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# Investigation of the response of new improved alfalfa cultivars to salinity in field conditions

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## Extended abstract

#### Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops and it produces high quality fodder for all types of livestock and alone can provide energy, protein, minerals and vitamins for livestock. Alfalfa is relatively sensitive to the salinity, but it has a high genetic diversity that can be used to select resistant cultivars. Therefore, the success of selection of alfalfa resistant cultivars requires exploitation of this genetic diversity that can produce more resistant plants to salinity compared to other forage plants, thus cultivar selection is important for alfalfa hay production. The aim of this project was to evaluate the tolerance of new alfalfa cultivars to salinity in field conditions.

## Material and methods

This research was carried out in 2017 and 2020 at the Research Farm of East-Azarbaijan Agricultural and Natural Resources Research and Education Center, Tabriz, Iran (Firuz Salar village located 4 km from Azarshahr city). In this study, two synthetic cultivars A and B along with a local ecotype as a control cultivar were compared in saline water and soil conditions. Synthetic A cultivar was selected from 11 ecotypes based on general combining ability test and evaluation of half-sib families, which eventually led to the production of Synthetic A cultivar. Synthetic cultivar B has been produced by selecting superior genotypes from five elite ecotypes. With polycross of selected genotypes in completely isolated conditions, synthetic cultivar B has been produced. Synthetic cultivars and common ecotype and local control were evaluated in a randomized complete block design with three replications. The field was irrigated with saline water of the experimental area. Chlorophyll index, plant height, stem diameter, leaf area index, fresh and dry forage yield in each harvest and their annual total, leaf to stem ratio, protein content, digestibility, ADF, NDF, and the chlorophyll content a and b were measured.

## **Results and discusions**

The results showed that there was a significant difference between the studied cultivars for all measured traits at the level of 1% probability and also the effect of harvest was significant for all traits except leaf to shoot ratio. The mean height in both synthetic cultivars A and B was higher than the control cultivar and both were in a statistical group. Similar results were observed for fresh and dry forage yield, leaf area index, stem diameter, leaf to shoot ratio and chlorophyll index and synthetic cultivars were superior to the control cultivar.

The average fresh forage yield of synthetic cultivars A and B in each harvest was 18.06 and 17.81 tons per hectare, respectively, which was significantly higher than the average fresh forage yield of the control

cultivar (15.24 tons per hectare). For dry forage yield was quite similar and dry forage yield of synthetic cultivars was significantly higher than the control cultivar. Synthetic cultivars were superior to control cultivar in terms of chlorophyll content. It seems that improving the photosynthetic ability of these cultivars is one of the reasons for the high forage yield in these cultivars.

#### Conclusions

The results showed that there was a significant difference between the studied cultivars for all measured traits. The average fresh forage yield of synthetic cultivars A and B in each crop was 18.06 and 17.81 tons per hectare, respectively, which was significantly higher than the average fresh forage yield of the control cultivar (15.24 tons per hectare). For dry forage yield, quite similar results were obtained and the dry forage yield of synthetic cultivars was significantly higher than the control cultivar. The mean height in both synthetic cultivars A and B was higher than the control cultivar. On average, during the three years of the study, the third and second harvest produced the highest fresh forage yields. It seems the cultivars that have been modified for normal conditions also appear to be better under stress conditions. According to the total results obtained, synthetic cultivars have the necessary potential for cultivation under salinity conditions similar to the conditions of this study.

Keywords: Chlorophyll, Forage quality, Forage yield

 Table 1. Physico-chemical properties of the 0-35 cm soil depth in the experiment

EC	pН	Organic Carbon	Ν	Р	K	Sand	Silt	Clay
dS m <sup>-1</sup>		%%		mg.kş	g <sup>-1</sup>		%	
5.3	7.5	1.29	12	51.85	285	37	50	13

 Table 2. Combined analysis of variance (Mean Squares) of quantitative traits measured in alfalfa cultivars under salinity conditions

		Plant	Fresh Forage	Dry Forage	Leaf Area	Shoot	Leaf to	Chlorophyll
S.O.V	d.f	Height	Yield	Yield	Index	Diameter	shoot ratio	index
Block (B)	2	5.331 <sup>ns</sup>	2.146 <sup>ns</sup>	0.166 <sup>ns</sup>	2.149 <sup>ns</sup>	0.315 <sup>ns</sup>	0.006 <sup>ns</sup>	30.623 <sup>ns</sup>
Cultivar (C)	2	424.440**	88.223**	10.104**	21.002**	$4.769^{*}$	$0.047^{*}$	364.675*
Error I	4	9.192	8.682	0.208	0.656	0.468	0.003	45.876
Year (Y)	2	25.029 <sup>ns</sup>	1.986 <sup>ns</sup>	0.670 <sup>ns</sup>	2.957 <sup>ns</sup>	0.171 <sup>ns</sup>	0.021*	2.155 <sup>ns</sup>
Y × B	4	3.406 <sup>ns</sup>	4.673 <sup>ns</sup>	0.164 <sup>ns</sup>	0.343 <sup>ns</sup>	0.088 <sup>ns</sup>	0.004 <sup>ns</sup>	14.090 <sup>ns</sup>
Y × C	4	3.184 <sup>ns</sup>	.468 <sup>ns</sup>	0.148 <sup>ns</sup>	0.838 <sup>ns</sup>	0.162 <sup>ns</sup>	0.003 <sup>ns</sup>	4.783 <sup>ns</sup>
Error II	8	6.771	6.013	0.434	1.101	0.256	0.002	26.832
Harvest (H)	3	326.834**	21.707**	4.956**	$7.080^{**}$	1.020**	0.003 <sup>ns</sup>	142.753**
B×H	6	33.878 <sup>ns</sup>	4.798 <sup>ns</sup>	0.240 <sup>ns</sup>	1.217 <sup>ns</sup>	0.227 <sup>ns</sup>	0.001 <sup>ns</sup>	16.065 <sup>ns</sup>
C×H	6	18.690 <sup>ns</sup>	2.036 <sup>ns</sup>	0.648 <sup>ns</sup>	0.386 <sup>ns</sup>	$0.524^{*}$	0.002 <sup>ns</sup>	27.281 <sup>ns</sup>
$\mathbf{H} \times \mathbf{Y}$	6	30.386 <sup>ns</sup>	20.015**	0.753 <sup>ns</sup>	1.495 <sup>ns</sup>	0.925**	$0.010^{*}$	28.399 <sup>ns</sup>
Error III	60	19.179	5.091	0.461	0.677	0.190	0.003	16.623

ns, \* and \*\*: Non significant, significant at the 5% and 1% probability levels, respectively

		Fresh forage	Dry forage	Leaf area	Shoot	Leaf to shoot	Chlorophyll
Cultivar	Plant height	yield	yield	index	diameter	ratio	index
	cm	t.ha	<sup>-1</sup>		mm		
Check	56.76 <sup>b</sup>	15.24 <sup>b</sup>	3.98 <sup>b</sup>	4.23 <sup>b</sup>	2.59 <sup>b</sup>	0.87 <sup>b</sup>	46.66 <sup>b</sup>
Synthetic B	62.83ª	17.81ª	4.79 <sup>a</sup>	5.49ª	3.19 <sup>a</sup>	0.93ª	52.06 <sup>a</sup>
Synthetic A	62.58ª	18.06 <sup>a</sup>	4.97 <sup>a</sup>	5.60 <sup>a</sup>	3.25ª	0.93ª	52.27ª
Mean	60.72	17.04	4.58	5.11	3.01	0.91	50.33

Table 3. Comparison of mean traits studied in synthetic and control alfalfa cultivars under salinity conditions during three years

Means followed by the same letter are not significantly different at p=0.05, according to Duncan test

#### Table 4. Comparison of the mean of studied traits in harvests and studied years under salinity conditions

			Fresh forage	Dry forage	Leaf area	Shoot	Leaf to	Chlorophyll
Year	Harvest	Plant height	yield	yield	index	Diameter	shoot ratio	index
		cm	t.h	a <sup>-1</sup>		mm		
	1	55.77	15.31 <sup>cde</sup>	4.01	4.40	2.53 <sup>e</sup>	0.99ª	48.74
	2	59.47	16.72 <sup>bcde</sup>	4.70	5.84	3.04 <sup>bcd</sup>	0.92 <sup>ab</sup>	51.81
2018	3	65.39	19.79 <sup>a</sup>	5.36	4.73	3.10 <sup>bc</sup>	0.91 <sup>ab</sup>	53.36
	4	59.46	17.38 <sup>bcd</sup>	4.87	4.41	3.07 <sup>bcd</sup>	0.91 <sup>ab</sup>	48.56
	Mean	60.02	17.30	4.73	4.85	2.94	0.93	50.62
	1	56.90	14.88 <sup>e</sup>	3.87	4.84	2.80 <sup>cde</sup>	0.91 <sup>ab</sup>	48.23
	2	63.47	18.03 <sup>ab</sup>	4.27	5.66	2.90 <sup>cde</sup>	0.89 <sup>ab</sup>	53.89
2019	3	63.42	17.27 <sup>bcde</sup>	5.27	5.76	3.60 <sup>a</sup>	0.89 <sup>ab</sup>	51.00
	4	58.29	17.79 <sup>ab</sup>	4.47	5.41	2.92 <sup>cde</sup>	0.87 <sup>b</sup>	47.68
	Mean	60.52	16.99	4.47	5.42	3.06	0.89b	50.20
	1	60.01	17.70 <sup>abc</sup>	4.56	4.52	3.19 <sup>abc</sup>	0.89 <sup>ab</sup>	52.08
	2	62.53	17.28 <sup>bcde</sup>	4.46	5.39	3.40 <sup>ab</sup>	0.93 <sup>ab</sup>	51.24
2020	3	66.64	17.20 <sup>bcde</sup>	4.89	5.91	3.02 <sup>bcd</sup>	0.96 <sup>ab</sup>	51.57
	4	57.40	15.18 <sup>de</sup>	4.30	4.48	2.61 <sup>de</sup>	0.93 <sup>ab</sup>	45.86
	Mean	61.65	16.84	4.55	5.08	3.06	0.93	50.19
	1	57.56°	15.96	4.14 <sup>c</sup>	4.59 <sup>b</sup>	2.84	0.93	49.69 <sup>b</sup>
	2	61.82 <sup>b</sup>	17.34	4.47 <sup>bc</sup>	5.63ª	3.11	0.91	52.31ª
Mean	3	65.15 <sup>a</sup>	18.09	5.17 <sup>a</sup>	5.47ª	3.24	0.92	51.97 <sup>a</sup>
	4	58.38°	16.78	4.54 <sup>b</sup>	4.77 <sup>b</sup>	2.87	0.90	47.36°
	Mean	60.72	17.04	4.58	5.11	3.02	0.92	50.33
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Means followed by the same letter are not significantly different at p=0.05, according to Duncan test.

 Table 5. Combined analysis of variance (Mean Squares) of traits measured in alfalfa cultivars under salinity conditions

				ADF	NDF		
				Acid	Neutral		
		Protein		detergent	detergent		
<b>S.O.V</b>	d.f	content	Digestibility	fiber	fiber	Chlorophyll a	Chlorophyll b
Block (B)	2	79.311 <sup>ns</sup>	5.975 <sup>ns</sup>	1.062 <sup>ns</sup>	10.541 <sup>ns</sup>	0.058 <sup>ns</sup>	0.269 <sup>ns</sup>
Cultivar (c)	2	193.274*	$234.140^{*}$	73.141**	366.630*	$2.868^{*}$	1.562 <sup>ns</sup>
Error I	4	26.082	26.585	2.929	31.740	0.188	0.301
Year (Y)	1	184.320**	2.722 <sup>ns</sup>	1.561 <sup>ns</sup>	14.401 <sup>ns</sup>	1.176 <sup>ns</sup>	$0.222^{**}$
Y×B	2	8.835 <sup>ns</sup>	71.047 <sup>ns</sup>	5.951 <sup>ns</sup>	28.687 <sup>ns</sup>	0.048 <sup>ns</sup>	0.172**
Y× C	2	5.318 <sup>ns</sup>	11.994 <sup>ns</sup>	7.021 <sup>ns</sup>	13.438 <sup>ns</sup>	0.161 <sup>ns</sup>	$0.077^{**}$
Error II	4	6.090	35.780	10.947	8.341	0.162	0.002
Harvest (H)	3	218.547**	179.978**	$27.962^{*}$	96.116**	1.557**	1.801**
H×B	6	3.490 <sup>ns</sup>	51.713 <sup>ns</sup>	6.785 <sup>ns</sup>	24.751 <sup>ns</sup>	0.138 <sup>ns</sup>	0.146 <sup>ns</sup>
H×C	6	27.972 <sup>ns</sup>	31.448 <sup>ns</sup>	3.191 <sup>ns</sup>	30.730 <sup>ns</sup>	0.206 <sup>ns</sup>	$0.382^{*}$
H×Y	3	14.356 <sup>ns</sup>	10.707 <sup>ns</sup>	14.309 <sup>ns</sup>	28.431 <sup>ns</sup>	0.410 <sup>ns</sup>	0.363 <sup>ns</sup>
Error III	36	27.096	21.747	9.021	21.958	0.229	0.133

ns, \* and \*\*: Non significant, significant at the 5% and 1% probability levels, respectively

Cultivar	Protein content	Digestibility	ADF Acid detergent fiber	NDF Neutral detergent fiber	Chlorophyll a	Chlorophyll b
		%			μg.n	1l <sup>-1</sup>
Check	18.17 <sup>b</sup>	27.53 <sup>b</sup>	13.71 <sup>b</sup>	46.28°	5.73 <sup>b</sup>	2.73 <sup>b</sup>
Synthetic B	22.63 <sup>a</sup>	33.65 <sup>a</sup>	17.01 <sup>a</sup>	54.08 <sup>a</sup>	6.38 <sup>a</sup>	3.18 <sup>a</sup>
Synthetic A	23.44 <sup>a</sup>	31.67 <sup>a</sup>	16.35 <sup>a</sup>	49.73 <sup>b</sup>	6.25 <sup>a</sup>	3.17 <sup>a</sup>
Mean	21.41	30.95	15.69	50.03	6.12	3.03

Table 6. Comparison of the mean of studied traits in harvests and studied	vears under salinity conditions
Table 0. Comparison of the mean of studied traits in harvests and studied	years under sammey conditions

Means followed by the same letter are not significantly different at p=0.05, according to Duncan test

Harvest	Protein content	Digestibility	ADF Acid detergent fiber	NDF Neutral detergent fiber	Chlorophyll a	Chlorophyll b
			%		μg.:	ml <sup>-1</sup>
1	26.03ª	26.96 <sup>b</sup>	14.16 <sup>b</sup>	46.89 <sup>b</sup>	5.75°	2.68°
2	20.83 <sup>b</sup>	29.86 <sup>b</sup>	15.41 <sup>ab</sup>	50.33ª	6.29 <sup>ab</sup>	3.10 <sup>b</sup>
3	21.23 <sup>b</sup>	33.43 <sup>a</sup>	16.05 <sup>ab</sup>	50.43ª	6.41 <sup>a</sup>	3.43 <sup>a</sup>
4	17.57 <sup>b</sup>	33.55ª	17.14 <sup>a</sup>	52.47ª	6.02 <sup>bc</sup>	2.90 <sup>bc</sup>
Mean	21.41	30.95	15.69	50.03	6.12	3.03

Table 7. Comparison of the mean of studied traits in different classes under salinity conditions

Means followed by the same letter are not significantly different at p=0.05, according to Duncan test.