Peroxidase responses of two contrasting Medicago ciliaris populations to cold stress in aerial and root systems

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Peroxidase responses of two contrasting *Medicago ciliaris* populations to cold stress in aerial and root systems

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Abstract. Effect of cold on antioxidant responses in aerial (shoots and leaves) and roots tissues of two contrasting accessions of *Medicago ciliaris* Krockers (Cil 126, tolerant and Cil 123, sensible) seedling were investigated. Ten-day-old grown seedlings were subjected at different periods of cold acclimation (4 °C) 2, 4, and 6 days. Peroxidase (EC 1.11.1.7) (POD) activities and isoenzymes expression of plantlets treated and control (23 °C) of the aerial (shoot + leaves) and root systems were carried. Global peroxidase activity under low temperature stress was higher in tolerant ecotype than sensible one. The same trend was also observed at expressed isoenzymes. On the other hand, whether in tolerant or sensitive, activity and isoenzymes peroxidase was more pronounced at the root than in aerial system.

Keywords. Peroxidase activity – Isoenzymes – Cold stress – *M. ciliaris.*

Activité antioxydante des peroxydases sous stress de froid au niveau du système aérien et racinaire chez deux populations de* M. ciliaris *Krockers

Résumé. *L'étude a porté sur l'activité antioxydante sous stress de froid au niveau des tissus aérien (feuille et tige) et racinaire chez deux populations de* M. ciliaris *Krockers (Cil 126, tolérant et Cil 123, sensible au froid). Des plantules âgées de 10 jours sont soumises à différentes périodes de stress de froid (4 °C) de 2, 4, et 6 jours. L'activité des peroxydases ainsi que l'expression isoenzymatique au niveau des parties aériennes et racinaires des jeunes plantes traitées et de leurs témoins respectifs ont été analysées. L'activité globale sous basse température est plus prononcée chez l'écotype tolérant Cil 126 que chez le sensible. L'expression isoenzymatique est plus importante au niveau du sytème racinaire qu'au niveau aérien (feuille + tige).*

Mots-clés. *Activité des peroxydases – Isoenzymes – Stress de froid* – M. ciliaris.

I – Introduction

Cold stress is an environmental factor limiting the geographic distribution of plants and crop production. Cold also leads to excessive production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and other dangerous derivatives oxygen, which cause progressive oxidative damage and as consequence cell death (Mohammadian *et al.,* 2012). During cold acclimation, increases of enzymatic changes were associated with cold tolerance by a significant capture of these harmful molecules. Antioxidant enzyme activities in plants are accepted as a good indicator of tolerance under stress conditions (Öklen *et al.,* 2008). Wang *et al.* (2009) suggested that peroxidase (POD) activity in normal conditions in two alfalfa cultivars was higher in root than in shoots. However, POD activities in shoot and root tissues of both cultivars showed similar level of under chilling stress. Keshavkant and Naithani (2001) reported that aerial parts of the chilling sensitive young sal (*Shorea robusta*) seedlings showed overproduction of reactive oxygen species. In order to determine the peroxidase response under cold stress during germination, we measured the enzymatic activity and peroxidase isoenzymatique pattern in aerial and root system of two *Medicago ciliaris* accessions contrasting in their cold tolerance.

II – Materials and methods

The study was carried on two annual *Medicago ciliaris* Krockers (Cil 126, tolerant and Cil 123, sensible) (Table 1). Ten seeds for each accession were germinated after scarification and disinfected by dipping in 70% (v/v) ethanol, at temperature room in Petri dishes containing universal compost imbibed with distiller water. At three days growth stage, seedlings were divided into two lots. Cold treated lot at 4 °C for three durations 2, 4 and 6 days (T2, T4 and T6) and control lot kept at 23 °C (T02, T04 and T06). The experimental layout was a completely randomized block design with 3 replications.

Aerial (shoot and leaves) and root tissues from control and treated plants were homogenized in homogenization buffer [10 mM Tris-KCl, pH 6.8, 10 % (w/v) saccharose and 1 mM PMSF]. Tissues were frozen in liquid nitrogen and ground in mortar on ice using homogenization buffer.

Peroxidase activity (EC 1.11.1.7) was assayed in reaction solution (3 ml) containing 0.2 M sodium acetate buffer pH 4.6, 1% o-dianizidine, 10 % H_2O_2 (Mac Adam *et a*l., 1992) modified. The reaction was started by adding 10 µl crude extract, and the enzyme activity is monitored for every 15 seconds for 3 minutes using a spectrophotometer at 460 as o-dianizidine oxidation, with 3 replications. Enzyme activity was expressed as µmol. of o-dianizidine oxidation. min^{-1}. g^{-1} of fresh matter.

The aerial (shoot and leaves) and root tissues peroxidase pattern were resolved by native in 10% separating polyacrylamide gel electrophoresis on vertical slab gel (Hoffer, USA) using the procedure of Laemmli (1970). Equal amounts of proteins (15 µl) were loaded on to each lane. The gel electrophoresis experiment was repeated tree times. To determine the pattern of peroxidase isoforms gels were staining and visualized by immersing the gels in 100 ml 0.2 M acetate buffer pH 4.6 added with 1% o-dianizidine (Sigma), dissolved in 2 ml 95% ethanol, and 200 µl 3% H_2O_2 at room temperature until the brown color appeared. Scanned Isozymes profiles gels were analyzed by Software GELANALYZER (Istvan Lazar Hungary Copyright 2010) to determine Rfs bands.

Table 1. Accessions analyzed for peroxidase antioxidant under cold stress with their origin and ecological description

Species	Accessions	Origin	Latitude	Longitude
M. ciliaris Krocker.	Cil 123	Algeria	36°46'02''N	8° 18' 9.57'' E
	Cil 126	Algeria	36° 28' 0'' N	7° 26' 0'' E

III – Results and discussion

Chilling tolerance or sensitivity in plants is well correlated with inherent antioxidant responses. Tolerant plant species generally have a better capacity to protect themselves from chilling-induced oxidative stress, via the enhancement of antioxidant enzyme activity. Liu *et al*. (2013) have observed an enhancement in activities peroxidase synthesis pathway during cold acclimation. In this study, we observed Increasing of patterns of antioxidant enzyme activities in both aerial and root system under cold stress comparing with control in tolerant and sensible ecotypes. Whereas, under these activities were more pronounced in root than in aerial (leaves and shoots) tissues (Fig. 1). The result was in accordance with study of Wang *et al*. (2009). Under cold stress the activities was better in root than in aerial tissues. In contrast, at 6 days treatment, Cil 126 exhibit slightly increased activity aerial system (Fig. 1B) than in root tissue (Fig. 1A).

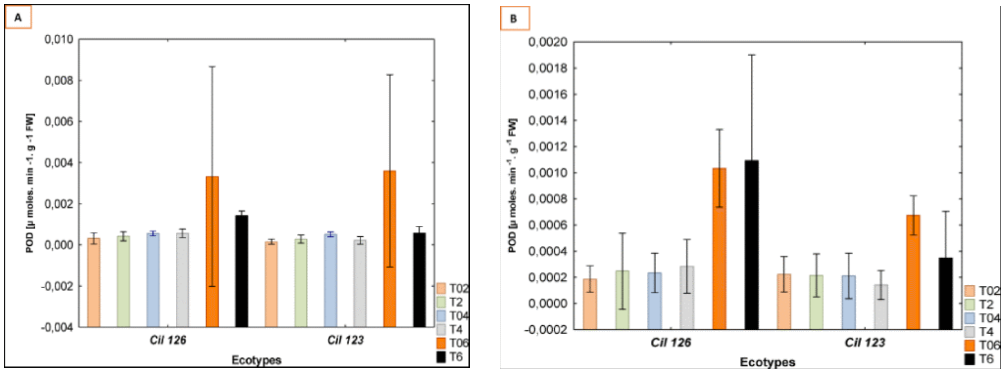

Fig 1. Peroxidase activity in *M. ciliaris* Krockers. (Cil 126 and Cil 123) seedlings untreated (controls: T02, T04, and T06) and treated with different durations (T2, T4, and T6) under cold stress. A: Root system. B: aerial system (shoot and leafs). Data are shown as mean ± SD of three independent measurement.

Comparing the peroxidase profiles between control plants and those treated under cold stress using native-page, the pattern of isoenzyme showed that cold treatment induced a high peroxidase activity. This activity increase slightly in aerial tissues (Fig. 1B) than in root system (Fig. 1A) in both ecotypes. The peroxidase activities were more pronounced when cold stress is maintained in the time. This trend was observed in tolerant accession Cil 126 when plantlets were subjected at 6 days of stress. In contrast, in sensible accession Cil 123 peroxidase activities were slightly maintained (Fig 1A and B). The same results were observed in chickpea (*Cicer orientum* L.) (Nazari *et al.,* 2012). It was found that the number of bands (named POD – Rfs) was higher (five) in tolerant than in sensible (four) ecotype in sensible population. Our results revealed that under cold treatment the intensity of bands were more pronounced in roots than in aerial tissues. As showed in Fig. 2 A the intensity of POD 5-Rf_0.89 of Cil 126 and in Fig. 3 A POD 4-Rf_0.69 of Cil 123 in root was increased in cold treated plants in comparison of aerial tissues in both tolerant and sensible accession.

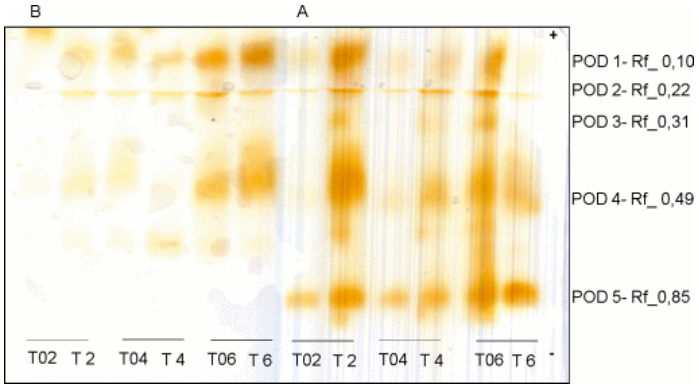

Fig 2. Peroxidase isoenzyme patterns in *M. ciliaris* Krockers. (Cil 126) seedlings untreated (controls: T02, T04, and T06) and treated with different durations (T2, T4, and T6) under cold stress. A: Root system. B: shoot system.

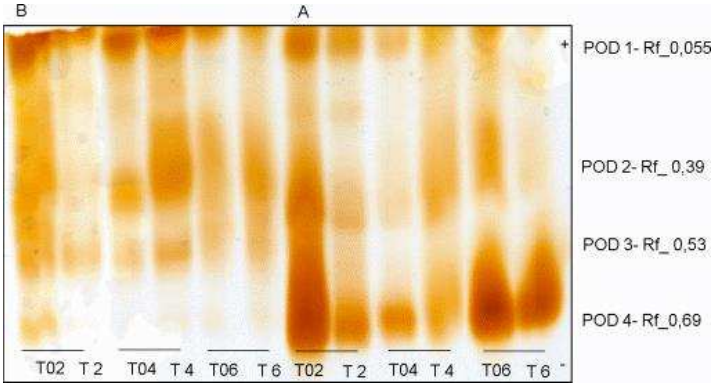

Fig 3. Peroxidase isoenzyme patterns in *M. ciliaris* Krockers. (Cil 123) seedlings untreated (controls: T02, T04, and T06) and treated with different durations (T2, T4, and T6) under cold stress. A: Root system. B: shoot system.

IV- Conclusion

Based on obtained results, it could be concluded that activities of peroxidase had different change trends with tolerant and sensible accession. The enhanced of scavening ability for H_2O_2 in tolerant was better than in sensible accession. Two accessions Cil 126 and Cil 123 has potentially better antioxidant potential in root tissues than in aerial tissues.

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