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COMPARATIVE PHYSIOLOGICAL RESPONSE OF SAINFOIN (*ONOBRYCHIS VICIAEFOLIA*) SEEDLINGS TO ALKALINE AND SALINE-ALKALINE STRESS

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ABSTRACT

Salt-alkali stress is a major environmental factor that seriously limits crops development and productivity worldwide. The objective of this study is to compare growth and physiological responses of sainfoin (*Onobrychis viciaefolia*) to alkali ($NaHCO_3$) and salt-alkali (NaCl: $NaHCO_3$) stress, by investigating biomass, photosynthetic pigments, antioxidant enzymes, inorganic ions, organic substances such as proline and sugars. It was shown that both alkaline and saline-alkaline stress treatments significantly reduced fresh weight (FW) and dry weight (DW), chlorophyll (Chl) contents, K^+ concentrations, antioxidant enzymes activity and soluble sugars contents, whereas clearly increased malondialdehyde (MDA), proline and Na^+ concentrations in sainfoin plants compared with control (no added NaCl and $NaHCO_3$). It is also observed that in the 150 mM $NaHCO_3$, peroxidase (POD) activity, soluble sugar contents and K^+/Na^+ ratio in plants under $NaHCO_3$ treatment were lower than those in plants under NaCl: $NaHCO_3$ treatment. These results suggested that the damages caused by alkaline stress on the growth of sainfoin plants are more serious than those caused by saline-alkaline stress treatments. The findings of the present work provide a basis for understanding the physiological responses to saline-alkaline stress in forage legumes.

Key words: Forage legume; antioxidant enzymes; proline; soluble sugars; malondialdehyde

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INTRODUCTION

Soil salinization is becoming a serious problem in agricultural systems and is one of major factors which limits crop productivity worldwide (Li *et al*., 2020). Saline stress is primarily caused by NaCl and/or Na_2SO_4 accumulation, while alkaline stress is mainly caused by $NaHCO_3$ and Na_2CO_3 accumulation (Zhang *et al*., 2019). In northwest China, more than 70% of the agriculture area is threatened by saline-alkaline stress (Jia *et al*., 2019). It is well-known that alkaline stress or saline-alkaline stress can inhibit plants growth and development more seriously than neutral-salt stress (Xu *et al*., 2019). Saline-alkali stress has a significant effect on chloroplast ultrastructure and photosynthetic components, which are considered to be non-stomatal factors that reduce photosynthetic capacity (Bejaoui *et al*., 2016; Sun *et al*., 2019). Salt and alkali stress also markedly decreased the chlorophyll (Chl) a and Chl b contents in the leaves of plants (Zhang *et al*., 2019). Generally, Chl contents were more sensitive to alkaline stress than saline stress at the same concentration (Zhang *et al*., 2019). Alkaline stress has also been shown to inhibit uptake of anions such as Cl^-, NO_3^-, SO_4^{2-} and $H_2PO_4^-$, and significantly reduce the selective uptake and transport for K^+ over Na^+ (Wang *et al*., 2011). To cope with saline-alkaline stress, plants have evolved a variety of adaptive mechanisms such as osmotic adjustment (OA), accumulation of organic substances, antioxidant enzyme, and so on (Jia *et al*., 2019). Soluble sugars, proline, malondialdehyde (MDA), superoxidase dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) play crucial roles in OA and might be of extreme importance in scavenging reactive oxygen species (ROS) when plants were subjected to salinity stress (Bejaoui *et al*., 2016; Khalid *et al*., 2020).

Sainfoin (*Onobrychis viciaefolia*), belonged to the *Leguminosae* family, is recognized as one of the most important forage legumes with high quality in the world (Bhattarai *et al*., 2018), and it is also praised as "the queen of forage". In China, sainfoin is mainly planted in the arid and semi-arid areas of northwest China, including Gansu province (Shen *et al*., 2019). Due to its high contents of condensed tannins, sainfoin has been documented to decrease parasites (such as roundworm and trematode) in ruminant digestive tracts and provides environmental benefits by decreasing emissions of methane from the ruminant animals (Bhattarai *et al*., 2018). Although sainfoin has good adaptability to abiotic environments, plants are sensitive to high salt conditions. The low concentrations (5–50 mM) of NaCl had no significant effect on plant growth of sainfoin, whereas high concentrations (100 and 200 mM) of NaCl significantly inhibited growth compared to control (Wu *et al*., 2017a). However, the studies on effects of alkaline stress on growth and physiological responses in sainfoin plants have been rarely performed.

The objective of this work is to compare

physiological responses of sainfoin to alkaline stress and saline-alkaline stress, by investigating biomass, photosynthetic pigments, antioxidant enzymes, inorganic ions, organic substances such as proline and sugars. The findings of the present study will provide a basis for understanding the physiological responses to saline-alkaline stress in forage legume species.

MATERIALS AND METHODS

Plant materials, growth conditions and stress treatments: The seeds of sainfoin (*O. viciaefolia* Scop.) cultivar 'GANSU' were purchased from Lanzhou Xinglong Grass Technical Service Co., Ltd, Gansu province, China. The uniform seeds were selected and sowed into vermiculite (4 seeds/container) irrigated with the sterile water. After 3 d of germination, seedlings were irrigated with the modified Hoagland nutrition solution containing 0.5 mM $NH_4H_2PO_4$, 2 mM KNO_3, 0.1 mM $Ca(NO_3)_2$, 60 µM Fe-Citrate, 0.25 mM $MgSO_4$, 92 µM H_3BO_3, 1.6 µM $ZnSO_4 \cdot 7H_2O$, 18 µM $MnCl_2 \cdot 4H_2O$, 0.25 µM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and 0.6 µM $CuSO_4 \cdot 5H_2O$. Solution was changed every 3 d. Seedlings are grown in a growth chamber with the temperature of 18 °C/24 °C (night/day), the light intensity of 400–500 µmol/m^2/s, and the relative humidity (RH) of 50–55% (Wu *et al*., 2017b).

According to our previous studies (Wu *et al*., 2019), four-week-old plants were challenged by alkali and salt-alkali stress treatments as follows: control (added neither salt nor alkaline, C), 50 mM $NaHCO_3$ (alkali stress 1, AS1), 100 mM $NaHCO_3$ (alkali stress 2, AS2), 150 mM $NaHCO_3$ (alkali stress 3, AS3), 25 mM NaCl+25 mM $NaHCO_3$ (salt-alkali stress 1, SAS1), 50 mM NaCl+50 mM $NaHCO_3$ (salt-alkali stress 2, SAS2), and 75 mM NaCl+75 mM $NaHCO_3$ (salt-alkali stress 3, SAS3). Salt composition of the treatments was shown in Table 1. Each treatment had eight biological repeats. Each repeat included three plants. Treatment solution was changed every 2 d. After 7 d of alkali and salt-alkali stress treatments, plants were used to test physiological parameters.

Table 1. Concentrations (mM) of various salts (NaCl, $NaHCO_3$) in treatments.

Treatment	NaCl (mM)	$NaHCO_3$ (mM)	Salinity (mM)	pH
C	0	0	0	6.85
AS1	0	50	50	8.59
AS2	0	100	100	8.60
AS3	0	150	150	8.62
SAS1	25	25	50	8.51
SAS2	50	50	100	8.53
SAS3	75	75	150	8.54

C, control; AS, alkali stress ($NaHCO_3$); SAS, salt-alkali stress (NaCl: $NaHCO_3$).

Determination of growth, Na^+ and K^+ concentrations, and chlorophyll contents: At the end of the alkali and salt-alkali stress treatments, to remove surface vermiculite, plant roots were washed four time with deionized water. Shoots and roots were separated and blotted; shoot and root fresh weight (FW) were measured immediately and then were dried in an oven at 80 °C for 72 h to determine dry weight (DW). Water content (WC) of tissue was calculated by following the formulas: WC (g/g DW) = (FW - DW)/DW. K^+ and Na^+ were extracted from dried sample in 100 mM acetic acid at 95 °C for 2 h. K^+ and Na^+ concentrations were assayed by using a flame spectrophotometer (2655-00, Cole-Parmer Instrument Co., Vernon Hills, USA). K^+/Na^+ ratio was measured according to methods of Wang *et al*. (2009). Chlorophyll **(**Chl**)** contents were determined by using the methods of Zhang *et al*. (2019) with the slight modification. Briefly, Chl a and Chl b were extracted from fresh leaves samples of 1.5 g by a mixture of ethanol, acetone, and H_2O with a volumetric ratio of 4.5:4.5:1. The absorbances at 665 and 649 nm (A_{665} and A_{649}, respectively) were determined by using a spectrophotometer (UV- 300 OPC, Mapada Co., Shanghai, China). The contents of Chl a and Chl b were calculated by the following formulas: Chl a (mmol/g FW) = $(13.95A_{665} - 6.88A_{649}) \times V/1000 \times$ FW, Chl b (mmol/g FW) = $(24.96A_{649} - 7.32A_{665}) \times V/1000 \times$ FW, where V is the volume of extraction, FW is fresh weight of leaves samples. The total Chl contents are sum of Chl a and Chl b.

Analysis of antioxidant enzymes and malondialdehyde: Superoxidase dismutase (SOD) was tested by measuring the capability of the enzyme to inhibit the photochemical decrease of nitro blue tetrazolium (NBT) reagent according to the methods described by Beauchamp and Fridovich (1971). Peroxidase (POD) was determined by monitoring the increase in absorbance at wavelength of 470 nm recorded 40 s after the additional H_2O_2 according to the methods of Sakharov and Ardila (1999). Catalase (CAT) was analyzed by measuring the amount of H_2O_2 consumed during the reaction process with the methods of Aebi (1984). Ascorbate peroxidase (APX) was determined by monitoring the increase in absorbance at 290 nm recorded 60 s after the additional H_2O_2 according to the methods of Nakano and Asada (1981) with the slight modification. Malondialdehyde (MDA) concentration was tested by following the methods described by Bao *et al*. (2009).

Determination of proline and soluble sugar contents: Proline concentrations were measured by using ninhydrin reagent based on the methods described by Bates *et al*. (1973). Soluble sugars were tested by using the anthrone ethyl acetate reagent according to the methods of Zhang *et al*. (2006) with the slight modification. The contents of sucrose, glucose, and fructose were tested by using the resorcinol, sodium arsenomolybdate, and resorcinol

reagents, respectively, according to the method of Liu *et al*. (2008).

Statistical analysis: All data were subjected to a one-way analysis of variance (ANOVA) using SPSS (v. 19.0, SPSS Inc., Chicago, IL, USA), and the mean differences were compared by with Duncan's multiple range test at $P < 0.05$. Data were represented as the mean ± standard error (SE) in the tables and figures.

RESULTS AND DISCUSSION

Effects of alkaline and salt-alkali stress treatments on growth of sainfoin: Phenotypically, growth of plants was remarkedly inhibited under either alkaline stress (SA) or salt-alkaline stress (SAS) compared to control (C), whereas SAS-treated plants grew relatively better than SA-treated plants (Fig. 1).

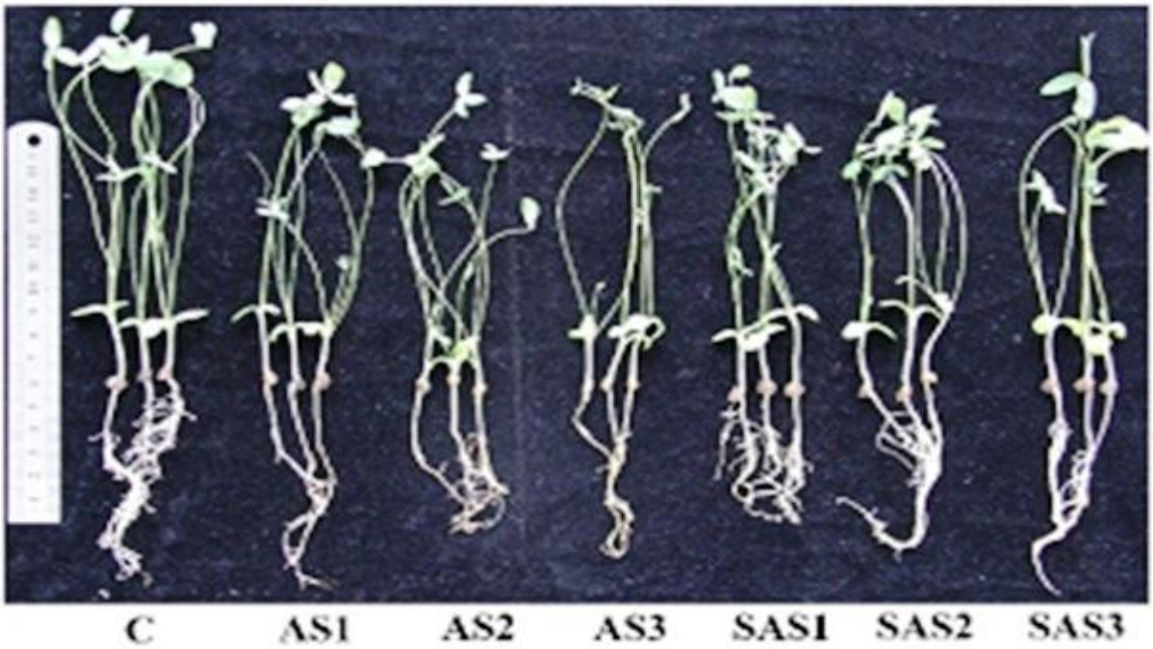

Fig. 1. Phenotypes of sainfoin under alkaline stress and salt-alkaline stress. Four-week-old seedlings were treated with modified Hoagland nutrient solution supplemented without (control, C) or with 50 mM $NaHCO_3$ (alkali stress 1, AS1), 100 mM $NaHCO_3$ (alkali stress 2, AS2), 150 mM $NaHCO_3$ (alkali stress 2, AS2), 25 mM NaCl+25 mM $NaHCO_3$ (salt-alkali stress 1, SAS1), 50 mM NaCl+50 mM $NaHCO_3$ (salt-alkali stress 2, SAS2), and 75 mM NaCl+75 mM $NaHCO_3$ (salt-alkali stress 2, SAS2) for 7 d.

Biomass accumulation on plants is an optimum parameter for evaluating plant stress (Alvarez-Acosta *et al*., 2018). Shoot fresh weight (FW) and dry weight (DW) were observably lower in plants subjected to both alkali and salt-alkali stresses, compared with control ($P < 0.05$). High alkali stress (150 mM $NaHCO_3$, AS3) significantly reduced shoot water content compared with control ($P < 0.05$), while salt-alkali stresses have no significant effects on shoot water content. Root FW was observably lower in plants exposed to AS2, AS3, SAS2 and SAS3 treatments than in control plants ($P < 0.05$). AS3 treatment also significantly decreased root DW compared to control ($P < 0.05$), whilst salt-alkali stresses did not have any effects on root DW compared with control (Table 2). Additionally, shoot water content in SA3-treated plants were lower than that in untreated plants (Table 2).

Chl is the major photosynthetic pigment, which plays critical roles in the absorption, transformation, and transmission of light (Zhang *et al*., 2019). In our study, the total Chl contents displayed a significant decrease in plants leaves under all the alkali and salt-alkali stresses compared with control ($P < 0.05$) (Fig. 2a). It may because stresses disturbed the balance between the biosynthesis and degradation of Chl, and thus limited Chl synthesis (Jia *et al*., 2019). This is in accordance with the findings in avocados (*Persea americana*) and Goji berry (*Lycium barbarum*) by Alvarez-Acosta *et al*. (2018) and Zhang *et al*. (2019). Besides, AS2, AS3 and SAS3 treatments also clearly reduced ratio of Chl a/Chl b in leaves of plants compared to control, and the value of Chl a/Chl b in plants under SAS2 treatment was 92.7% higher than that in plants under AS2 treatment ($P < 0.05$) (Fig. 2b).

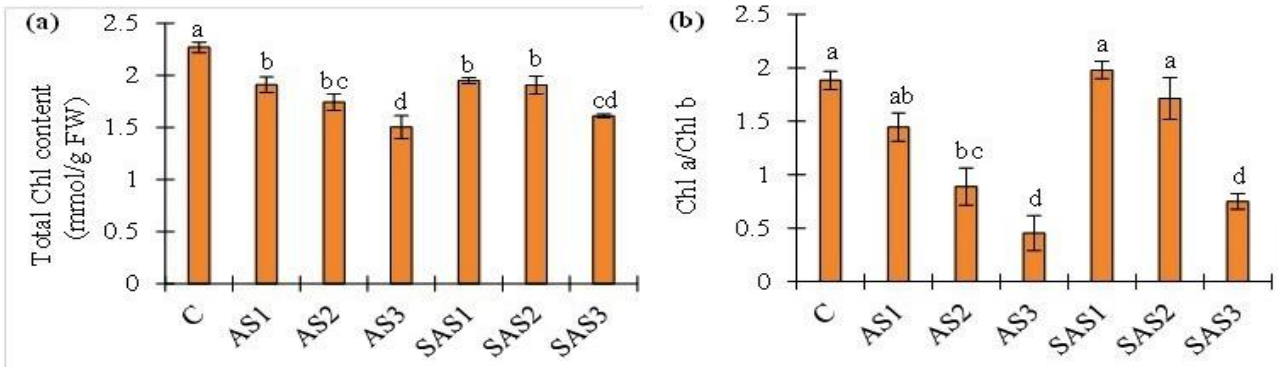

Fig. 2. Effects of alkaline and salt-alkaline stresses on total Chl content (a) and Chl a/Chl b ratio (b) in shoot of sainfoin. Four-week-old seedlings were treated with modified Hoagland nutrient solution supplemented without (control, C) or with 50 mM $NaHCO_3$ (alkali stress 1, AS1), 100 mM $NaHCO_3$ (alkali stress 2, AS2), 150 mM $NaHCO_3$ (alkali stress 2, AS2), 25 mM NaCl+25 mM $NaHCO_3$ (salt-alkali stress 1, SAS1), 50 mM NaCl+50 mM $NaHCO_3$ (salt-alkali stress 2, SAS2), and 75 mM NaCl+75 mM $NaHCO_3$ (salt-alkali stress 2, SAS2) for 7 d. Three plants were pooled in each replicate. Values are means ± SE (n = 8) and bars represent SE. Columns with different letters represent significant differences at $P < 0.05$ (Duncan's test). FW - fresh weight.

This may because high-pH will disorder the ion homeostasis and plants do not uptake metal ions (Fe^{2+} and Mg^{2+}) needed to synthesize Chl (Jia *et al*., 2019). These results implied that harmful effects of alkaline on sainfoin plants are stronger than those of salt-alkali stress.

Effects of alkaline and salt-alkali stress treatments on antioxidant enzymes and MDA of sainfoin: Under stressed condition, plants will produce a lot of ROS, causing damage to membrane lipid peroxidation. Plants have developed a ROS-scavenging system that includes antioxidant substances and antioxidant enzymes to alleviate oxidative stress (Gong *et al*., 2013). SOD is an important antioxidant enzyme for the removal of ROS, which can dismutase $O_2^{\bullet-}$ to H_2O_2 and oxygen, then CAT and POD turn H_2O_2 into water and oxygen (Sun *et al*., 2019). APX, another important antioxidant enzyme, uses ascorbate as the electron donor for the decrease of H_2O_2 (Gong *et al*., 2013). In the present study, AS1, AS2, AS3, SAS2 and SAS3 significantly reduced activities of SOD, POD, CAT and APX in leaves compared to control ($P < 0.05$) (Fig. 3a, b, c, d).

These effects because alkaline and salt-alkali stresses block the pathway of enzymes synthesis and reduce the activities of enzymes (Zhang *et al*., 2019). Interestingly, in 150 mM $NaHCO_3$, activity of POD was significantly higher in plants under SAS3 treatment than that under AS3 treatment ($P < 0.05$) (Fig. 3b), thus indicating that POD activity of sainfoin plants has stronger adaptability to saline-alkaline stress compared to alkaline stress. MDA is a key sign of membrane lipid peroxidation damage in plants (Bao *et al*., 2009). In the treatment of 150 mM $NaHCO_3$, both AS3) and SAS3 treatments significantly increased leaf MDA concentration by 3.4- and 3.1-fold compared to control ($P < 0.05$). However, in the treatment of 100 mM $NaHCO_3$, MDA concentration was significantly increased by alkaline stress (AS2) ($P < 0.05$), but not salt-alkali stress (Fig. 4a), which showed that sainfoin plants suffered severe oxidative damage under alkaline stress.

Effects of alkaline and salt-alkali stress treatments on proline and soluble sugar contents of sainfoin salt-alkali: Under abiotic stresses, plants can accumulate compatible solutes such as proline and sugars to regulate osmotic balance and enhance plant tolerance to stressed conditions (Sperdouli and Moustakas, 2012). In the present study, both AS3 and SAS3 treatments remarkably enhanced proline concentrations in leaves compared to control ($P < 0.05$) (Fig. 4b). Similar results were found in *Malus halliana* (Jia *et al*., 2019), where proline was significantly accumulated in leaves of plants under alkaline and salinity-alkaline stresses, indicating that the accumulation of proline was a product of damage from stresses. It was found that soluble sugars concentration of shoot was significantly decreased in plants subjected to AS3 and SAS3 treatments compared to control, and the values in plant under SAS3 treatment were remarkably higher than in plants under SA3 treatment (Fig. 5a). Similar reduction of sugars concentration was observed in sainfoin plants subjected to salt stress (Wu *et al*., 2017b). Four treatments (AS2, AS3, SAS2, and SAS3) significantly reduced the contents of sucrose compared to control (Fig. 5b). Furthermore, the contents of fructose were observably lower under AS1, AS2, AS3, SAS2, and SAS3 treatment than those under control condition (Fig. 5c). Either alkaline or salt-alkali stresses significantly decreased glucose contents compared to control (Fig. 5d).

These results suggested that alkaline and salt-alkali stresses may inhibit sugars synthesis and reduce

sugars accumulation. In conclusion, our results showed that both alkaline stress and saline-alkaline stress significantly reduced FW and DW, Chl contents, K^+ concentration, antioxidant enzymes activity and sugars contents, while clearly increased MDA, proline and Na^+ concentrations in sainfoin plants compared with control. It is also observed that in the 150 mM $NaHCO_3$, POD activity, soluble sugars contents and K^+/Na^+ ratio in plants under $NaHCO_3$ treatment were lower than those in plants under NaCl: $NaHCO_3$ treatment. These results suggested that the damages caused by alkaline stress were more serious than those caused by saline-alkaline stress.

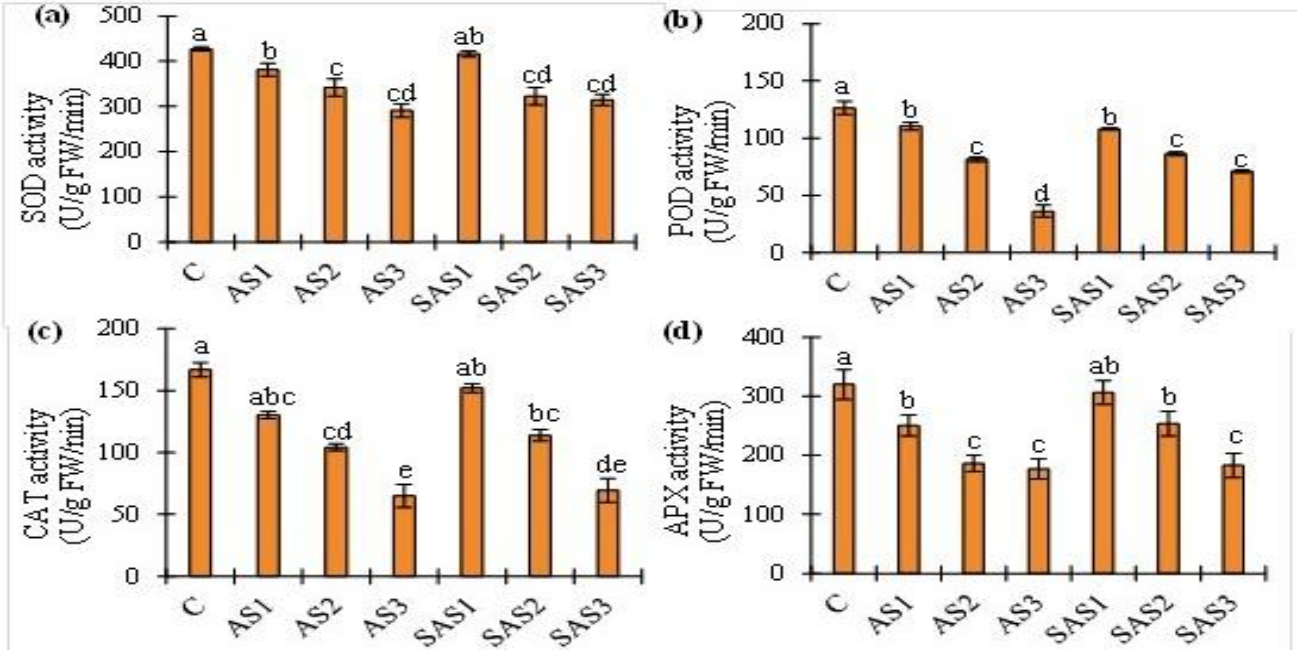

Fig. 3. Effects of alkaline and salt-alkaline stresses on SOD (a), POD (b), CAT (c), and APX (d) activity of shoot in sainfoin. Four-week-old seedlings were treatment with modified Hoagland nutrient solution supplemented without (control, C) or with 50 mM $NaHCO_3$ (alkali stress 1, AS1), 100 mM $NaHCO_3$ (alkali stress 2, AS2), 150 mM $NaHCO_3$ (alkali stress 3, AS3), 25 mM NaCl+25 mM $NaHCO_3$ (salt-alkali stress 1, SAS1), 50 mM NaCl+50 mM $NaHCO_3$ (salt-alkali stress 2, SAS2), and 75 mM NaCl+75 mM $NaHCO_3$ (salt-alkali stress 3, SAS3) for 7 d. Three plants were pooled in each replicate. Values are means ± SE (n = 8) and bars represent SE. Columns with different letters represent significant differences at P < 0.05 (Duncan's test). FW - fresh weight.

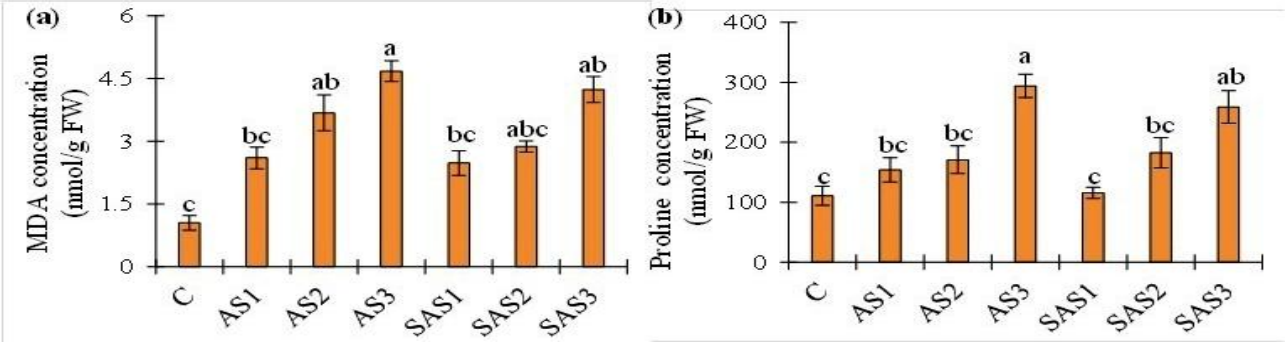

Fig. 4. Effects of alkaline and salt-alkaline stresses on MDA (a) and proline (b) concentrations of shoot in sainfoin. Four-week-old seedlings were treated with modified Hoagland nutrient solution supplemented without (control, C) or with 50 mM $NaHCO_3$ (alkali stress 1, AS1), 100 mM $NaHCO_3$ (alkali stress 2, AS2), 150 mM $NaHCO_3$ (alkali stress 3, AS3), 25 mM NaCl+25 mM $NaHCO_3$ (salt-alkali stress 1, SAS1), 50 mM NaCl+50 mM $NaHCO_3$ (salt-alkali stress 2, SAS2), and 75 mM NaCl+75 mM $NaHCO_3$ (salt-alkali stress 3, SAS3) for 7 d. Three plants were pooled in each replicate. Values are means ± SE (n = 8) and bars represent SE. Columns with different letters represent significant differences at P < 0.05 (Duncan's test). FW - fresh weight.

Table 2. Effects of alkaline and salt-alkali stresses on fresh weight, dry weight and water content of shoot and root in sainfoin. Four-week-old seedlings were treated with modified Hoagland nutrient solution supplemented without (control, C) or with 50 mM (alkali stress 1, AS1), 100 mM $NaHCO_3$ (alkali stress 2, AS2), 150 mM $NaHCO_3$ (alkali stress 3, AS3), 25 mM NaCl+25 mM $NaHCO_3$ (salt-alkali stress 1, SAS1), 50 mM NaCl+50 mM $NaHCO_3$ (salt-alkali stress 2, SAS2), and 75 mM NaCl+75 mM $NaHCO_3$ (salt-alkali stress 3, SAS3) for 7 d. Three plants were pooled in each replicate. Values are means ± SE (n = 8). Within each column, means followed by different letters are significantly different at $P < 0.05$ (Duncan's test).

Treatment	Shoot			Root		
	Fresh weight (mg/plant)	Dry weight (mg/plants)	Water content (g/g DW)	Fresh weight (mg/plant)	Dry weight (mg/plants)	Water content (g/g DW)
C	205.03±9.81 a	19.05±1.07 a	9.83±0.25 a	74.64±10.97 a	3.55±0.37 a	21.11±2.06 a
AS1	163.45±4.94 bc	16.56±0.89 bc	9.12±0.73 ab	61.27±4.22 abc	3.16±0.22 ab	19.10±2.07 a
AS2	149.22±3.11 cd	15.17±0.45 bc	8.89±0.32 ab	50.45±0.99 bcd	2.83±0.12 ab	17.17±1.08 a
AS3	126.85±4.19 e	14.45±0.49 c	7.86±0.44 b	43.95±2.75 d	2.60±0.22 b	17.24±2.50 a
SAS1	171.10±7.06 b	16.84±0.51 b	9.17±0.33 ab	65.29±3.41 ab	3.30±0.42 ab	21.16±1.97 a
SAS2	153.05±4.67 bcd	16.11±0.66 bc	8.65±0.54 ab	56.33±2.76 bcd	3.19±0.24 ab	17.23±1.28 a
SAS3	135.09±6.17 de	15.07±4.56 bc	8.03±0.50 ab	47.93±4.33 cd	2.95±0.31 ab	16.03±1.65 a

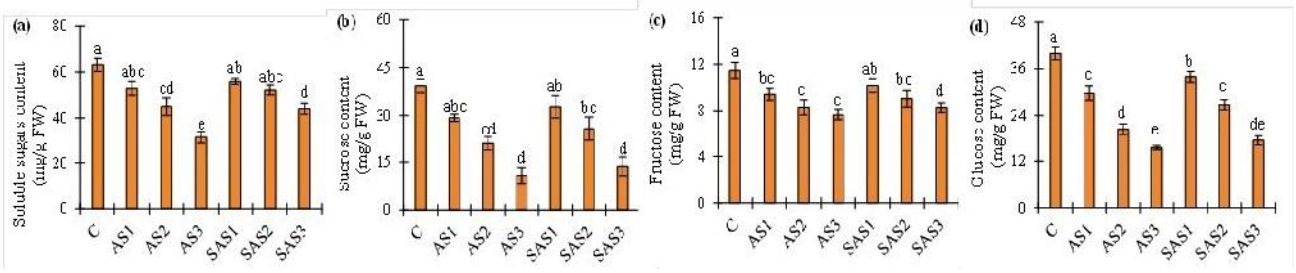

Fig. 5. Effects of alkaline and salt-alkaline stresses on soluble sugars (a), sucrose (b), fructose (c), and glucose (d) contents of shoot in sainfoin. Four-week-old seedlings were treatment with modified Hoagland nutrient solution supplemented without (control, C) or with 50 mM $NaHCO_3$ (alkali stress 1, AS1), 100 mM $NaHCO_3$ (alkali stress 2, AS2), 150 mM $NaHCO_3$ (alkali stress 3, AS3), 25 mM NaCl+25 mM $NaHCO_3$ (salt-alkali stress 1, SAS1), 50 mM NaCl+50 mM $NaHCO_3$ (salt-alkali stress 2, SAS2), and 75 mM NaCl+75 mM $NaHCO_3$ (salt-alkali stress 3, SAS3) for 7 d. Three plants were pooled in each replicate. Values are means ± SE (n = 8) and bars represent SE. Columns with different letters represent significant differences at $P < 0.05$ (Duncan's test). FW - fresh weight.

Table 3. Effects of alkaline and salt-alkaline stresses on Na^+ and K^+ concentrations, and K^+/Na^+ ratio of shoot and root in sainfoin. Four-week-old seedlings were treated with modified Hoagland nutrient solution supplemented without (control, C) or with 50 mM $NaHCO_3$ (alkali stress 1, AS1), 100 mM $NaHCO_3$ (alkali stress 2, AS2), 150 mM $NaHCO_3$ (alkali stress 3, AS3), 25 mM NaCl+25 mM $NaHCO_3$ (salt-alkali stress 1, SAS1), 50 mM NaCl+50 mM $NaHCO_3$ (salt-alkali stress 2, SAS2), and 75 mM NaCl+75 mM $NaHCO_3$ (salt-alkali stress 3, SAS3) for 7 d. Three plants were pooled in each replicate. Values are means ± SE (n = 8). Within each column, means followed by different letters are significantly different at $P < 0.05$ (Duncan's test). DW - dry weight.

Treatment	Shoot			Root		
	K^+ (mmol/g DW)	Na^+ (mmol/g DW)	K^+/Na^+ ratio	K^+ (mmol/g DW)	Na^+ (mmol/g DW)	K^+/Na^+ ratio
C	0.91±0.05 a	0.39±0.09 f	2.84±0.34 a	0.94±0.05 a	0.45±0.09 c	2.33±0.29 a
AS1	0.81±0.03 ab	0.71±0.06 de	1.17±0.10 ab	0.74±0.04 ab	0.69±0.07 bc	1.19±0.21 b
AS2	0.63±0.05 c	1.06±0.07 bc	0.62±0.08 bcd	0.61±0.05 bcd	0.82±0.08 abc	0.79±0.10 bc
AS3	0.41±0.02 d	1.47±0.10 a	0.29±0.02 d	0.34±0.05 d	1.16±0.23 a	0.31±0.02 d
SAS1	0.83±0.04 ab	0.61±0.06 ef	1.41±0.10 ab	0.76±0.04 ab	0.62±0.07 bc	1.19±0.15 b
SAS2	0.66±0.05 bc	0.88±0.06 cd	0.76±0.06 bc	0.63±0.04 bc	0.66±0.05 bc	0.98±0.10 b
SAS3	0.43±0.03 d	1.16±0.10 b	0.38±0.04 cd	0.41±0.03 cd	0.93±0.03 ab	0.45±0.05 cd

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