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EFFECTS OF CADMIUM AND SALINITY ON GROWTH AND PHOTOSYNTHESIS PARAMETERS OF *BRASSICA* SPECIES

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Abstract

Hydroponic experiment was conducted to investigate the sole and combined effects of cadmium and salinity on growth and photosynthesis of *Brassica napus* (cultivar Abasin) and *Brassica juncea* (cultivar NIFA-Raya). Cadmium (as $CdCl_2$; 3 and 6 µmol L^{-1}) and NaCl (100 and 200 mM) were added either alone or in combination in Hoagland solution along with non-treated controls. Cd and NaCl treatments imposed significant negative effects ($p \leq 0.05$) on shoot and root length, shoot and root fresh and dry weights, shoot and root water content, number of leaves plant^{-1}, chlorophyll content (SPAD value), photosynthesis and stomatal conductance compared to control. The lone application of either Cd or NaCl on these parameters had comparatively less drastic effects than the combined application of both. Of the two species tested, *B. juncea* was found comparatively less influenced by Cd and NaCl.

Introduction

Many human activities are directly or indirectly polluting the environment. These pollutants are mainly heavy metals such as silicon, lead and cadmium (Zhou & Qiu 2005). Heavy metals originate mainly from industrial emissions, mining activity, disposal of wastes, fertilizer, pesticide use and through automobile exhausts. The increasing influx of heavy metals into water bodies from industrial, agricultural and domestic activities is of global concern because of their well documented toxic effects on human and ecosystem (Mataka *et al.*, 2006). The toxicity of heavy metals is a problem for ecological, evolutionary and environmental reasons (Nagajyoti *et al.*, 2008).

Cadmium is a heavy metal with high toxicity and has an elimination half-life of ten to thirty years (Jan, *et al.*, 1999). People are exposed to Cd by intake of contaminated food or by inhalation of tobacco smoke or polluted air (Järup *et al.*, 1998). High concentrations of cadmium in soils present a potential threat to human health because it is incorporated in the food chain mainly by plant uptake (Alvarez-Ayuso, 2008), whereas soil receives Cd through application of sewage sludge, city waste, and Cd-containing fertilizers causes the increase of Cd content in soils (Williams & David, 1973). Cd is easily taken up by plants and then enters the food chain, resulting in a serious health issue for humans such as renal tubular disease (Watanabe *et al.*, 1998). Hence, the accumulation of heavy metals into soil and then food chain has been a matter of great concern around the globe. The presence of excessive amount of Cd in soil causes many toxic symptoms in plants such as reduction of growth (Weigel & Jäger, 1980), disturbances in mineral nutrition and carbohydrate metabolism (Moya *et al*., 1993), and may, therefore, strongly reduce biomass production. The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis (Padmaja *et al*., 1990) and photosynthesis (Baszynski *et al*., 1986). Studies have reported a marked reduction in photosynthetic rate for different plant species when exposed to Cd stress (Satyakala, 1997).

Salinity is one of the major abiotic stresses around the world and causes heavy losses to crop plants. The problem of salinity becomes even more severe when plants are exposed to Cd stress (Shafi *et al.,* 2009, 2010). Salt stress may also results in an oxidative stress (Hernandez & Alamansa 2002). It has been well documented that NaCl and Cd stress in combination causes higher plasma membrane permeability and enhances the production of oxygen radicals and H_2O_2 in plants (Muhling & Lauchli 2003).

Environmental restoration of metal /salinity polluted soils by traditional physical and chemical methods demands large investments of economic and technological resources (Susarla *et al.,* 2002). In contrast, methods based on plants are becoming more popular as these methods are safer and cheaper. Phytoremediation refers to the use of green plants, soil amendments and agronomic techniques to remove, contain or render the pollutants harmless (Chaney *et al.,* 1997) and phytoextraction refers to the use of pollutant-accumulating plants that can extract and translocate contaminants to the harvestable parts. For phytoextraction, a high biomass and a very high metal accumulation in the plant tissues are expected (Chaney *et al.,* 1998). Recently, novel strategies for bioremediation of heavy metal-polluted soils and waters by phytoextraction and/or phytomining with (hyper) accumulator plants have led to a surge of interest in the physiology of (hyper) accumulating plant species (Haag-Kerwer, *et al.*, 1999). In this context, *Brassica juncea* has been identified as a high biomass-producing crop with the capacity to take up and accumulate heavy metals such as Cd, Ni, Zn, Pb, and Se (Kumar *et al.*, 1995; Salt *et al.*, 1995, 1997). For the investigation of phytoextraction, mostly hydroponic experiments are conducted, which have increased the understanding of metal uptake by hyperaccumulator, accumulator and tolerant plants (Zhao *et al.*, 2000). This type of experiment has also been important for determining the uptake efficiency and metal tolerance of potential phytoremediation species.

At present, little is known about how growth, photosynthesis and transpiration are affected when Cd and NaCl are accumulated in *Brassica* plants. Thus, the aim of the present study was to compare the Cd and NaCl effects on growth rate, photosynthesis, CO_2 assimilation and transpiration in two *Brassica* species.

Materials and Methods

The experiment was conducted in the greenhouse of College of Agriculture and Biotechnology, Huajiachi campus, Zhejiang University, Hangzhou, China using two brassica species *Brassica napus* (cultivar Abasin) and *Brassica juncea* (cultivar NIFA-Raya). Five hundred seeds each of these cultivars were surface sterilized with 0.1% H_2O_2 for 20 min, rinsed thoroughly with deionized water and soaked overnight in sterile water at room temperature. The sterilized seeds were germinated in moist quartz sand. At two leaf stage, plants of uniform height were selected and transplanted into plastic pots containing 5 L half strength Hoagland nutrient solution (Hoagland & Arnon, 1950). Pots were covered with lids containing 48 evenly spaced holes with 1 seedling hole^{-1}. Seedlings were fixed in holes with the help of cotton/foam plugs. Cadmium in the form of $CdCl_2$ was added into the solution to obtain three Cd levels; 0, 3 and 6 µM and salinity was added as NaCl in three levels (0, 100 and 200 mM) after 4 days of seedling transformation. The experiment was arranged as a split-split plot design and was replicated thrice. The nutrient solution was continuously aerated and renewed after every 3 days.

Chlorophyll content (SPAD value) was recorded on the fourth fully expanded leaves using a SPAD meter (Minolta SPAD-502) and photosynthesis related data i.e., net photosynthesis and conductivity was recoded with portable photosynthesizer 30 days after the initiation of treatments. On the same day, plants were harvested and washed thoroughly with sterile water. Plants were separated into roots and shoots. Roots and shoots lengths and fresh weights were recorded. These samples were then oven dried at 80°C for 48 hours and root and shoot dry weights were then recorded. Root and shoot water contents were determined by the formula as suggested by Barrs & Weatherley (1962). Data were statistically analyzed by analysis of variance (ANOVA) (Steel & Torrie, 1984). The significance of difference among means was compared by using DMR test (Duncan, 1955).

Results and Discussion

The effect of Cd and NaCl on plant growth was evaluated by examining root and shoot length, root and shoot fresh and dry weights, root and shoot water content, number of leaves plant^{-1}, chlorophyll content (SPAD value), net photosynthesis and conductivity in two *Brassica* species.

There was significant ($p \leq 0.05$) decrease in all the growth parameters, water content and number of leaves plant^{-1} when exposed to Cd and NaCl stresses compared to control (Tables 1-5). Root and shoot length (Tables 1-2), shoot fresh and dry weights (Table 3), root and shoot water contents (Table 4) and number of leaves (Table 5) of *Brassica juncea* and *napus* gradually decreased with the increase in concentration of Cadmium and NaCl when compared with control. The combination of two stresses (NaCl and Cd) led to a further significant ($p \leq 0.05$) reduction in these growth traits when compared with sole treatment of Cd or NaCl. Both *Brassica* species used in the present study responded differently to Cd and NaCl levels. On average, *B. juncea* was comparatively less affected when exposed to NaCl and Cd treatments in comparison to *B. napus* cultivar. Lesser affects of Cd and NaCl on *B. juncea* suggests that tolerance to these hazardous material is species specific. Seedling growth inhibition by heavy metals has also been reported (Shafiq & Iqbal, 2005; Shafi *et al.*, 2009, 2010). Similarly, many reports indicated the presence of excessive amount of Cd in soil caused many toxic symptoms in plants which could finally reduce biomass production (Weigel & Jäger, 1980; Moya, *et al.*, 1993), Significantly ($p \leq 0.05$) decreased root elongation and leaf chlorophyll contents of *Brassica chinensis* and *Brassica pekinensis* in response to Cd concentration has been reported (Liu *et al.*, 2004). Cd and NaCl imposed stresses in wheat significantly reduced dry matter weight by reducing shoot and root dry weights in wheat genotypes (Shafi *et al.*, 2011). Anjum *et al.*, (2008) also reported significant decrease in rapeseed plant biomass in response to Cd-exposed at different stages. According to Li *et al.*, (2008), cadmium is a highly toxic contaminant that affects many plant metabolic processes. Cadmium can also affect root metabolism and shows sensitivity to Cd^{2+} toxicity by a reduction in lateral root size (Wójcik &Tukendorf, 1999) which could be due to reductions in both new cell formation and cell elongation of the root (Liu, *et al.*, 2004).

Table 1. Effects of cadmium and salinity on root and shoot length of *Brassica* species.

Cd (µM)	NaCl (mM)	Root length (cm)			Shoot length (cm)		
		B. juncea	*B. napus*	Mean	*B. juncea*	*B. napus*	Mean
0	0	11.63	11.67	11.65	16.95	15.84	16.39
	100	10.22	7.25	8.74	14.39	9.93	12.15
	200	6.36	6.67	6.51	13.16	8.22	10.68
3	0	10.39	11.03	10.71	16.47	16.18	16.32
	100	7.36	6.92	7.14	13.32	8.58	10.94
	200	4.88	4.75	4.82	11.94	6.72	9.33
6	0	11.44	10.30	10.87	15.88	18.09	16.98
	100	6.57	4.17	5.37	10.56	10.18	10.36
	200	3.99	3.67	3.84	9.56	6.09	7.82
	Means	8.09a	7.38b		13.58a	11.09b	

Root length $LSD_{(0.05)}$ for Cd and NaCl 0.63, Cd x NaCl 1.10, G x C x NaCl 1.55
Shoot length $LSD_{(0.05)}$ for Cd and NaCl 0.78, Cd x NaCl 1.34, G x C x NaCl 1.90

Table 2. Effects of cadmium and salinity on root fresh and dry weights of *Brassica* species.

Cd (µM)	NaCl (mM)	Root fresh weight (gm)			Root dry weight (gm)		
		B. juncea	*B. napus*	Mean	*B. juncea*	*B. napus*	Mean
0	0	0.91	0.45	0.68	0.13	0.08	0.11
	100	0.51	0.37	0.44	0.09	0.06	0.08
	200	0.32	0.27	0.26	0.03	0.03	0.03
3	0	0.52	0.76	0.64	0.07	0.05	0.06
	100	0.41	0.43	0.42	0.05	0.04	0.05
	200	0.28	0.21	0.25	0.02	0.02	0.02
6	0	1.12	0.75	0.94	0.15	0.11	0.13
	100	0.52	0.54	0.53	0.06	0.06	0.06
	200	0.32	0.41	0.37	0.04	0.03	0.04
	Means	0.55	0.47	0.51	0.07a	0.05b	

Root fresh weight $LSD_{(0.05)}$ for Cd & NaCl 0.30, Cd x NaCl 0.42, G x C x NaCl 0.73
Root dry weight $LSD_{(0.05)}$ for Cd & NaCl 0.02, Cd x NaCl 0.03, G x C x NaCl 0.05

Table 3. Effects of cadmium and salinity on shoot fresh and dry weights of *Brassica* species.

Cd (µM)	NaCl (mM)	Shoot fresh weight (gm)			Shoot dry weight (gm)		
		B. juncea	*B. napus*	Mean	*B. juncea*	*B. napus*	Mean
0	0	8.03	4.95	6.50	0.45	0.38	0.42
	100	5.66	4.17	4.92	0.27	0.28	0.28
	200	3.15	3.88	3.52	0.25	0.24	0.24
3	0	6.34	5.94	6.14	0.39	0.44	0.42
	100	4.76	4.79	4.78	0.29	0.31	0.3
	200	2.74	1.71	2.23	0.18	0.08	0.13
6	0	4.92	4.27	4.60	0.47	0.41	0.44
	100	3.59	3.10	3.35	0.31	0.28	0.30
	200	2.19	1.33	1.76	0.22	0.1	0.16
	Means	4.60a	3.79b		0.31a	0.28b	

Shoot fresh weight $LSD_{(0.05)}$ for Cd & NaCl 0.56, Cd x NaCl 0.79, G x C x NaCl 1.37
Shoot dry weight $LSD_{(0.05)}$ for Cd & NaCl 0.07, Cd x NaCl 0.09, G x C x NaCl 0.17

Table 4. Effects of cadmium and salinity on root and shoot water content of *Brassica* species.

Cd (µM)	NaCl (mM)	Root water content (%)			Shoot water content (%)		
		B. juncea	*B. napus*	Mean	*B. juncea*	*B. napus*	Mean
0	0	91.03	94.68	92.86	94.55	93.78	94.17
	100	86.45	91.38	88.92	94.17	93.24	93.70
	200	84.32	77.70	81.01	93.06	92.35	92.70
3	0	93.10	92.74	92.92	94.54	95.41	94.97
	100	87.93	91.67	89.80	93.96	93.40	93.67
	200	87.02	90.15	88.59	92.37	92.54	92.46
6	0	92.92	86.81	89.87	95.46	93.23	94.34
	100	92.47	85.53	89.00	93.34	92.78	93.06
	200	81.91	75.06	78.49	92.07	91.31	91.69
	Means	88.57	87.30		93.72a	93.12b	

Root water content $LSD_{(0.05)}$ for Cd & NaCl 2.87, Cd x NaCl 4.06, G x C x NaCl 7.04
Shoot water content $LSD_{(0.05)}$ for Cd & NaCl 0.59, Cd x NaCl 0.84, G x C x NaCl 1.45

Table 5. Effects of cadmium and salinity on number leaves plant^{-1} and SPAD of *Brassica* species.

Cd (µM)	NaCl (mM)	Leaves per plant			SPAD		
		B. juncea	*B. napus*	Mean	*B. juncea*	*B. napus*	Mean
0	0	7.50	6.33	6.92	31.59	32.95	32.269
	100	6.92	6.58	6.75	25.68	27.10	26.39
	200	4.42	5.67	5.05	25.95	23.30	24.625
3	0	6.75	6.25	6.50	30.33	28.65	29.49
	100	6.25	5.25	5.75	28.31	22.75	25.533
	200	5.25	5.00	5.13	21.53	19.60	20.565
6	0	7.25	6.67	6.96	29.56	28.60	29.077
	100	6.59	5.26	5.92	24.15	25.83	24.988
	200	5.25	4.50	4.88	22.88	14.10	18.49
	Means	6.24a	5.72b		26.66a	24.76b	

Leaves per plant $LSD_{(0.05)}$ for Cd & NaCl 0.44, Cd x NaCl 0.65, G x C x NaCl 1.08
SPAD $LSD_{(0.05)}$ for Cd & NaCl 2.07, Cd x NaCl 2.09, G x C x NaCl 5.07

Table 6. Effects of cadmium and salinity on net photosynthesis and conductivity of *Brassica* species.

Cd (µM)	NaCl (mM)	Photosynthesis			Conductivity		
		B. juncea	*B. napus*	Mean	*B. juncea*	*B. napus*	Mean
0	0	27.13	20.58	23.86	0.49	0.41	0.45
	100	20.75	15.78	18.27	0.45	0.33	0.39
	200	18.23	13.22	15.73	0.39	0.27	0.33
3	0	22.58	18.50	20.54	0.57	0.34	0.46
	100	17.20	16.85	17.03	0.24	0.17	0.21
	200	14.63	11.20	12.92	0.19	0.11	0.15
6	0	19.35	18.98	19.17	0.39	0.38	0.39
	100	16.13	11.75	13.94	0.20	0.13	0.17
	200	12.30	8.94	10.62	0.18	0..103	0.18
	Means	18.70a	15.09b		0.34a	0.27b	

Photosynthesis $LSD_{(0.05)}$ for Cd & NaCl 1.68, Cd x NaCl 2.37, G x C x NaCl 4.11
Conductivity $LSD_{(0.05)}$ for Cd & NaCl 0.55, Cd x NaCl 0.78, G x C x NaCl 1.35

Table 5 shows that both Cd and NaCl stresses caused significant ($p \leq 0.05$) negative impact on chlorophyll content of leaves. Both Cd and NaCl alone had detrimental effects on SPAD, however, the degree of decline in SPAD value was found greater in the presence of combined Cd and NaCl treatments than lone application. Between the two *Brassica* species, *B. napus* was significantly ($p \leq 0.05$) more affected by Cd and NaCl either applied alone or together. These results are in agreement with the earlier reports of Larson, *et al.*, (1998) who found significantly ($p \leq 0.05$) decreased root dry weight, leaf area, total chlorophyll content, carotenoid content and the photochemical quantum yield of photosynthesis in *Brassica napus* L., in response to cadmium exposure. Similarly, Shafi *et al.,* (2009, 2011) also reported reduced chlorophyll content in response to Cd and NaCl imposed stresses in wheat genotypes.

Net photosynthesis was significantly ($p \leq 0.05$) reduced by the addition of Cd and NaCl either alone or in combination on *Brassica* species (Table 6). *B. napus* was more sensitive to these compounds than *B. juncea*. The sole application of Cd or NaCl was comparatively less destructive than combined treatments. Stomatal conductance (conductivity) was non-significantly ($p \geq 0.05$) reduced by the addition of both compounds in the nutrient medium (Table 7), however, *B. juncea* was less affected in comparison to *B. napus*. Ouzounidou, *et al.,* (1997) reported that the inhibition of photosynthesis by Cd stress resulted from the indirect effect of either decreased chlorophyll content or decreased stomatal conductance. Reduction of biomass by Cd toxicity due to reduction in chlorophyll synthesis and photosynthesis have also been reported by Padmaja, *et al*., (1990) and Baszynski, *et al*., (1986). In soybean, Cd and salt stress drastically reduced photosynthesis by reducing total chlorophyll content and stomatal conductance (Kang, *et al.,* 2007). Similarly, decline in growth, chlorophyll and photosynthesis have been documented (Satyakala, 1997; Sepehr & Ghorbanli 2006) in response to Cd and NaCl stresses. However, according to Haag-Kerwer *et al*., (1999), photosynthesis in *Brassica juncea* was not affected even when it was exposed to 25 μ mol L^{-1} Cd, while transpiration showed significant ($p \leq 0.05$) decline particularly under lower light conditions. The discrepancies could possibly be due to the differences the gentic make of these genotypes.

In conclusion Cd and NaCl either alone or in combination decreased growth by reducing root and shoot fresh and dry weights, number of leaves, chlorophyll content and photosynthesis in both *Brassica napus* and *Brassica juncea*. However, the combined effect of Cd and NaCl was more negative on these parameters than the sole effect of Cd and NaCl. *Brassica juncea* was comparatively less affected by these stresses indicating that CD and NaCl tolerance could be species specific.

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