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EFFECT OF ZINC ON FREE RADICALS AND PROLINE IN *BRASSICA* AND *CAJANUS*

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Key Word Index—*Brassica juncea*; Brassicaceae; *Cajanus cajan*; Fabaceae; proline; malondialdehyde; free radicals.

Abstract—Investigations were carried out to study the effect of zinc on growth, free radical production and proline accumulation in shoots of *Brassica juncea* (Brassicaceae) and *Cajanus cajan* (Fabaceae). Seedlings were raised in modified B_5 medium supplemented with zinc sulphate under controlled aseptic conditions. In general, small concentrations of zinc (up to 0.1 mM) promoted growth of seedlings in both plant species. In contrast, production of free radicals (measured in terms of malondialdehyde) and the level of proline were low in the seedlings raised in the presence of these concentrations of zinc. However, zinc at higher concentrations significantly reduced growth, but promoted generation of free radicals as well as the accumulation of proline. Irrespective of the concentration of zinc sulphate, the shoots of *C. cajan* showed significantly higher levels of malondialdehyde as well as proline as compared with those of *B. juncea*. These results suggest the existence of a correlation between the generation of free radicals and the accumulation of proline. In this communication we propose that accumulation of proline is related to non enzymatic detoxification of free radicals that are generated excessively under stress.

INTRODUCTION

Zinc is an essential mineral nutrient which is required at low concentrations for normal growth and development of plants [1, 2]. However, its presence at higher concentration is highly detrimental for all types of living organisms [3, 4]. Phytotoxicity of zinc has been recently reviewed by Chaney [5]. In spite of considerable literature available, the fundamental biochemical mechanism of zinc phytotoxicity has not yet been identified for any plant [5].

Plants exposed to various stresses including heavy metal stress, exhibit an increase in lipid peroxidation due to excessive generation of free radicals [6–8]. Although zinc deficiency stress has also been reported to promote lipid peroxidation [2], no authentic reports exist regarding the status of free radical generation under zinc toxicity. Earlier, we had reported, for the first time, that proline accumulates in plants exposed to heavy metal stress, including zinc toxicity [4]. We have also shown that proline can reduce the high light intensity-promoted free radical generation from isolated thylakoids [9] and further, it was proposed that one of the adaptive roles of proline, in plants exposed to stress, is to reduce free radical generation [9, 10]. Therefore, the present investigations were undertaken in order to see (i) whether zinc toxicity promotes free radical generation and (ii) if there exists any correlation between the changes in the levels of proline and generation of free radicals under zinc toxicity in two popular crop plants of India, namely *Brassica juncea* (oil crop) and *Cajanus cajan* (pulse crop).

RESULTS AND DISCUSSION

Growth (measured in terms of length and fresh weight of the shoots) of both *Brassica juncea* as well as *Cajanus cajan* was promoted at low concentrations of zinc. However, at higher concentrations, zinc caused significant suppression in growth of both species (Table 1). It is well established that zinc stimulates growth at low concentrations by regulating a number of important metabolic processes responsible for growth and development of the plants [11, 12]. The reduction in growth observed at higher concentrations of zinc is probably a consequence of its interference with certain essential metabolic events, as has been reported earlier [11, 13–15]. Free radical generation is one of the initial cytochemical responses of plants to stress [8, 16]. Malondialdehyde (MDA) is a major cytotoxic product of lipid peroxidation and acts as an indicator of free radical production [17]. The level of MDA was low in shoots of *C. cajan* as well as *B. juncea* raised in the presence of low concentrations of zinc sulphate compared with those grown in its absence. Zinc deficiency is known to cause peroxidative damage of

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Table 1. Length and fresh weight of shoots of *Brassica juncea* and *Cajanus cajan* grown on B_5 medium supplemented with varying concentrations of zinc sulphate

Zinc sulphate (mM)	*Brassica juncea*		*Cajanus cajan*	
	Length (cm)	Fr. wt (mg)	Length (cm)	Fr. wt (mg)
0.00	7.4^{b}	108^{b}	11.6^{b}	150^{c}
0.01	8.1^{a}	111^{b}	13.1^{a}	164^{b}
0.05	8.5^{a}	117^{a}	14.2^{a}	200^{a}
0.10	8.0^{a}	101^{c}	13.8^{a}	150^{c}
0.50	7.1^{bc}	95^{d}	12.9^{ab}	147^{c}
1.00	6.4^{c}	81^{e}	11.2^{b}	118^{d}
2.50	4.7^{d}	57^{f}	9.8^{c}	75^{e}
5.00	3.4^{e}	46^{g}	8.1^{d}	53^{f}
10.00	1.9^{f}	28^{h}	5.7^{e}	40^{g}

Data represent mean of 24 replicates. Mean values followed by the same letters within a column do not differ significantly at $P \leqslant 0.05$ level (Duncan's multiple range test) [26].

biological membranes and enzymes, and its presence is reported to protect them against the attack by toxic oxygen species [2, 18]. In both plant species, a significant increase in the level of MDA was recorded when raised in the presence of higher concentrations of zinc sulphate and the extent of enhancement in the level of MDA increased with an increase in the concentration of zinc sulphate (Table 2). These results clearly suggest that higher levels of zinc promote free radical generation and hence, lipid peroxidation. The increase in free radical production could be due to interference of zinc with normal functioning of electron transport chains of mitochondria and chloroplasts. Heavy metals including zinc have been reported to suppress electron transport chains associated with these organelles [14, 19]. Just like MDA, levels of proline in the shoots of plants raised in B_5 medium supplemented with low concentrations of zinc sulphate were found to be comparatively lower than those raised in B_5 medium alone (Table 2). At the same time, seedlings raised in medium with higher concentrations of zinc sulphate showed a significant increase in the level of proline. Interestingly, as in the case of MDA, the extent of enhancement in the level of proline increased with increase in the concentration of zinc in the medium (Table 2). Comparatively little change in the production of either MDA or proline in shoots of *B. juncea* and *C. cajan*, at a low zinc concentration, and an increase at its higher concentration shows the existence of a correlation between free radical generation and proline accumulation. The level of MDA in shoots of *C. cajan* was considerably greater than that in *B. juncea* at all the concentrations of zinc sulphate used. The level of proline was also similarly higher in the former than the latter, again suggesting that there is a definite correlation between MDA and proline levels.

Proline accumulation under zinc toxicity has been reported previously [4, 20]. Although, proline has been suggested to play an important role in: osmoregulation, protection of enzymes, stabilization of the machinery of protein synthesis, regulation of cytosolic acidity, etc. (see ref. [4]), the actual reason behind proline accumulation remains controversial. We have shown earlier that the presence of proline can reduce the level of free radicals being generated by chloroplasts when exposed to high light intensities [9, 10]. Smirnoff and Cumbes [21] also noted a decline in xanthine oxidase-promoted free radical generation in the presence of proline. The correlative changes observed in the present findings along with our earlier findings, wherein we had observed a significant reduction in the strong white light-promoted free radical generation in the presence of proline [9, 10], strongly make us believe that proline plays an important role in non-enzymatic free radical detoxification mechanism(s),

Table 2. Malondialdehyde content and proline content in shoots of *Brassica juncea* and *Cajanus cajan* grown on B_5 medium supplemented with varying concentrations of zinc sulphate

Zinc sulphate (mM)	*Brassica juncea*		*Cajanus cajan*	
	Malondialdehyde (μmol g^{-1} fr. wt)	Proline ($\mu g\ g^{-1}$ fr. wt)	Malondialdehyde (μmol g^{-1} fr. wt)	Proline ($\mu g\ g^{-1}$ fr. wt)
0.0	2.3^{a}	80^{b}	6.5^{b}	117^{c}
0.01	2.1^{a}	79^{b}	6.0^{b}	90^{b}
0.05	2.1^{a}	70^{a}	4.6^{a}	77^{a}
0.1	2.3^{a}	75^{b}	6.0^{b}	115^{c}
0.5	2.7^{b}	79^{b}	7.2^{c}	119^{c}
1.0	3.7^{c}	92^{c}	9.1^{d}	162^{d}
2.5	4.7^{d}	106^{d}	11.4^{e}	248^{e}
5.0	5.9^{e}	136^{e}	16.3^{f}	542^{f}
10.0	7.4^{f}	185^{f}	24.5^{g}	1094^{g}

Data represent mean of eight replicates. Mean values followed by the same letters within a column do not differ significantly at $P \leqslant 0.05$ level (Duncan's multiple range test) [26].

possibly in a manner somewhat similar to other biological molecules such as ascorbate, glutathione, tocopherol and glucose [6, 22].

No significant change in growth and levels of proline or MDA was observed in the presence of magnesium sulphate at concentrations as high as 10 mM (in addition to levels already present in B_5 medium) (data not shown), suggesting that the effects seen with zinc sulphate are purely because of zinc rather than sulphate.

EXPERIMENTAL

Seeds of *B. juncea* cv. Dira 367 and *C. cajan* cv. BS-15, procured from Indian Agricultural Research Institute (New Delhi, India), were surface sterilized and used for raising seedlings on modified B_5 [23] medium supplemented with 0, 0.05, 0.1, 0.5, 1, 2.5, 5 and 10 mM $ZnSO_4$ as described earlier [3]. Shoots excised from 10-day-old seedlings were used for all investigations.

Growth of the seedlings was measured in terms of length and fr. wt of shoots. The MDA content in the shoots was determined by using thiobarbituric acid reaction [24]. In brief, about 1 g of tissue was homogenized in 10 ml of 5% trichloroacetic acid (TCA) and the homogenate was centrifuged at 12000 *g* for 15 min at room temp. The supernatant was mixed with an equal vol. of thiobarbituric acid (TBA) reagent (0.5% in 20% TCA) and the mixt. was boiled for 25 min at 100° and then centrifuged for 5 min at 7500 *g* to clarify the soln *A* of supernatant was measured at 532 nm and corrected for non specific turbidity by subtracting the *A* at 600 nm. The amount of malondialdehyde was calcd using an extinction coefficient of 155 $M^{-1} cm^{-1}$. Proline content was measured according to the procedure of ref. [25].

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