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Interactive Effects of Cd and NaCl on Growth and Mineral Nutrition in Hordeum vulgare L. (Var. Raihane)

Imen Ayachi[1]; Ameni ben Zineb[2]; Rim Ghabriche[1]; Mohsen Hanana[1]; Chedly Abdelly[1]; Tahar Ghnaya[1,3]

[1]Laboratory of Extremophile plants, Biotechnology center of BorjCedria, BP 901, Hammam-Lif 2050 Tunis Tunisia

[2]Laboratory of Plant Molecular Physiology, Centre of Biotechnology of Borj-Cedria, BP 901, Hammam-Lif 2050, Borj-Cedria, Tunisia

[3]Higher Institute of Arts and Crafts of Tataouine, University of Gabes, Rue Omarr Eben khattab 6029 Zerig–Gabes, Tunisia

Corresponding Author: imenayachi31@gmail.com

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Abstract:

In this work, we investigated the combined effect of salt and cadmium on plant nutrition and Cd accumulation in the most cultivated barley variety in Tunisia, Raihane. Seedling were hydroponically subjected to four different treatment: the control without salt and Cd, 50mM NaCl alone, 10µM Cd alone and to the combination of Cd (10 µM) and NaCl (50 mM) during 1 month. At the harvest, plant growth, Cd content in shoots and roots and tissue nutrient concentrations in shoots(Ca, Fe, Mn and Zn) were measured. 50 mM NaCl alone does not significantly affected the morphology and the biomass of plants. However, when subjected to 10 µM Cd alone, plants of barley were less developed and produced low biomass as compared to control ones. NaCl addition to the Cd-treated plants further reduces the development and biomass production.

With respect to nutrient acquisition results showed that NCl alone reduced Ca^{2+} and Fe^{2+} concentration in the shoots but not affected that of Zn and Mn. But Cd, applied alone or combined with NaCl disturbed all measured element concentration in the shoots. However, the addition of salt to the Cd-containing medium reduced significantly Cd accumulation in the roots and the shoots of plants.

In conclusion, salt in the water irrigation is able to reduce Cd accumulation in this barley variety but it accentuated in the same time the toxic effect of this heavy metal in barley.

Keywords: Barley, Cadmium, Salinity, Growth, Cd content, minerals

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1. Introduction

The exploitation of mining sites has become over time an important economic activity as the industrialization of societies has taken place. With a continuous increase in the demand for metals, mining activity has begun to affect the environment more seriously through large changes in the landscape and in the volumes of rock it (Agboola et al., 2020; Falagán et al., 2017). Indeed, the mining activity can unbalance the natural environments in several ways; by the deposition of solid waste, metals, the release of toxic liquid and atmospheric effluents and finally the transformation of landscapes, thus considerable damage to the environment (Falagán et al., 2017). In Tunisia, about twenty mines of Lead, Zinc, Nickel, Iron, Chromium and Cadmium were in operation until the early sixties, the majority of which are located in the Medjerda Basin. Indeed, these sites were the source of the generation of large stocks of mining residues consisting mainly of Cadmium whose concentrations reach 143-ppm existing surroundings of barley (Sahraoui and Hachicha, 2016). These discharges thus constitute a great danger for the agricultural lands located around these mines by the clearing of the surface layer of the soil and the destruction of the vegetation. In addition, to ameliorate saline soils quality biosolids, that may contain Cd, are added to soils particularly in dry areas(Weggler-Beaton et al., 2000), phosphorous fertilizers are applied to these salted soils to reduce the effect of salinity on crops(Dey et al., 2021). Furthermore the use of poor quality water (saline water and not treated wastewater) for irrigation caused high levels of salinity and an increase of cd concentration(Abbas et al., 2019; Mukhopadhyay et al., 2021).Thus, when there is a risk of exposure to these two stresses, their interaction should not be neglected.

The aim of this work is to understand the salt stress impact on cd-stressed plants whether it is enhance or alleviates cd tolerance in barley.

2. Materials and Methods

2.1. Growth conditions

Seedlings of the Tunisian genotype of barley (*Hordeum vulgare* L.) were germinated in sterilized perlite. When seedlings grew the second leaf (Ten days after germination), they were selected for uniformity and transplanted onto hydroponic container which was covered with plastic plate with spaced holes filed with 5L Hoagland‘s nutrition solution and placed in a greenhouse. After ten days, plants were randomly subjected to four treatments during 1 month: Control, 50mM NaCl, 10µM $CdCl_2$ and the combination of the 2 treatments.

2.2. Physiological measures and Cd content assay

At the harvest, the dry weight of shoots and roots was determined after 48 h of desiccation in an oven at 60°C. Cadmium content and minerals concentration was measured by using microwave plasma atomic emission spectroscopy (MP-AES, Agilent 4200, Agilent Technologies) associated with an auto sampler. Samples were dried and ground into fine powder using a porcelain mortar and a pestle. Around 50 mg of samples was completely mineralized first 2 mL of HNO3according to the following temperature cycle 80 °C for the

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first hour, 100 °C for the second hour, and 120 °C for 6 hours, then 1 mL of H_2O_2. The mineralized samples were diluted in Milli-Q water to a final volume of 20 mL.

2.3. Statistical analysis

All data were analyzed using the statistical software of SPSS 20.0. One-way ANOVA was used to determine the significant difference of among mean values of treatments by Duncan's test ($P \leq 0.05$).

3. Results and Discussion

Table 1: Interaction of NaCl and Cd on biomass production of the Tunisian barley genotype Raihane. Values are means ± SD (n=5), values followed by different letters are statistically different ($p < 0.05$).

Genotype	Treatments	Roots DW g/plant	Shoots DW g/plant
Raihane	**Control**	1.061 ± 0.26**a**	3.547 ± 0.55**a**
	NaCl	0.921 ± 0.25 **a**	3.683 ± 0.40 **a**
	Cd	0.508 ± 0.07**b**	2.871 ± 0.30 **b**
	Cd+NaCl	0.432 ± 0.09**bc**	1.893 ± 0.44**c**

The effect of different treatments on barley growth was evaluated based on shoots and roots dry biomass production. Under the stress of 50mM sodium chloride, the biomass of barley plants was not affected. Hence, cadmium only significantly decrease shoots DW.
Compared with Cd alone, the addition of 50mM of NaClled to significant decline in dry biomass of the roots and shoots.

The cadmium accumulation capacity of the tested plants was estimated based on total amounts of cadmium in shoots and roots. The effect of exposure of barley plants to cadmium alone or combined with NaCl on the amount of Cd in shoots and roots is presented in figure 1.
This parameter, which represents the product of shoots or roots dry biomass and their Cd concentrations showed that alone or combined with 50mM of sodium chloride, Cadmium was predominantly accumulated in the roots, which is a characteristic behavior of a non-hyperaccumulated species.
The roots accumulate around 800µg Plant^{-1}(Figure 1) while the Cd content in the shoots did not exceed 250µg Plant^{-1}. The addition of NaCl in the Cd-containing medium led to significant reduction of Cd accumulation in both the roots and shoots of the tested plants.

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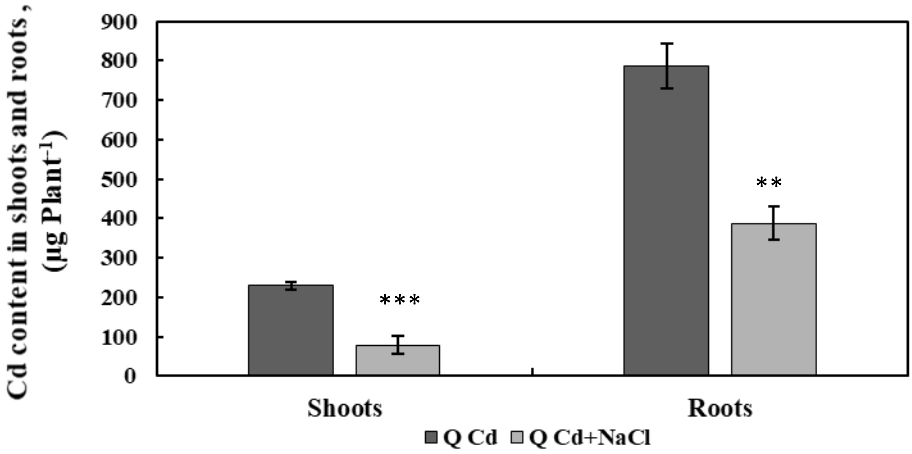

Figure 1: Variation of Cd content (µg/Plant) in the shoot and roots of the Tunisian barley genotype Raihane. Means of five replicates. Bare marked with the same letter are not significantly different at P=0.05.

The effect of Cd and NaCl alone or combined on minerals concentrations in resumed in the table 1.Under the effect of NaCl and Cd alone the Ca concentration decreased significantly in roots and shoots of tested plants. In the presence of both Cd and NaCl, Ca concentration did not differ from that under NaCl alone.

Cadmium decreased Fe concentration in the shoots of Raihane plants.

The application of 50mM NaCl showed no effect on the Mn concentration in the straws of the tested genotype, while Cd and NaCl+Cd stresses significantly decreased it (Table 1). With the presence of 10µM of Cd Zn concentration of straws significantly increase while the combination NaCl+Cd slightly decreaseZn concentration.

Table 2: Mineral concentration of Ca, Fe, Mn and Zn inthe shoots of the Tunisian barley genotype Raihane. Values are means ± SD (n=5), values followed by different letters are statistically different (p <0.05).

Genotype	Shoots	Ca mg/g DW	Fe mg/g DW	Mn mg/g DW	Zn mg/g DW
Raihane	Control	9.042 ± 0.751**a**	0.100 ± 0.017**a**	0.051 ± 0.013**a**	0.019 ± 0.004 b
	NaCl	4.053 ± 0.774**c**	0.049 ± 0.007**c**	0.063 ± 0.010**a**	0.019 ± 0.005 b
	Cd	5.093 ±0.433**b**	0.068 ± 0.007**b**	0.033 ± 0.003**b**	0.029 ± 0.002 a
	NaCl+Cd	3.026 ±0.307**c**	0.065 ± 0.005**bc**	0.022 ± 0.002**b**	0.025 ±0.006 ab

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Cadmium (Cd) is one of the heavy metals present in soils from both natural and anthropic sources (Akhtar et al., 2016; Choppala et al., 2014). In hydroponics and soil conditions, Cd plays no biological function and originate high toxicity even at low concentrations, thus causing many changes to plant physiology such as decreasing total biomass, fresh and dry weight, root length and leaf size(Akhtar et al., 2016; Rizwan et al., 2017; Yousaf et al., 2016).Cadmium stress also affect nutrient absorption and translocation by competition (Li et al., 2012; J. G. Liu et al., 2003; Pereira de Araújo et al., 2017).It has been reported that plants can counter Cd toxicity with several strategies including reducing Cd entry into their roots (Rizwan et al., 2016), binding Cd to cell walls (Parrotta et al., 2015), Chelatation (such as complexification of Cd with metallothionein and phytochelatin)(Ferri et al., 2017) vacuole compartmentation (Sharma et al., 2000) antioxidant enzyme system(Mao et al., 2014). Even though the mechanisms of the above strategies are unclear and the strategies of plants differ widely depending on species and growing conditions including the composition of the substrate(Ghnaya et al., 2007; Han et al., 2012).Despite the lack of clarity regarding the mechanism, salinity is known to have a great impact on Cd concentrations in crops (Helal et al., 1999; Smolders and McLaughli, 1996; Weggler-Beaton et al., 2000).

In the present study, we were interested in the evaluation of the combination of moderate salinity (50 mM) and cadmic stress of barley in a hydroponic medium. According to the results of our study, adding NaCl to the medium has improved the Cd tolerance of barley by a decrease of Cd accumulation in roots and shoots.

The additive stress (Cd+NaCl) declined the dry weight significantly. A similar response was Numerous studies proved the same heavier growth inhibition with the additive toxic effect of NaCl and Cd in glycophytes (Mühling and Andre, 2003; Smykalova and Zamecnikova, 2003).

Using hydroponic culture, studies of the impact of NaCl on Cd translocation in plants did not find consistent results. *Atriplex halimus* and *Kosteletzkya virginica* were less likely to translocate Cd with the presence of NaCl (Han et al., 2012; Lefèvre et al., 2009) although with *Sesuvium portulacastrum* the addition of NaCl increase Cd translocation and shoot accumulation(Ghnaya et al., 2007)(Mariem et al., 2014)(Wali et al., 2015).

Literature has identified numerous mechanisms explaining how salinity impacts Cd bioavailability. In soil solution, a higher concentration of Na+ increases Cd^{2+} chemical activity via cation exchange with soil colloids or organic matter dissolved in soil (Smolders et al., 1998). Further with the presence of NaCl, Cd is mobilized through the formation of soluble inorganic chlorides and through desorption in soil(Weggler-Beaton et al., 2000). Cd uptake is enhanced by $CdCl_2$ in soil, through direct uptake either by plants or through diffusion of Cd^{2+} around root uptake sites(Smolders and McLaughli, 1996). It remains unclear how could $CdCl^+$ be easily absorbed by plants. Even though Ca^{2+} nonselective transporters can readily absorb cd^{2+}, the size of $CdCl^+$ ions makes such absorption inefficient.

Helal et al.(1999) suggest that root selectivity can be compromised by osmotic stress and root cell membrane damage, especially in glycophytes, resulting in non-selective entry of

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$CdCl^+$ ions. It may be possible to experiment the effect of NaCl on Cd absorption separately from Cd bioavailability in hydroponics medium, which will minimize factors that can influence results from soil-based studies caused by salt addition. Studies of Ghnaya et al.(2007) and Lefèvre et al. (2009)on hydroponics have nevertheless shown that NaCl reduces the detrimental effects of Cd on plant growth while also enabling a slower accumulation of Cd in plants.

Due to the fact that free Cd^{2+} ions are the preferred species for plant uptake, the formation of complex $CdCl_n^{2-n}$ in nutrient solution decreases Cd^{2+} activity, unlike in soils(Smolders and McLaughli, 1996). Accordingly, adding NaCl to Cd-hydroponic solutions decrease Cd concentration in plants thanks to the decreased, rather than increased, Cd availability in solutions(Ghnaya et al., 2007; Han et al., 2012; Lefèvre et al., 2009; Mariem et al., 2014; Wali et al., 2015).

However, some findings have been in conflict, (McLaughlin et al., 1994) reported that salinity often caused increased Cd accumulation in potato tubers with low amounts of cadmium. Conforming to the results of Mühling and Andre (2003)and Sepehr and Ghorbanli, (2006), adding NaCl increase Cd concentration with salt sensitive species of wheat and *Zeamays*.

There is evidence that, other than changes in Cd^{2+} activity in solutions, NaCl affects Cd uptake and translocation through additional mechanisms. According to the research of(Huang et al., 2007) using hydroponic solution with four genotypes of barley, the addition of NaCl in Cd stressed solution resulted in decrease of Cd concentration in both roots and shoots due to the weakening of the capacity of roots ion uptake caused by the high salinity.

Regarding the consequences of Cd toxicity on nutrient uptake and accumulation in plants, previous works has provided opposite results, which were likely due to differences in growth methods, species, organs, and conditions like medium concentration, growth period, and temperature. J. Liu et al.(2003) reported that significant positive correlations between Cd and Fe, Cd and Zn, existed in rice in terms of their concentrations in roots and shoots. In contrast other studies(Metwally et al., 2005)indicated that toxic levels of Cd inhibited the uptake of nutrients. In addition, they found that the uptake of nutrients such as Ca, Zn and Mn by plants in an organ- and genotype-specific manner in *Pisum sativum*. In barley Cd toxicity also affected concentration of some nutrients(Wu et al., 2003). According to(Huang et al., 2007), Cd stress reduced Ca and Mg concentrations in shoots of barley, but had non-significant effect on Cu, Fe and Mn concentrations in shoot.

Cd, NaCl and NaCl+Cd stresses decreased Ca and Mg concentration, and increased K and Cu concentration in roots of all barley genotypes as compared to the control(Huang et al., 2007). Different stresses resulted in marked reduction in shoot Ca and had no significant effect on Fe and Mn concentration. Moreover, the effect of Cd, NaCl and NaCl+Cd stresses on shoot Zn concentration varied with the stress type and genotype, (Jalil et al., 1994)found that Cd application decreased the concentration of Zn, and Mn in roots and shoots of durum wheat, while the Fe and Cu concentrations in shoots and roots were not affected.

Mingjie et al.(1998)reported that the addition of Cd to decreased the accumulation of Fe, Mn and Ca in cabbage, ryegrass, maize and white clover.

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In short, the effect of cadmium on nutrient uptake and accumulation was different from that of salinity stress, and the effect of the combination of NaCl+Cd stress does not mean the simple addition of Cd and NaCl stress alone.

4. Conclusion

Our results showed that NaCl addition to the Cd-treated plants reduces the development and biomass production in barley. NCl alone reduced Ca^{2+} and Fe^{2+} concentration in the shoots but not affected that of Zn and Mn. Cadmium applied alone or combined with NaCl disturbed all measured element concentration in the shoots. However, the addition of salt to the Cd-containing medium reduced significantly Cd accumulation in the roots and the shoots of plants. Salt in the water irrigation is able to reduce Cd accumulation in Raihane barley genotype but it accentuated the toxic effect of this heavy metal in barley.

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A Brief Author Biography

Imen Ayachi, Tunisian doctoral student at the National Agronomic Institute of Tunisia (http://www.inat.tn/fr), I am conducting my research at the Biotechnology Center of Borj-Cedria (CBBC) -Laboratory of Extremophilic Plants (http://www.cbbc.rnrt.tn/index.php?choix=7&ident=1). My research project focuses on cadmium accumulation in barley (*Hordeum vulgare* L.): Physiological and molecular aspects.