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Physiological behaviour of four rapeseed cultivar (*Brassica napus* L.) submitted to metal stress

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Abstract

Eliminating heavy metals in the environment by phytoremediation is a method that uses, generally, plants with a low biomass yielded and feeble depth of root system. For the purpose of improving this technique, we have tested four varieties of productive specie with high yields, the rapeseed (*Brassica napus* L.). In particular, we have studied metal stress effect on biomass, growth, and endogenous Zn and Cd content. Metal treatment caused significant dry weight differences between metal-treated and control plants. A significant genotypic difference has been noticed between the four cv. For two varieties, Jumbo and Drakkar, the accumulation is more important in the stems and petioles, whereas, this accumulation is at a maximum level in the root system for the two varieties, Cossair and Pactol. Chlorophyll and carotenoïd content, as well as lipid peroxidation, known as stress markers, were also evaluated. Metal treatment led to an increase in the amount of malondialdehyde (MDA) in the leaves. However, the increase of Zn and Cd levels in the tissue culture was followed by a decrease in the photosynthetic pigments. ***To cite this article: A. Ben Ghnaya et al., C. R. Biologies 332 (2009).***

Résumé

Comportement physiologique de quatre variétés du colza (*Brassica napus* L.) soumises au stress métallique. L'élimination des métaux toxiques dans l'environnement par phytoremédiation est une méthode qui utilise en général, des plantes de faible biomasse avec un système racinaire de faible profondeur. Dans le but d'améliorer cette technique, on a testé quatre variétés d'une espèce productrice d'une forte biomasse, le colza (*Brassica napus* L.). En particulier, on a étudié l'effet de stress métallique sur la biomasse, la croissance et sur la teneur du Zn et du Cd. Une différence significative a été observée entre les variétés. Pour les deux variétés Jumbo et Drakkar, l'accumulation est la plus importante dans les tiges et les pétioles alors que cette accumulation est maximale dans les racines pour les deux variétés, Cossair et Pactol. Les teneurs en chlorophylle, en caroténoïdes et en malondialdehyde connus comme marqueurs de stress ont été également évaluées. Les métaux entraînent une augmentation de la teneur

Abbreviations: cultivar: cv; ANOVA: analysis of variance; $A\lambda$: absorbance at λ (nm); C_a: chlorophyll a; C_b: chlorophyll b; C_{a+b}: total chlorophyll; C_{x+c}: carotenoïds; HM: Heavy metal; MDA: malondialdehyde; PAR: photosynthetically active radiations.

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en malondialdehyde dans les feuilles. Tandis que l'augmentation de la teneur en zinc et en cadmium dans les tissus entraîne une diminution de la teneur en pigments photosynthétiques. ***Pour citer cet article : A. Ben Ghnaya et al., C. R. Biologies 332 (2009).***

Keywords: Soil; Zinc; Cadmium; Phytoremediation; Pigments; Lipid peroxidation

Mots-clés : Sol ; Zinc ; Cadmium ; Phytoremédiation ; Pigments ; Peroxidation des lipides

1. Introduction

Soil contamination due to human activity is a major concern throughout the world, particularly in the case of soils surrounding special activities such as mines and heavy industry. As a consequence, underground water pollution and contamination of agriculture products are so harmful that measures must be taken to fight this phenomenon. In Tunisia, soils around open-cast mining exhibit a very high content of Pb, Zn, and Cd [1]. The average content of Pb, Zn, and Cd detected in the plants of these mines was, respectively, 0.7%; 0.9% and 0.003% [2]. These metals may have an influence on the physical, chemical, and biological environment of the plants. Therefore, there is an urgent need to decontaminate these polluted sites.

Decontamination of polluted sites is a complex problem which may be solved through the use of expensive physicochemical methods which often produce sterile residues [3]. Consequently, their use is limited to the most contaminated soils. The search for alternative methods has since then attracted more interest and has led to the development of new approaches such as phytoremediation [4].

Phytoremediation is based on the ability of plants grown on a soil to take up minerals in general and metals in particular. Indeed, some plants are known to have a high capacity to tolerate and accumulate metals, concentrate them in their roots, and finally translocate them to the aerial parts [5]. Although most of the tolerant plants accumulate heavy metals in their root systems and only a minimal proportion reaches the aerial parts, the hyperaccumulating plants can store 10 to 500 times more metals in their stems and leaves than normal species [6]. Phytoextraction relies on this potential of hyperaccumulators. So far, most of the research carried out has focused on one hyperaccumulator species *Thlaspi caerulescens*. Most investigations aimed at understanding the mechanisms of heavy metal absorption [7,8], their translocation [9], and their storage [10,11]. However, the biomass of *T. caerulescens* is reduced and its root system is superficial. Other plant species may also be of interest, especially to study the physiological mechanisms associated with metal hyperaccumulation, these hyperaccumulators must then be domesticated (adaptation to soils, climate, nature of the contamination, and genetic improvement of the wild species).

Therefore, the present study was designed to understand the physiology of *Brassica napus* L., a plant with an important biomass and a deep root system, known for its capacity to tolerate metals. The ability of four genotypes of rapeseed to extract Zn and Cd and the effect of the metals on chlorophyll, caretonoid and malondialdehyde (MDA) contents were investigated.

2. Material and methods

2.1. Plant material

Four *Brassica napus* L. cultivars were used in this study. Seeds of cv. Drakkar, Cossair and Pactol were graciously provided by the Institut National de Recherches Agronomiques de Tunis (INRAT, Tunis, Tunisia) while those of cv. Jumbo were obtained from Institut National de la Recherche Agronomique (INRA, Rennes, France).

Seeds were surface-sterilized in 70% ethanol for 30 s, followed by immersion in calcium hypochlorite (5% w/v) during 30 min with two drops of Tween-20. The seeds were rinsed 3 times with sterile water, sown in plastic pots containing sterile compost (Twenty seeds per pot) and allowed to germinate in the greenhouse at 23 °C with 80% relative humidity and a 16:8 photoperiod at 50 μmol PAR. $m^{-2}\,s^{-1}$. Plants were water every day alternatively with distilled water or Hoagland's nutrient solution [12]. After 15 days seedlings were thinned to five plants per pot and watered daily with Hoagland's solution. Plants were further cultivated for approximately one month and were used for experiments as they had 16 leaves.

2.2. Metal treatments

Plants were submitted to metal by adding zinc sulphate ($ZnSO_4$, 2000 μM) or cadmium chloride ($CdCl_2$, 250 μM) to the nutrient solution and watering three consecutive days with this solution. Every sample was repeated 6 times. One day later, plants were harvested; the