Available online at www.sciencedirect.com
ScienceDirect
C. R. Biologies 332 (2009) 363–370
COMPTES RENDUS BIOLOGIES

Physiology / Physiologie

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

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Received 30 August 2008; accepted after revision 3 December 2008

Available online 29 January 2009

Presented by Philippe Morat

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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1631-0691/$ – see front matter
doi:10.1016/j.crvi.2008.12.001

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

5 plants from each pot were pooled and used for analysis of metal or pigment contents (plants from 3 pots for each type of analysis).

2.3. Metal accumulation

The plants were harvested three days after the watering with the metallic solutions ($ZnSO_4$) and ($CdCl_2$). These plants were uprooted from the pots with the help of fine jet of water, causing minimum damage to the roots, washed thoroughly with running deionized water (the roots were rinsed three times in 500 ml of deionized water), and dried. Different plant parts were separated in limbs (L); petioles and stems (S + P); and roots (R). They were, manually cut in small pieces, dried in filter papers and the fresh weight (FW) of different these organs measured. The various organs were then heated at 80 °C for three days to determine dry weight (DW) of each organ, before crushing and storage in small flasks for the extraction of zinc and cadmium.

The metal content was determined using Electrothermal Atomic Absorption Spectrometry using a SIMAA 6100 Atomic Absorption Spectrometer (PerkinElmer) and the programme recommended by the manufacturer. To this effect, 200 mg of dried plant material was digested by a mixture of HNO_3, HF and H_2O_2 (4 ml/1 ml/3 ml) until dryness at 100 °C followed by 3 hours in 4 ml HNO_3. The results were expressed in $\mu g\, g^{-1}$ DW.

2.4. Estimation of photosynthetic pigments

The pigments were extracted from leaf discs in 80% acetone (three limbs of three different plants from each cultivar were randomly selected). The different extracts were then centrifuged at 5000 tours/min/rotor during 10 min at 4 °C. The chlorophyll and carotenoïd contents were determined using the following equations [13]:

$$C_a = 12.25A663 - 2.79A645 \ (\text{mg L}^{-1}\ \text{FW}),$$

$$C_b = 21.50A645 - 5.10A663 \ (\text{mg L}^{-1}\ \text{FW}),$$

$$C_{a+b} = 7.15A663 + 18.71A645 \ (\text{mg L}^{-1}\ \text{FW}),$$

$$C_{x+c} = \frac{1000A470 - 1.82C_a - 85.02C_b}{198} \ (\text{mg L}^{-1}\ \text{FW}),$$

where C_a = chlorophyll a; C_b = chlorophyll b; C_{a+b} = total chlorophyll; C_{x+c} = carotenoïds A_x = absorbance at x (nm).

2.5. Estimation of lipid peroxidation (MDA)

Lipid peroxidation of the control and treated plants was estimated by spectrophotometric determination of malondialdehyde (MDA) [14,15]. About 1 g fresh leaf tissue was homogenized with 6 ml of 5% metaphosphoric acid and 120 µl of 2% butyl hydroxytoluene (in ethanol). After 30 min at 4 °C, homogenate was centrifuged at 5000 rpm for 20 min. The chromogen was formed by mixing 4 ml supernatant with 400 µl 2% butyl hydroxytoluene, 2 ml of 1% (w/v) thiobarbituric acid (in 50 mM NaOH) and 2 ml of 25% HCl. The reaction mixture was heated for 30 min at 95 °C and then cooled in ice rapidly. The chromogen was extracted by adding 1.5 ml of 1-butanol. After 30 s vortexing, the organic phase was separated by centrifugation (5000 rpm, 5 min) and the thiobarbituric acid reactive-substances (TBARS) determined by measuring the absorbance at 532 nm. The concentration of TBARS was calculated by using the extinction coefficient of 155 $mM^{-1}\ cm^{-1}$.

2.6. Statistical analysis

The whole experiment was set up at random. To confirm the variability of data and validity of results, all the data were subjected to an analysis of variance (ANOVA) and the differences among the averages (5% level of significance) were tested by Duncan's Multiple Range Test using StatGraphics Plus 5.1.

3. Results

3.1. Plant growth

A significant decrease of the FW and the DW was noted for all organs of Drakkar cultivar, Cossair, and Pactol. In most cases, the Cd had a more depressing effect than the Zn (Table 1). Only Jumbo cultivar showed a significant increase in both FW and DW for zinc treatment (Table 1) while with Cadmium, the FW and the DW of all organs of Jumbo cultivar decreased significantly (Table 1).

3.2. Cadmium and Zinc accumulation

The initial results of metal accumulation showed that Cd and Zn accumulation varied according to the organ and the cultivar (Tables 2 and 3).

3.2.1. Cadmium accumulation

The Cd concentration was more important for the plants treated with the solution of Cadmium chloride than for the untreated control plants whatever the variety and the organ.

Table 1
Effect of $ZnSO_4$ and $CdCl_2$ treatments on plant growth in roots, stems and petioles and limbs for four cultivars of *Brassica napus* L.

Cultivars	Metal concentration (μM)	Plant growth					
		Fresh weight (g)			Dry weight (g)		
		L	S + P	R	L	S + P	R
Jumbo	0	9.31^b	8.02^b	5.29^b	0.74^b	0.90^b	0.32^b
	$ZnSO_4$ (2000 μM)	15.50^a	13.39^a	6.94^a	1.28^a	1.38^a	0.60^a
	$CdCl_2$ (250 μM)	6.11^c	6.05^c	2.81^c	0.54^c	0.77^c	0.21^b
Drakkar	0	17.63^a	18.94^a	6.53^a	1.87^a	1.72^a	0.86^a
	$ZnSO_4$ (2000 μM)	14.33^b	13.38^b	5.54^a	1.48^b	1.30^b	0.51^b
	$CdCl_2$ (250 μM)	10.93^c	9.56^c	3.43^b	1.26^c	0.89^c	0.20^c
Cossair	0	14.85^a	13.94^a	5.39^a	2.91^a	1.95^a	0.77^a
	$ZnSO_4$ (2000 μM)	10.09^b	9.46^b	4.01^b	1.82^b	1.03^b	0.48^b
	$CdCl_2$ (250 μM)	8.11^c	6.37^c	2.1^c	1.18^c	0.91^c	0.12^c
Pactol	0	13.28^a	12.6^a	4.91^a	2.20^a	1.82^a	0.67^a
	$ZnSO_4$ (2000 μM)	11.19^b	10.72^b	3.65^b	1.46^b	1.05^b	0.40^b
	$CdCl_2$ (250 μM)	8.64^c	7.91^c	1.1^c	1.01^c	0.74^c	0.11^c

L: limbs, S + P: stems and petioles; R: roots.
The results were calculated from three replicated experiments. For each variety, and in each column, the values with different letters are significantly different according to ANOVA and Duncan's test at the level of 5%.

Table 2
Effect of $CdCl_2$ treatment on accumulation of cadmium in roots, stems and petioles and limbs for four cultivars of *Brassica napus* L.

Cultivars	$CdCl_2$ (μM)	Cd accumulation ($\mu g\,g^{-1}$ DW)		
		L	S + P	R
Jumbo	0	0.125^b	0.124^b	0.22^b
	250	8.02^a	64.75^a	44.5^a
Drakkar	0	0.2^b	0.175^b	0.085^b
	250	5.45^a	51.77^a	41.05^a
Cossair	0	0.085^b	0.075^b	0.19^b
	250	8.27^a	17.77^a	31.25^a
Pactol	0	0.055^b	0.06^b	0.155^b
	250	7.04^a	20.05^a	34.97^a

L: limbs, S + P: stems and petioles; R: roots.
The results were calculated from three replicated experiments. For each variety, and in each column, the values with different letters are significantly different according to ANOVA and Duncan's test at the level of 5%.

Table 3
Effect of $ZnSO_4$ treatment on accumulation of zinc in roots, stems and petioles and limbs for four cultivars of *Brassica napus* L.

Cultivars	$ZnSO_4$ (μM)	Zn accumulation ($\mu g\,g^{-1}$ DW)		
		L	S + P	R
Jumbo	0	24.8^b	69.6^b	27.4^b
	2000	42.95^a	142.85^a	69.32^a
Drakkar	0	29.75^b	83.15^b	33.5^b
	2000	59.5^a	133.80^a	73.85^a
Cossair	0	20.6^a	31.65^a	54.6^b
	2000	22.25^a	33.9^a	117.52^a
Pactol	0	14.5^b	26.6^a	76.5^b
	2000	27.13^a	30.15^b	107.75^a

L: limbs, S + P: stems and petioles; R: roots.
The results were calculated from three replicated experiments. For each variety, and in each column, the values with different letters are significantly different according to ANOVA and Duncan's test at the level of 5%.

Jumbo variety: The stems and petioles (S + P) accumulated the most Cd (64.75 $\mu g\,g^{-1}$ DW) followed by the roots (R) (44.5 $\mu g\,g^{-1}$ DW) while the limbs (L) displayed the lowest amount of Cd (8.02 $\mu g\,g^{-1}$ DW) (Table 2).

Drakkar variety: As observed for the Jumbo, the most important content of accumulated Cd was measured in the stems and petioles (S + P) (51.77 $\mu g\,g^{-1}$ DW). The roots (R) accumulated almost the same amount as did the roots of Jumbo cultivar (41.05 $\mu g\,g^{-1}$ DW). Also, the Cd level of the limbs was the weakest (5.45 $\mu g\,g^{-1}$ DW) (Table 2).

Cossair variety: The most important content of Cd (31.25 $\mu g\,g^{-1}$ DW) was registered in the roots whereas it was less significant at the level of the stems and petioles (S + P) and limbs (L) with respective contents of 17.75 $\mu g\,g^{-1}$ DW and 8.27 $\mu g\,g^{-1}$ DW (Table 2).

Pactol variety: The stems and petioles accumulated 20.05 μg g^{-1} DW whereas the roots contained 34.97 μg g^{-1} DW. A slight reduction was noted for the Cd content of the limbs in comparison with the Cossair cultivar (7.04 μg g^{-1} DW) (Table 2).

According to these initial results, the amount of Cd accumulated differed according to the organ and the variety of plant. The stems and petioles concentrated most Cd for Jumbo and Drakkar while the roots accumulated the best Cd for Cossair and Pactol (Table 2).

3.2.2. Zinc accumulation

The Zn accumulation was more important for the plants treated with Zinc sulphate solution than for the control plants whatever the variety and the organ (Table 3).

Jumbo variety: The accumulated Zn reached 42.95 μg g^{-1} DW in the limbs of the treated plants against 24.8 μg g^{-1} DW for the untreated control. Stems and petioles of the treated plants accumulated the most important amount of Zinc (142.85 μg g^{-1} DW) whereas the untreated contained 69.6 μg.g^{-1} DW (Table 3). The roots of the treated plants accumulated more than twice the Zn uptake of untreated control (respectively 69.32 μg g^{-1} DW and 27.4 μg g^{-1} DW).

Drakkar variety: An important accumulation in the limbs of the treated plants (59.5 μg g^{-1} DW) was measured representing more than the double of the Zn concentration of the untreated plants (29.75 μg g^{-1} DW) (Table 3). The same trend was observed for roots with 73.85 μg g^{-1} DW for the treated plants and 33.5 μg g^{-1} DW for the untreated ones (Table 3). Like Jumbo, the accumulation of zinc was found maximum in stems and petioles (133.80 μg g^{-1} DW) (Table 3).

Cossair variety: The roots retained the greatest quantity of Zn (117.52 μg g^{-1} DW) which decreased in the stems and petioles (33.9 μg g^{-1} DW) and in the limbs (22.25 μg g^{-1} DW) (Table 3). For the limbs, no significant difference was observed for Zn accumulation between the treated and the control plants with, respectively, 22.25 μg g^{-1} DW and 20.6 μg g^{-1} DW (Table 3). On the other hand, a significant difference was noted between the control plants (54.6 μg g^{-1} DW of Zn) and the treated ones (117.8 μg g^{-1} DW of Zn). For the stems and petioles, this amount was almost the same with 33.9 μg g^{-1} DW for the treated plants and 31.65 μg g^{-1} DW for the untreated ones.

Pactol variety: The roots accumulated 107.75 μg g^{-1} DW whereas the stems and petioles contained 31.15 μg g^{-1} DW (Table 3). The limbs of the treated plants accumulated nearly twice the level of the control plants (27.13 μg g^{-1} DW and 14.5 μg g^{-1} DW, respectively). The stems and petioles of the control plants accumulated less than the treated ones with only 26.6 μg g^{-1} DW.

According to these results, the amount of Zn accumulated differed according to the organ and variety of plant. The stems and petioles showed the most important content of Zn for Jumbo and Drakkar while roots accumulated the most Zn for Cossair and Pactol (Table 3).

3.3. Chlorophyll and carotenoïd analysis

For the four cultivars, the results presented in Table 4 indicates that the chlorophyll *a* content (C_a) decreased from the control to the treated plants. The Cd had a more depressing effect than the Zn. The same trend was noted for the chlorophyll *b* content (C_b) after the treatment with Zinc sulphate and with Cadmium Chloride (Table 4). The carotenoïd content decreased from the control to the treated plants and the Cd was found to have a more depressing effect than the Zn (Table 4). The results confirmed that these cultivars were more sensitive to the stress of Cd then Zn.

3.4. Effect on lipid peroxidation

The application of Zn and Cd treatments to plants, involved a significant increase of MDA compared to the control (Table 5). This increase was observed for the four cultivar. In different cases, Cd was found to have a more depressing effect than Zn (Table 5). The most significant level of MDA content was measured for Jumbo (Table 5).

4. Discussion

The present studies report data on the effect of metallic stress of Zn and Cd on the plants physiological state. In response to metallic stress, the plants reacted by decreasing their FW and DW. In this respect, the Cd had a more depressing effect than the Zn. The inhibition of growth has already been reported in literature for zinc on other species [16]. Jumbo cultivar showed an increase in the FW and DW due to zinc stress but a decrease in biomass with Cd treatment (Table 1). Thus, the influence of the metallic stress depends not only

Table 4
Effect of $CdCl_2$ and $ZnSO_4$ treatment on the pigment content (Chlorophyll and Carotenoid content) for four cultivars of *Brassica napus* L.

Cultivars	Metal concentration (μM)	Pigment limbes (mg g^{-1} FW)			
		Chlorophyll			Carotenoïd
		C_a	C_b	C_{a+b}	C_{x+c}
Jumbo	0	0.747[a]	0.283[a]	1.03[a]	0.217[a]
	$ZnSO_4$ (2000 μM)	0.614[b]	0.181[b]	0.796[b]	0.167[b]
	$CdCl_2$ (250 μM)	0.512[c]	0.115[c]	0.627[c]	0.127[c]
Drakkar	0	1.017[a]	0.301[a]	1.318[a]	0.264[a]
	$ZnSO_4$ (2000 μM)	0.787[b]	0.200[b]	0.987[b]	0.197[b]
	$CdCl_2$ (250 μM)	0.645[c]	0.141[c]	0.786[c]	0.157[c]
Cossair	0	0.942[a]	0.344[a]	1.285[a]	0.257[a]
	$ZnSO_4$ (2000 μM)	0.704[b]	0.236[b]	0.940[b]	0.188[b]
	$CdCl_2$ (250 μM)	0.404[c]	0.163[c]	0.567[c]	0.113[c]
Pactol	0	0.748[a]	0.360[a]	1.108[a]	0.222[a]
	$ZnSO_4$ (2000 μM)	0.586[b]	0.243[b]	0.829[b]	0.166[b]
	$CdCl_2$ (250 μM)	0.424[c]	0.149[c]	0.573[c]	0.115[c]

C_a = chlorophyll *a*; C_b = chlorophyll *b*; C_{a+b} = total chlorophyll and C_{x+c} = carotenoid. The data were calculated from three replicated experiments. For each variety, and in each column, the values with different letters are significantly different according to ANOVA and Duncan's test at the level of 5%.

Table 5
Effect of $CdCl_2$ and $ZnSO_4$ treatment on the MDA content for four cultivars of *Brassica napus* L.

Cultivars	Metal concentration (μM)	MDA (nmol g^{-1} FW)
Jumbo	0	14.09[c]
	$ZnSO_4$ (2000 μM)	21.21[b]
	$CdCl_2$ (250 μM)	34.79[a]
Drakkar	0	9.91[c]
	$ZnSO_4$ (2000 μM)	18.63[b]
	$CdCl_2$ (250 μM)	24.22[a]
Cossair	0	7.91[c]
	$ZnSO_4$ (2000 μM)	10.63[b]
	$CdCl_2$ (250 μM)	17.22[a]
Pactol	0	2.58[c]
	$ZnSO_4$ (2000 μM)	5.05[b]
	$CdCl_2$ (250 μM)	11.83[a]

The results were calculated from three replicated experiments. In each column, the values with different letters are significantly different according to ANOVA and Duncan's test at the level of 5%.

on the nature of the metal but also on the cultivar under test. As far as the growth evolution with different doses of Zn and Cd is concerned, we noted that the different parts of the rapeseed reacted differently, with the aerial parts (L) and (S + P) being slightly more sensitive. These results corroborate those reported by other studies [17]. In addition, the amount of accumulated Zn or Cd varied according to the organ, the metal, and the cultivar studied. The accumulation of Cd and Zn was more important at the level of the aerial parts than in the roots for Jumbo and Drakkar cultivars. However, for Cossair and Pactol these metals were mainly stored in the roots. With regard to the effect of the metallic stress on chlorophylls and carotenoïd contents, for all cultivar the stress's origin was a reduction of the assimilator pigment content. Decreased chlorophyll contents observed in various plant species were due to heavy metals [18], especially zinc which often inhibit metabolic processes through the inhibition of the action of enzymes. Decreased chlorophyll contents associated with heavy metal stress may be the result of the inhibition of enzymes responsible for chlorophyll biosynthesis [19].

In this study, all cultivars revealed a reduction of chlorophyll and carotenoïd content after metallic stress with Zn and Cd. But, in different cases, Cd treatment was found to have a more depressing effect than Zn treatment. This drastic reduction of carotenoïd may be the result of a strong production of ROS.

MDA is a major cytotoxic product of lipid peroxidation and acts as an indicator of free radical production. An enhanced level of lipid peroxidation (high MDA production) was observed in the leaves after different Zn and Cd treatments. This higher production of MDA was positively correlated with metal concentration in leaves. Jumbo showed the highest raise of MDA production. Indeed, these results proved that the genotype effect should be taken into account with regard to MDA production. All variations in the growth, the accumulation of metals in different organs, and the pigment

content suggested structural changes in plants, mainly in the leaves, were induced by the treatment with metals [17]. Indeed, it has been shown that Cd provoked in tomatoes a reduction of the CO_2 photosynthetic fixation and an increase of its concentration in internal foliar tissues [20]. The sum of these modifications can be at the origin of a reduction of the assimilator pigment content and the availability of the photoassimilats in different organs which can affect, in turn, the growth of the aerial parts [21,22]. Hence, it is highly probable that the oxidative stress remains an important way that enables Zn and Cd to apply their toxic effects in different parts of the plants, in particular in the chloroplasts. More precisely, the membrane of the chloroplasts can be sensitive to this type of radicalic reactions since at one hand, 50% to 80% of the fat acids that constitutes them are polysatured (C18:3) [22] and at the other hand, the oxygen resulting from the photosynthesis accumulates essentially in the thylakoidal membranes.

5. Conclusion

The results obtained in this study brought out the variable behaviour of the four rapeseed cultivars in reaction to metallic stress, indicating a cultivar effect. The nature of the response to stress of each cultivar depended on the metal. $ZnSO_4$ and $CdCl_2$ led to a variation of the biomass, growth, chlorophyll and carotenoïd content, and metal accumulation. These results showed that some cultivars (Cossair and Pactol) were sensitive to metallic stress while others were resistant (Jumbo and Drakkar). Therefore, in the conditions of study, the two last cultivars (Jumbo and Drakkar) seemed more efficient in phytoextraction since both showed a significant increase in Zn and Cd accumulation in all parts of the plants. When compared to the control, they accumulated nearly the double at the level of the aerial parts (L) and (S + P). Moreover, to better understand the mechanisms that rapeseed may develop in response to metallic stress of Zn and Cd, more research is needed on the behaviour of the same cultivars *in vitro* and their ability to accumulate heavy metals under sterile and controlled conditions. The selection pressure carried out during the neoformation phase *in vitro* helped in obtaining tolerant and eventually hyperaccumulative plants which would be physiologically, biochemically, and genetically studied. This study has also proved that the choice of the specie plays a major role for the improvement of the phytoremediation and particularly the phytoextraction.

Acknowledgements

We thank the Institut national de recherches agronomiques de Tunis (INRAT, Tunis, Tunisia) for kindly providing seeds of *Brassica napus* var. Drakkar, Cossair and Pactol and we thank the Institut national de la recherche agronomique (INRA, Rennes, France) for graciously providing seeds of *Brassica napus* var. Jumbo. We are grateful to Professor Michel Couderchet (Directeur du Laboratoire des Plantes, des pesticides et du développement durable URVVC – EA 2069 Université de Reims Champagne-Ardenne, France) for revising the English.

References

[1] A. Sebei, F. Chaabani, M.K. Ouerfelli, Impacts of mining wastes on the soil and plants in the Boujaber area (NW. Tunisia). Chemical fractionation of heavy metals in soils, Geo-Eco-Trop. 29 (2005) 37–50.
[2] A. Sebei, F. Chaabani, M.K. Ouerfelli, S. Abdeljaoued, Evaluation de la contamination des sols par des métaux lourds dans la région minière de Fedj Lahdoum (NW de la Tunisie), Revue Méditerranéenne de l'Environnement (2006) 1–12.
[3] M.S. Kambhampati, G.B. Begonia, M.F.T. Begonia, Y. Bufford, Phytoremediation of a lead-contaminated soil using Morning Glory (*Ipomoea lacunosa* L.): effects of a synthetic chelate, Bulletin of Environmental Contamination and Toxicology 71 (2) (2003) 379–386.
[4] L.H. Wu, H. Li, Y.M. Luo, P. Christie, Nutrients can enhance phytoremediation of copper-polluted soil by Indian mustard, Environmental Geochemistry and Health 26 (2004) 331–335.
[5] S.D. Cunningham, W.R. Berti, Remediation of contaminated soils with green plants: An overview, In vitro Cellular and Development Biology (1993) 207–212.
[6] R.R. Brooks, R.D. Reeves, T. Jaffré, Detection of nickeliferous rocks by analysis of *herbarium specimens* of indicator plants, Journal of Geochemical Exploration 7 (1977) 49–57.
[7] S.P. Mc Grath, S.J. Dunham, Potential phytoextraction of zinc and cadmium from soils using hyperaccumulator plant, in: Proceedings of 4th International Conference on the Biochemistry of Trace Elements, Ann Arbor Press, Berkeley, 1997.
[8] M.M. Lasat, A.J.M. Baker, L.V. Kochian, Physiological characterization of root Zn^{2+} absorption and translocation to shoots in Zn hyperaccumulator and non accumulator species *Thlaspi*, Plant Physiology 112 (1996) 1715–1722.
[9] Z.G. Shen, F.J. Zhao, S.P. McGrath, Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*, Plant Cell and Environment 20 (1997) 898–906.
[10] M.D. Vàzquez, C.H. Poschenrieder, J. Barcelo, A.J.M. Baker-Hatton, G.H. Cope, Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens*, J. & C. Presl. Bot. Acta 107 (1994) 243–250.
[11] H. Küpper, F.J. Zhao, S.P. McGrath, Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*, Plant Physiology 119 (1999) 305–311.
[12] D.E. Jharna, B.L.D. Chowdhury, M.A. Haque, M.R.H. Bhuiyan, M.M. Husain, Biochemical screening of some groundnut

(*Arachis Hypogaea* L.) genotypes for drought tolerance, Online Journal of Biological Science 1 (2001) 1009–1011.

[13] H.K. Lichtenthaler, Chlorophylls and carotenoïds: Pigments of photosynthetic biomembrane, Methods in Enzymology 148 (1987) 350–381.

[14] Minotti, S.D. Aust, The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide, Journal of Biological Chemistry 262 (1987) 1098–1104.

[15] I. Iturbe-Ormaetxe, P.R. EscuredoArrese-Igor, C.M. Becana, Oxidative damage in pea plants exposed to water deficit or Paraquat, Plant Physiology 116 (1998) 173–181.

[16] C. Gisbert, R. Clemente, J. Navarro-Avino, C. Baixauli, A. Ginés, R. Serrano, D.J. Walker, F. Monnet, N. Vaillant, P. Vernay, A. Coudret, H. Sallanon, A. Hitmi, Relationship between PSII activity, CO_2 fixation, and Zn, Mn and Mg contents of *Lolium perenne* under zinc stress, Journal of Plant Physiology 158 (2001) 1137–1144.

[17] W. Djebali, L. Boulila, W. Chaïbi, Remaniements structuraux induits par le cadmium au niveau de la tige et des feuilles chez *Lycopersicon esculentum*, Revue de la Société des Sciences Naturelles de Tunisie 31 (2005) 62–73.

[18] I. Oncel, Y. Keles, A.S. Ustun, Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings, Environmental Pollution 107 (2000) 315–320.

[19] A.K. Stobart, W.T. Griffiths, I. Ameen-Bukhari, R.P. Sherwood, The effects of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley, Plant Physiology 63 (1985) 293–298.

[20] A. El Alami, Contribution à la compréhension du comportement de la tomate cultivée en présence de cadmium par l'étude de paramètres physiologiques et biochimiques, Thèse de Doctorat de 3ème cycle, Département de Biologie, Faculté des Sciences de Tunis, Tunisie, 1998.

[21] M. Gerger, G. Bertell, Effects of Ca^{2+} and Cd^{2+} on carbohydrate metabolism in sugar beet (*Beta vulgaris*), Journal of Experimental Botany 43 (1992) 167–173.

[22] D. Malik, I.S. Sheran, R. Singh, Carbon metabolism of cadmium treated wheat seedlings, Plant Physiology and Biochemistry 30 (1992) 223–229.