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Role of native *Trifolium isthmocarpum* populations in saline soils in Morocco

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Abstract. The potential of previously unexploited crop legume species need to be investigated where environmental and biological stresses constrain the use of more conventional forage crops or where these species are better suited to the needs of sustainable agriculture. The present study have recorded *Trifolium isthmocarpum* Brot., in many different habitats ranging from healthy agricultural lands to abandoned saline areas. The plants maintained nitrogenase activities average 2.04 μmol C_2H_4 $plant^{-1}$ h^{-1} in different habitats. Shoot systems of plants collected from salt-affected soils exhibited higher concentrations of Na^+ and Cl^- than those collected from healthy soils, whereas relative water content was reduced by only 8%. This study suggests recommending the cultivation of *T. isthmocarpum* in moderately salt-affected soils, which are widespread and pose a problem for the farmers of Morocco.

Keywords. Mediterranean – Pasture – Crop – Stress.

Rôle des populations locales de* Trifolium *dans les sols salins affectés par le changement climatique

Résumé. *Le potentiel d'espèces de légumineuses cultivées auparavant inexploitées est à étudier dès lors que des contraintes environnementales et biologiques limitent l'utilisation de cultures fourragères plus conventionnelles ou lorsque ces espèces sont mieux adaptées aux besoins d'une agriculture durable. La présente étude a enregistré les caractéristiques de* Trifolium isthmocarpum *Brot. dans de nombreux habitats différents, allant de terres agricoles en bonne santé à des zones salines abandonnées. Les plantes ont maintenu des activités de nitrogénase moyennes de 2,04 μmol de plante* $C_2H_4^{-1}$ h^{-1} *dans différents habitats. Les systèmes de pousses de plantes prélevées dans des sols affectés par le sel présentaient des concentrations de* Na^+ *et de* Cl^- *plus élevées que celles recueillies dans des sols sains, alors que la teneur en eau relative n'était réduite que de 8%. Cette étude suggère de recommander la culture de* T. isthmocarpum *dans des sols modérément affectés par le sel, qui sont répandus et posent un problème aux agriculteurs marocains.*

Mots-clés. *Méditerranée – Pâturage – Culture – Stress.*

I – Introduction

The Mediterranean Basin is feeling the effects of climate change more than ever. Climate change affects agriculture, reduces fertilizer efficiency and increases evapotranspiration and soil salinity. More than 5% of Morocco's land area is already affected by salinization to varying degrees and 20% of irrigated land produces less (Najib *et al.*, 2017). *Trifolium* is one of the most important forage legumes among the genera of the Fabaceae family, both in terms of its agricultural value and the number of species (Sabudak and Guler, 2009). Several species of *Trifolium* are cultivated in intensive agricultural systems in association with companion grass species in simple or complex seeds mixtures (Lamont *et al.*, 2001). *Trifolium isthmocarpum* Brot., Moroccan clover, occurs as a weed in different habitats (Bennani et Bendaou, 2015; Beale *et al.*, 1993). It grows in moderately saline areas, where traditional forage legumes cannot be cultivated Beale *et al.*, 1993); however, it has not been widely studied despite its good palatability.The aim of our study was to examine the performance of *Trifolium isthmocarpum* in different habitats to evaluate its potential for use as a fodder crop in salt-affected soil in Morocco.

II – Materials and methods

1. Choice of habitats and collection of samples

Two different habitats were chosen. Healthy arable soil (including barley fields in coastal land) and salt affected soils (Fig. 1, Table 1). For the vegetation surveys, a simplified method describing species presence was performed for the different habitats. Homogeneous stands (10×10 m²) were selected, 20 in healthy soils and 20 in salt-affected soil.

Fig. 1. Different sampling locations in Morocco.

Table 1. Soil physicochemical characters of two habitats where *T. isthmocarpum* was recorded

	Healthy soil	Salt affected soil
K (mg/kg)	921 ± 26.8	998.5 ± 12
Na (mg/kg)	411 ± 31,2	2476 ± 78
Mg (mg/kg)	671 ± 17.5	1022 ± 24
Ca (mg/kg)	181.2 ± 11.3	198 ± 18
Fe (mg/kg)	26.5 ± 2.2	57.9 ± 1.9
SO_4^{2-} (mg/kg)	2026 ± 11.1	2999 ± 12
Cl (mg/kg)	2441 ± 22.3	4867 ± 15.9
EC (dS/m)	0.66 ± 0.02	6.1 ± 0.7
pH	7.08 ± 0.2	7.5 ± 0.11
Soil texture	Clay loamy	Clay loamy / Sandy
Rainfall average	360 mm	300 mm

2. Nodulation status and nitrogenase activity

The plants collected from each of the different habitats were analysed to determine nodulation percentage and to enumerate nodule number. The nitrogen- fixing activity (nitrogenase activity) of the legume–Rhizobium symbiosis was determined according to the methods described by Witty and Minchin (1988).

3. Chemical analysis and relative water content

Na^+, K^+ and Cl^- were analysed at the end of the experimental period in the shoots of plants. The concentration of Cl^- was measured following titrametric method. The concentrations of K^+ and Na^+ were determined using a flame photometer (Jenway Ltd, model PFP7; Essex, UK). Relative water content was calculated using: RWC = (FM-DM)/ (TM-DM) ×100, fresh mass (FM), fresh mass at full turgor (TM), measured after immersion of leaf petioles in distilled water for 48 h in the dark, and dry mass (DM), measured after oven drying at 70 °C for 24h to constant weight.

4. Recovery of germination

Recovery of germination upon transfer of seeds from NaCl solution to pure water attributes the initial repression of germination and its subsequent recovery to osmotic factors.

After 7 days, ungerminated seeds from the high concentrations of salt treatments (140 mM and 220 mM) were rinsed three times in distilled water and transferred in sterile Petri dishes with two discs of filter paper saturated with distilled water to study recovery of germination for 20 days.

The recovery percentages (RP) was determined by: **RP = [(*a*- *b*}(*c*- *b*)]100**, where ***a*** is the total number of seeds germinated after being transferred to distilled water, ***b*** is the total number of seeds germinated in saline solution, ***c*** is the total number of seeds.

III – Results and discussion

Table 1 shows variations in soil physicochemical properties of the investigated *T. isthmocarpum*

habitats, which ranged from sandy to clay loamy soils, and from healthy (EC = 0.66 dS/m) to salt affected soils (EC = 6.1 dS/m). Salt-affected soil showed higher concentrations of Na^+, Cl^-, Mg^{2+} et SO_4^{2-} than healthy soils by 6, 2, 1.5, and 1.4 fold respectively.

The studied plants showed high nodulation percentages (ranging between 60% and 97%) and nitrogenase activities (average 2.04 μmol C_2H_4 $plant^{-1}$ h^{-1}) at different habitats (Table 2).

Table 2. Nodulation, nitrogenase activity, shoot ion content and protein content of *T. isthmocarpum* collected from different habitats

	Healthy soil	Salt affected soil
Nodule $plant^{-1}$	69 ± 4.2	56 ± 1.8
Nodulation (%)	97 ± 7.2	80 ± 3.6
Nitrogenase activity (mol C_2H_4 $plant^{-1}h^{-1}$)	3.1 ± 0.02	1.00 ± 0.07
Protein (g/kg dry wt.)	267 ± 9.8	318 ± 5.5
Na^+ (mmol/g DM)	1.14 ± 0.01	2.48 ± 0.18
Cl^-(mmol/g DM)	0.68 ± 0.12	1.7 ± 0.09
K^+ (mmol/g DM)	1.87 ± 0.06	1.33 ± 0.05

The highest values of protein content were recorded in plants collected from salt-affected soils . Shoot systems of plants collected from salt affected soils exhibited higher concentrations of Na^+ and Cl^- than those collected from healthy soils by more than two fold, and showed a reduction in K^+ content of about 30% (Table 2). Various authors have mentioned that *T. isthmocarpum* is found in a broad range of landscapes in Morocco and it is known to be found in coastal areas and to tolerate moderately saline clay soils.

The nodulation percentage varied among individuals collected from different habitats. Sulieman (2014) provides a general overview of environmental constraints to nodulation and nitrogen fixation, as indicative of the importance of environmental stresses to rhizobia. This variation can be also explained by the different prevailing environmental conditions. One of the interesting findings in this study was the important nodulation percentage and nitrogenase activity recorded in the *T. isthmocarpum* plants, which gives the species economic importance as it can be used to enhance soil fertility. The lowest nodulation recorded at the salt affected soil, is likely to be due to decreases in population levels of rhizobia in this habitat. Under severe stress, the requirement for certain essential elements, such as calcium and phosphorus, increases and elevated levels of these elements enhance nodulation and N_2 fixation.

Recovery germination (Fig. 2) for all seed types was significantly affected by 220 mM NaCl compared with 140 mM NaCl ($P < 0.001$). The results show that the effects are initially osmotic, due to the recovery of germination once the salt stress has been removed. The ability to recover germinability has implications for seedling establishment in saline environments, particularly when initial rainfall is insufficient to flush salts from the soil surface. In such situations, imbibed seeds may be able to survive until subsequent rains permit completion of germination. However, this mechanism can only play a role in situations where the soil surface remains moist. Observations of delayed germination in highly saline, relative to less saline, areas of *Melilotus siculus* plots (Nichols *et al*., 2010) suggest this mechanism has importance in this species.

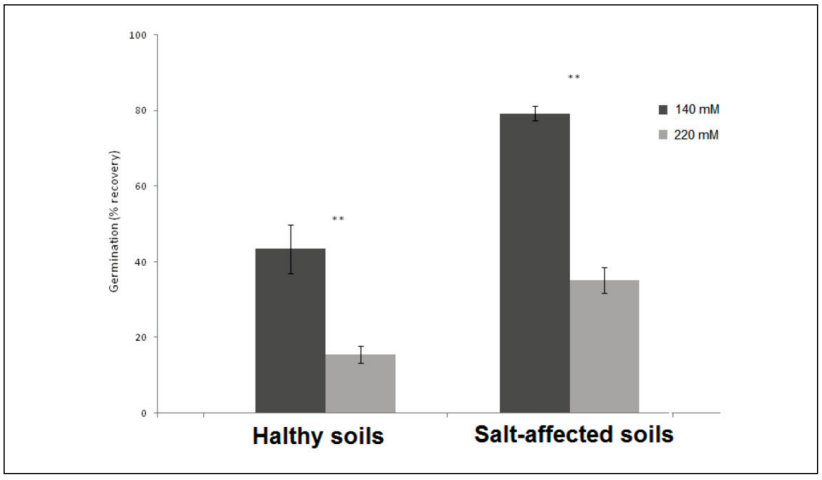

Fig. 2. Percentage of recovery germination, *T. isthmocarpum* seeds, when osmotic stress due to different concentrations of NaCl (140 mM and 220 mM) is alleviated (P < 0.001 according to ANOVA, Tukey test).**

IV – Conclusions

This study suggest recommending the cultivation of *T. isthmocarpum* in moderately salt-affected soils, which are widespread and pose a problem for the farmers of Morocco. However, further work is required to determine the adaptive significance of this trait.

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