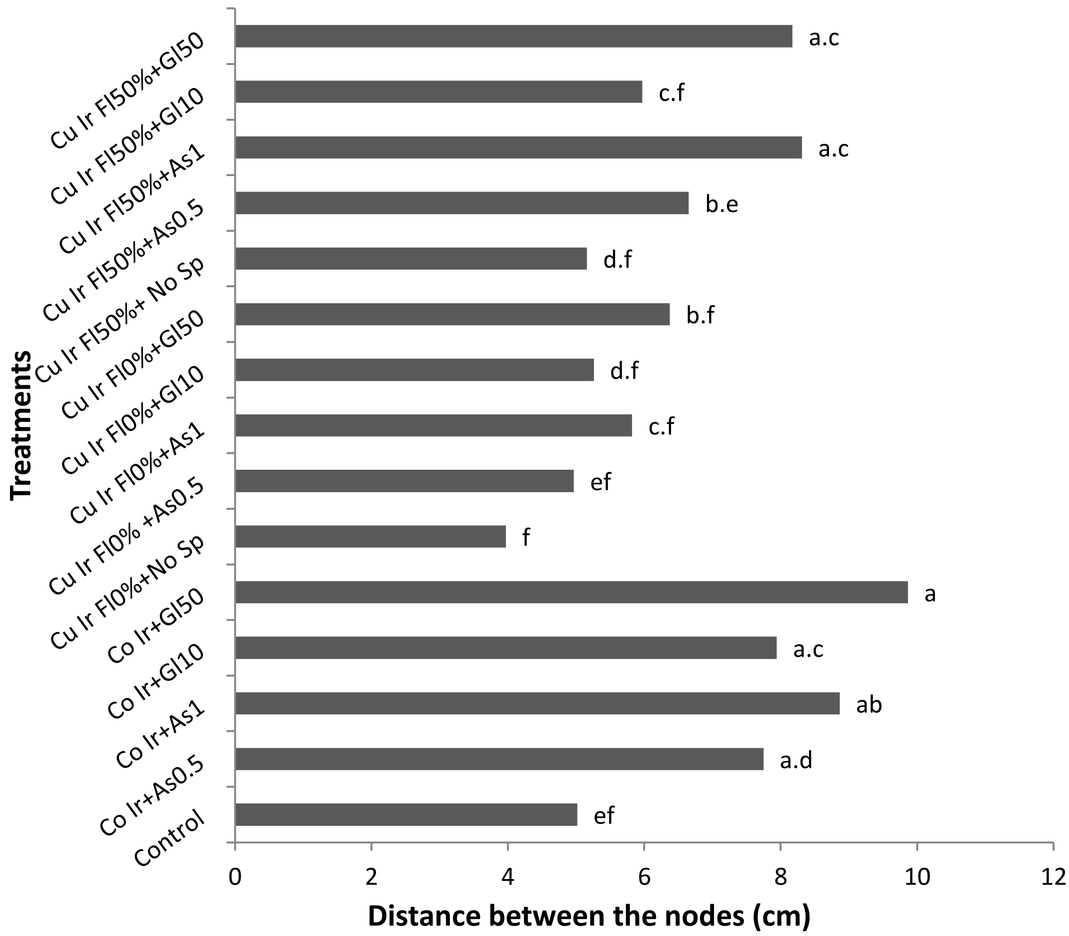

Fig. 5. Comparison of the average effect of irrigation and foliar application of stress reducing agents on distance between the nodes using Duncan test at 5% probability level.

4.6. Correlation between traits

As expected, wet forage yield showed a positive and significant correlation with dry forage yield and number of stems per plant. Wet forage yield showed a negative and significant relationship with the distance between nodes. This indicates that the forage weight has also increased as the internode spacing has decreased and the stem has become more compact. Also, as expected, there was no significant correlation between the number of stems per plant and the internode distance. The correlation between traits by Pearson method is shown in table 4.

5. CONCLUSION

The results showed that drought stress had an adverse effect on the studied traits and the use of stress reducing agents can reduce the destructive effects. Among different irrigation treatments, the effect of stopping irrigation at the beginning of flowering stage had the most negative effect on the studied traits.

In the study of stress reduction treatments, it was found that glycine-betaine has a more important role than salicylic acid in reducing the adverse effects of stress. In relation to wet and dry forage yield, which is the ultimate goal of planting any forage plant such as alfalfa, the simultaneous use of full irrigation and glycine was the best treatment. As no significant difference was observed between different concentrations of glycine, the use of 10 mM concentration is recommended to achieve the desired result. In both stress treatments (cessation of irrigation from the beginning of flowering and cessation of irrigation from 50% of flowering) when the stress-reducing treatments were used, the effect of stress was reduced. The reduction of stress effect was quite significant and significant in the treatments that used stress reducer.

Table 4. Correlation between traits

Treatment	Wet forage yield	Dry forage yield	Number of stems per plant	Distance between the nodes
Wet forage yield	1	0.99**	0.92**	**-0.93***
Dry forage yield		1	0.95*	**-0.90***
Number of stems per plant			1	**-0.73ns**
Distance between the nodes				**1**

ns, * and **: non-significant, significant at 5% and 1% of probability level, respectively.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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