COLD HARDINESS VARIATION IN ANNUAL *MEDICAGO* SPECIES AT THE SEEDLING STAGE

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ABSTRACT
The study was carried out on three annual *Medicago* species (*M. polymorpha, M. aculeata* and *M. ciliaris*) each represented by five populations. Cold tolerance of the accessions was tested using a laboratory chilling test on seedling growth and soluble protein content of acclimated and non-acclimated seedlings. Lots of 3-d-old seedlings were submitted to cold treatment for 48 and 96 hours. For each treatment, response was determined by measuring seedlings' weight and height, and radicle length for both acclimated and non-acclimated seedling lots. Seedling growth was adversely affected by low temperature, and resistant cultivars had a better rate of seedling growth than sensitive cultivars. *M. aculeata* genotypes were more resistant than those of *M. polymorpha* and *M. sativa* cv. Orca, and *M. ciliaris* populations were intermediate.

KEY WORDS
Annual *Medicago*, variability, cold hardiness, protein content.

INTRODUCTION
Improving breeding pastures constitutes one of the important tasks in North-Africa. The rotation of temporary prairies-cereal crops, involves the availability of adapted auto-generating forage legumes (Cremer-Bach,1992). Local annual *Medicago* species present, in proportion to commercial cultivars, a good adaption to local conditions (Francis, 1989).

PLANT MATERIAL AND METHODS
Fifteen ecotypes of annual *Medicago* species originating from different geographical sites (Table 1) and cultivar from *M. sativa* were used for evaluating temperature effect on seedling growth. Ciliaris 80, Aculeata 80, and Polymorpha Tah were used as control. Ten seeds for each ecotype and each repetition were germinated at 19°C in Petri dishes (12 cm diameter) containing compost imbibed with distilled water. When the seedlings were 3 d-old they were divided into two lots. Cold treatment was performed by placing the seedlings at 4°C (acclimated lot) for 48 or 96 h. Non-treated control seedlings were kept at 19°C (non-acclimated lot). The Petri dishes were arranged in a randomized complete block design with 10 replications.

Seedlings growth measurement. For each treatment, (48 and 96 h) response was determined by measuring seedlings' weight and height, and radicle length for both acclimated and non-acclimated seedling lots.

Protein extraction. All steps of protein extraction were carried out on ice-trays. Five seedlings per lot (from acclimated and non-acclimated ones) were homogenized in buffer containing 25 mM Tris-Hcl (pH 6.8), 10 mM Kcl, 20 mM $Mgcl_2$, glycerol 10%, ß-mercaptoethanol 5%, SDS 2%, EDTA 50 mM and insoluble PVP 0,5% (w/v) (Mohapatra *et al.,* 1987 modified). The homogeneate was centrifuged for 30 min at 14 000 trs/min to obtain supernatant expected to contain soluble proteins. Aliquots of this supernatant were used to determine protein concentration by Lowry method (1951) with BSA as standard.

RESULTS AND DISCUSSION
Seedling growth. Differences in seedling weight, seedling length and radicle length between acclimated and non-acclimated lots were significant at the two durations of treatment time. The range in growth among the 16 populations at low temperatures provides evidence of wide genetic variability for all characters. Studies show that seedling growth is also adversely affected by low temperature, and that resistant ecotypes have a better rate of seedling growth than sensitive cultivars (Maheswaran and Subramanian, 1989; Nykiforuk and Johnson-Flangan, 1994). In our study it is apparent that *M. aculeata* ecotypes were more resistant than those of *M. polymorpha* and *M. sativa* cv. Orca, whereas *M. ciliaris* populations were intermediate. The relation between high altitude origin and ecotype resistance found by Bounejmate *et al.* (1993) is clearly established in this work.

Protein content. Many authors demonstrated that protein content increase during cold acclimatation within resistant cultivars (Mohapatra *et al.,* 1987; Perras and Sarhan, 1989; Monroy *et al.,* 1993). Our results showed that the protein content seems to have a tendency to increase with increases in ecotype resistance (Aculeata 212, Aculeata 209, and Aculeata 203 for example) but this increase was not statistically significant. However, it was significant for Ciliaris 11, Aculeata 80 Polymorpha 46 and Polymorpha 226 at 46 hrs and Ciliaris 80 at 96 h.

CONCLUSION
The relationships between cold tolerance and seedling growth characters could provide useful markers for selection. Accessions displaying a high rate of seedling growth represent a promising source for breeding cold tolerant ecotypes. Whereas protein content variation as response to cold tolerance require perhaps much more time of acclimation to appear.

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Table 1
Ecotypes studied, origin and altitude of their sites of collection.

Species	Ecotypes	Origin	Site altitude(m)
M.ciliaris	ciliaris 02	Algeria	810
	ciliaris 11	Algeria	80
	ciliaris 13 C	Algeria	370
	ciliaris 56	Algeria	565
	ciliaris 80	Syria	-
M.polymorpha	polymorpha 46	Algeria	700
	polymorpha 22	Algeria	1120
	polymorpha 214	Algeria	1100
	polymorpha 226	Algeria	450
	polymorpha Tah	Syria	-
M.aculeata	aculeata 212	Algeria	1050
	aculeata 203	Algeria	710
	aculeata 209	Algeria	500
	aculeata 231	Algeria	910
	aculeata 80	Syria	-
M.sativa	cv. Orca	France	-

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