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Trifolium isthmocarpum Brot: biosaline agriculture for forage and livestock production

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Abstract. *Trifolium isthmocarpum* Brot. (*Leguminosae)*, occurs in different habitats in Morocco. It grows in moderately saline areas where traditional forage legumes cannot be cultivated. However, its existence has not been widely studied despite its good palatability. The salt tolerance was studied between natural field conditions and greenhouse. The extensive field studies have recorded the species in many different habitats ranging from healthy agricultural lands to abandoned saline areas. The plants maintained high nodulation capacity (ranging between 70% and 97%) and nitrogenise activities (average 2.05 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. Greenhouse experiments showed that germination percentage and vigor value of the studied species was not significantly ($P > 0.05$) affected at 160 mM NaCl, and that 25% of the germination ability was maintained when growing on substrates containing 220 mM NaCl. The growth rate of seedlings was not significantly affected by 160 mM NaCl but was reduced by 38% under 220 mM NaCl. This study recommends the cultivation of *T. isthmocarpum* in salt-affected soils, which are widespread and pose a problem for the farmers of Morocco and other countries in the world's arid belt.

Keywords. Pasture crop – Salt-affected land – *Trifolium isthmocarpum* Brot. – Wild Legumes.

Le* Trifolium isthmocarpum *Brot : culture sur sols salins pour la production de fourrage et l'élevage

Résumé. *L'espèce* Trifolium isthmocarpum *Brot., est une légumineuse présente dans différents habitats au Maroc. Elle pousse dans les zones modérément salines, où les légumineuses fourragères traditionnelles ne peuvent pas être cultivées. Cependant, elle n'a pas été largement étudiée malgré sa bonne appétence. La tolérance au sel a été étudiée dans les conditions naturelles et en serre. Des études de terrain ont recensé l'espèce sur différents habitats, allant de terres agricoles saines aux zones salines. Sur les habitats étudiés, l'espèce a maintenu une capacité de nodulation élevée (entre 70% et 97%) ainsi que son activité de nitrogénase (en moyenne 2,05 μmol C_2H_4 $plant^{-1}$ h^{-1}). Les plantes prélevées dans les sols salins présentent des concentrations plus élevées en Na^+ et Cl^- que celles prélevées dans les sols non salins. Les expériences en serre ont montré que le pourcentage de germination et la vigueur de l'espèce étudiée n'ont pas été significativement ($P > 0,05$) affectées à 160 mM de NaCl, et que 25% de la capacité de germination a été maintenue lors de la croissance sur des substrats contenant 220 mM de NaCl. Le taux de croissance n'a pas été significativement affecté par 160 mM de NaCl. Toutefois, il a été réduit de 38% sur 220 mM de NaCl. Cette étude suggère de recommander la culture de* T. isthmocarpum *sur des sols salins, qui sont largement répandus et posent un problème pour les agriculteurs du Maroc ainsi que d'autres pays des zones arides.*

Mots-clés. *Pâturage – Terres salinisées –* Trifolium isthmocarpum *Brot. – Légumineuse sauvage*

I – Introduction

Morocco is one of the countries which is seriously affected by salinity; 33% of its cultivated land is salinized (Cherkaoui *et al*., 2007). However, an understanding of the range of salinity that various legumes can tolerate is central to their use in programs for re-vegetation of saline lands (Behdani *et al*., 2008). The present study focused on salt tolerance of annual legumes, which can

offer economic and environmental benefits. Moreover, they avoid salinity concentrations which reach the peak in summer and autumn in Mediterranean environment. *Trifolium* is a leguminous genus, characterized by high seed yields, high nitrogen fixation rates, and important value in crop rotations. Generally, *Trifolium* species are recognised as being salt-sensitive. However, research undertaken on a limited number of species (Gibberd *et al*., 2001) suggests that species do vary in their response and that further research, may be beneficial in identifying species, that are suited to mild or moderate saline. *Trifolium isthmocarpum* Brot (annual clover) is one of these clovers that have not been researched largely. It is grown in moderately saline areas where traditional forage legumes cannot be grown in different habitats in Morocco. Few authors mentioned *T. isthmocarpum* in their studies and generally occurred in laboratory. For example, Rogers and West (1993) noted the superior tolerance of *T. isthmocarpum* compared to *T. subterraneum* L. and *T. purpureum* Loisel. However, salt tolerance may differ between laboratory or greenhouse and natural field conditions owing to the complex interaction of a number of edaphic and climatic factors. The present work investigates the performance of *T. isthmocarpum* under both field and laboratory conditions to evaluate its potential for use as a fodder crop in salt-affected soil in Morocco.

II – Materials and methods

The seeds of *T. isthmocarpum* used were from the seed bank of the National Institute of Agronomic Research of Rabat. The study was conducted in two sites: the experimental station Guich in Rabat (34º03' N, 06°46' O) on sandy soil, pH 7.3, organic matter (1.5%), phosphorus (65 ppm) and potassium (159 ppm) (determined by atomic absorption spectrometry Perkin-Elmer Corp., Norwalk, CT, USA). Subhumid bioclimatic domain. Average rainfall is 500 mm. This site was used as a control essay, with unsalted irrigation water: 0.56 dS/m. The second site was Benabid, affected by the problem of salinity, 10.9 dS/m of irrigation water (wells). This site is an agricultural area, subjected to a continental and oceanic influence. It is located at 33°51' N, -07°81'on sandy loam soil, pH 7.5, moderately rich in organic matter (2.28%), with sufficient phosphorus content (222.54 ppm) and potassium (386.5 ppm). The average annual rainfall was 480 mm when the experiment was carried out (December 8, 2010). The experimental design was a randomized complete block design with three replications, the basic plots are 1 m² each containing 24 plants. The space between the plots was 1 meter and the space between the two blocks was two meters. The irrigation frequency was: 10 days at the beginning of the culture and twice per week from the beginning of the four leaf stage. The plants were analysed to determine nodule number and nodulation percentage. They were determined at the beginning of flowering, from germination at 10% of heading. Nitrogenase activity of the legume-*Rhizobium* symbiosis was determined according to the methods described by Witty and Minchin (1988). The concentrations of soil minerals Na^+, K^+, Fe^{3+}, Ca^{2+}, and Mg^{2+} were determined using a Perkin 403 atomic absorption spectrophotometer (Anghel *et al*., 1999). Cl^- was quantified following titrametric method (Begum *et al*., 1997). The germination rate was measured to determine the ability of the plant to reproduce after its culture under saline conditions. The seeds of *T. isthmocarpum* were placed in sterilized Petri dishes with 5 ml of the treatment solution: 0 (control), 80, 160, and 220 mM of NaCl. Three replicates of 20 seeds were used in each treatment. The germination rate is expressed as the ratio of number of germinated seeds on the total number of seeds (TG = (n / N) x 100) where n: number of germinated seeds, N: total number of seeds placed in germination. Germination speed (vigor value) was calculated using the following formula: V = (a/1 + b/2 + c/3 + d/4 +…+ x/n) 100/S, where a, b, c,…x, respectively, represent the number of seeds that germinated after 1, 2, 3,…n days of incubation, and S is the total number of germinated seeds. The data were analysed using the Statistical Analyses System software. Significant differences between treatment means were determined using LSD test at the 0.05 probability level.

III – Results and discussion

The studied plants showed high nodulation percentages (ranging between 70% and 97%) and nitrogenase activities (average 2.05 µmol C_2H_4 plant^{-1} h^{-1}) at two different habitats (Table 1). The highest values of protein content were recorded in plants collected from salt-affected soil (Benabid). The nodulation percentage varied among individuals collected from different habitats. Giller (2001) 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 finding 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. Shoot systems of plants collected from salt-affected soil exhibited higher concentrations of Na^+ and Cl^- than those collected from healthy soil by more than twofold, and showed a reduction in K^+ content of about 30% (Table 1). Nutrient deficiencies can occur in plants when high concentrations of Na^+ in the soil reduce the amounts of available K^+, Mg^{2+}, and Ca^{2+} (Al-Abdoulhadi, 2012) or when Na^+ displaces membrane-bound Ca^{2+}. In addition, Na^+ may have a direct toxic effect, such as when it interferes with the function of potassium as a cofactor in various reactions. Many of the harmful effects of Na^+, however, seem to be related to the structural and functional integrity of membranes (Hasegawa *et al*., 2000).

Table 1. Average of nodulation, nitrogenase activity, shoot ion content and protein content of *T. isthmocarpum* collected from different habitats. (Five plants per replication)

	Guich site	Benabid site
Nodule plant^{-1}	69 ± 4.2	56 ± 1.8
Nodulation (%)	97 ± 7.2	80 ± 3.6
Nitrogenase activity (µmol C_2H_4 plant^{-1}h^{-1})	3.1 ± 0.02	2.02 ± 0.07
Protein (g/kg dry weight)	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

There is no significant difference on seed yield between the two sites. However, the germination capacity of harvested seeds during the experiment was sensitive to salinity. It was highly dependent ($p < 0.001$) to culture conditions. The ability of seeds native of Benabid, to germinate in NaCl treatment was important than those native of Guich. Germination percentage and vigor value of seeds native of Benabid was not significantly ($P > 0.05$) affected at 80 or 160 mM NaCl, whereas, 26% germination ability was maintained at 220 mM NaCl (Table 2). The selection of species tolerant to salinity needs first to study the behavior of their seeds during germination.

Table 2. Effect of NaCl on germination, vigor value (germination speed) and seed yield of *T. isthmocarpum*

		0 mM	80 mM	160 mM	220 mM	LSD
Guich site	Germination %	100	90	40	26	0.4
	Vigor value	30	28	15	11	0.03
	Seed yield per plant (g)	24.2	19.1	17.2	13.0	0.78
Benabid site	Germination %	98	89	78	45	0.01
	Vigor value	30	30	26	15	0.26
	Seed yield per plant (g)	24.5	22.2	18.4	16.1	0.15

The high germination percentage and vigor value (germination speed) of *T. isthmocarpum* recorded under salinity treatment is a very important character from the ecological point of view. Nichols *et al*. (2008) suggest that annual pasture legumes adapted to saline environments must have high salinity tolerance as seedlings or mechanisms to avoid germination at times of high salinity. The ability to germinate and establish seedlings on saline land is particularly important for annual pasture legumes, which must repeat this process each year. The seeds were collected from plants living in saline soils, which would be expected to exhibit salt tolerance during the germination stage, as a result of natural selection (Hameed and Ashraf, 2008).

IV – Conclusions

The high ability of the studied species to germinate, grow, and fix nitrogen under salt stress in both field and laboratory studies recommends its cultivation as a fodder crop and as a soil amelioration plant on salt-affected soils. More long-term studies with a wider taxonomic base would be needed to reach general conclusions on the natural selection in response to salinity.

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