

Supplementary Material

Integration of seed priming with nano-sized chitosan-proline and biochar application improves salt tolerance in differentially responding genotypes of alfalfa (*Medicago sativa*)

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Table S1. The physiochemical properties of the biochar used in the study

Parameters	Values
pH	9.5
EC (dS m ⁻¹)	1.3
OC (g kg ⁻¹)	68.4
CEC (cmol kg ⁻¹)	20.3
Ca (g kg ⁻¹)	0.0012
Cl (g kg ⁻¹)	1.39
HCO ₃ (g kg ⁻¹)	0.84
Total N (g kg ⁻¹)	5.8
NH ₄ (g kg ⁻¹)	
NO ₃ ⁻ (g kg ⁻¹)	
Total P (g kg ⁻¹)	13.97
Total K (g kg ⁻¹)	1.48
SSA (m ² g ⁻¹)	21.3
BD (g cm ⁻¹)	0.14
Porosity (%)	85

OC= Electrical conductivity; OC=Organic carbon; CEC= Cation exchange capacity; Ca= Calcium; Cl=Chloride; HCO₃= Bicarbonate; SSA= Specific surface area; BD=Bulk density

Table S2. Effect of NsCP seed priming and biochar application on photosynthetic rate, and carboxylation capacity of alfalfa genotypes subjected to salt stress (SP × G)

Seed priming (SP)	Carboxylation capacity (μmol /m ² /s)/(μmol /mol)		
	OMA-285	OMA-84	Mean (SP)
Dry seeds-NBC	0.0324ef	0.0311f	0.0317E
Hydropriming-NBC	0.0406cde	0.0362def	0.0384CD
NP-priming-NBC	0.0494ab	0.0416bcd	0.0455AB
Dry seeds-BC	0.0370c-f	0.0366def	0.0368DE
Hydropriming-BC	0.0451bc	0.0409cd	0.0430BC
NP-priming-BC	0.0539a	0.0430bcd	0.0484A
Mean (G)	0.0431A	0.0382B	
HSD (5%)	SP=0.0051; G= 0.0019; SP × G=0.0084		

BC=Biochar; NBC=No biochar; NP=Seed priming with nano-sized chitosan-proline; SP=Seed priming; G= Genotype; SS=Salt stress

For a given parameter, no significant differences ($p \leq 0.05$) exist among means sharing the same letter, both for interactions and main effects. Lowercase letters indicate comparisons among interaction means, while uppercase letters represent overall means.

Table S3. Effect on Na and Cl concentration of alfalfa genotypes subjected to salt stress (SP × SS)

	Na ⁺ concentration (mg kg ⁻¹)			Cl ⁻ concentration (mg kg ⁻¹)		
	OMA-285	OMA-84	Mean (SS)	OMA-285	OMA-84	Mean (SS)
Control	23.97 d	31.43 b	27.70 B	8.38 d	11.22 b	9.80 B
Salinity	30.20 c	34.77 a	32.48 A	10.73c	12.24 a	11.49 A
Mean (G)	27.09 B	33.10 A		9.56 B	11.73 A	
HSD (5%)	SS=0.43; G= 0.43; SS × G=0.80			SS=0.23; G= 0.23; SS × G=0.43		

For a given parameter, no significant differences ($p \leq 0.05$) exist among means sharing the same letter, both for interactions and main effects. Lowercase letters indicate comparisons among interaction means, while uppercase letters represent overall means.

Table S4. Effect on MDA content of alfalfa genotypes subjected to salt stress (SP × SS)

	MDA		
	OMA-285	OMA-84	Mean (SS)
Control	11.52d	17.78b	14.65B
Salinity	15.21c	23.38a	19.29A
Mean (G)	13.36B	20.58A	
HSD (5%)	SS=0.77; G= 0.77; SS × G=1.44		

For a given parameter, no significant differences ($p \leq 0.05$) exist among means sharing the same letter, both for interactions and main effects. Lowercase letters indicate comparisons among interaction means, while uppercase letters represent overall means.