

## Copper toxicity tolerance in *Aegilops* and *Haynaldia* seedlings

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### Abstract

The seedling response to high Cu concentrations (1 and 10  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ ) was studied in *Aegilops triuncialis*, *Ae. geniculata*, *Ae. cylindrica* and *Haynaldia villosa*. The negative effect of Cu on the root growth was recorded at both concentrations, while the shoot growth was inhibited at 10  $\mu\text{M}$ . The most tolerant was *Ae. triuncialis*, followed by *Ae. geniculata*. *Ae. cylindrica* and *H. villosa* were more sensitive.

*Additional key words:* excess copper, root growth inhibition, wheat improvement.

Copper (Cu) is essential micronutrient as a co-factor in a large number of metallo-enzymes and is easily absorbed by plants. Its optimal content in plant tissues is reported to be 5 - 20  $\mu\text{g g}^{-1}$  (f.m.) (Fernandez and Henriques 1991). At the same time plants are very sensitive to Cu contents slightly above the optimal levels, displaying metabolic disturbances (Moustakas *et al.* 1997, Bogoeva 1998, Fargašová 2001), chromosome aberrations (Jiang *et al.* 2001), root growth inhibition (Fargašová 2001, Jiang *et al.* 2001), which, in turn, results in reduced productivity. Cu phytotoxicity is one of the yield-limiting factors in some areas in Bulgaria, where the content of the naturally occurred Cu in the soils is above the world average and the industrial heavy metal contamination is significant (Tchuldjian 1978).

The excess Cu is mostly retained in the roots, and its content in seeds is relatively low. Wheat, in particular, belongs to the group of crops with very low heavy metal accumulation in the seeds. That is why it is important to increase the tolerance of wheat to excess Cu by utilizing the potential of wild relatives, which, in addition to the conventional soil melioration, can benefit the use of the polluted lands. Breeding for Cu tolerance in wheat through wheat-alien gene transfers requires as a first step identification of donors among the related species. The aim of our work was to study the effects of high Cu concentrations on the seedlings growth and the genotypic variation in the Cu toxicity tolerance in four wild cereals - *Aegilops triuncialis*, *Ae. geniculata*, *Ae. cylindrica* and

### *Haynaldia villosa*.

A total of 41 accessions belonging to *Ae. triuncialis* L., genome constitution UUCC (10), *Ae. geniculata* Roth, UUMM (8), *Ae. cylindrica* Host, CCDD (14), and *Haynaldia villosa*, 2n=14, VV (9) were screened for Cu tolerance at the seedling stage.

For each accession 150 seeds were germinated on moist filter paper for 3 d in the dark at 18 °C. Seedlings of root length 2.5 cm were placed in a solution of  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  at two concentrations (1 and 10  $\mu\text{M}$ ) for another 6 d at 18 °C. Tap water was used for the control group. Plant growth was estimated by measuring the length and fresh mass of roots and shoots. For each accession, measurements were performed in at least 30 seedlings per variant. Tolerance index was calculated for the root length ( $\text{TI}_{rl}$ ), root mass ( $\text{TI}_{rm}$ ), shoot length ( $\text{TI}_{shl}$ ) and shoot mass ( $\text{TI}_{shm}$ ) according to Macnair (1993) as the ratio between root or shoot length (mass) in Cu-stressed plants and root or shoot length (mass) in control plants.

There was a 23.3 % reduction in the root length, and a 28.0 % reduction in the root fresh mass at 1  $\mu\text{M}$ , on average over all genotypes. At 10  $\mu\text{M}$  the root growth was considerably inhibited - 71.1 % length reduction and 64.8 % fresh mass reduction (Table 1). At both concentrations the lowest depression in the root growth (length and fresh mass) was recorded in the accessions of *Ae. triuncialis*, followed by *Ae. geniculata* (Table 1). The root growth was more affected in *Ae. cylindrica* and

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Table 1. Tolerance index, TI [%] (mean and coefficient of variation, *CV*), and growth reduction [%], calculated for the parameters of root and shoot growth after treatment with 1 and 10  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  at seedling stage (*n* - number of accessions; values within columns followed by a different letter are significantly different from each other as determined by Behrens-Fisher (*t'*) test.

Species	Genome	Root length		Root mass		Shoot length		Shoot mass		
		1 $\mu\text{M}$	10 $\mu\text{M}$							
		<i>n</i>	mean	<i>CV</i>	mean	<i>CV</i>	mean	<i>CV</i>	mean	<i>CV</i>
<i>Ae. triuncialis</i>	UC	10	86.2a	8	32.0a	11	84.2	29	44.7a	21
<i>Ae. geniculata</i>	UM	8	77.9a	20	35.4a	24	63.5	21	35.9b	16
<i>Ae. cylindrica</i>	CD	14	66.3b	6	24.5b	9	66.1	35	31.0b	41
<i>H. villosa</i>	V	9	76.3a	15	23.8b	20	74.2	26	29.1b	38
Average TI			76.7		28.9		72.0		35.2	
Reduction			23.3		71.1		28.0		64.8	

*H. villosa*. The interspecific differences in the  $\text{TI}_{\text{rl}}$  were significant at both concentrations, and in the  $\text{TI}_{\text{rm}}$  - at 10  $\mu\text{M}$  (Table 1).

The shoot length and fresh mass were slightly reduced or slightly stimulated at 1  $\mu\text{M}$ . However, Cu ions had toxic effect on shoot growth at 10  $\mu\text{M}$ . There was a 28.3 % reduction in the shoot length and 34.5 % reduction in the shoot mass (Table 1). Genotypes did not differ significantly in  $\text{TI}_{\text{shl}}$  and  $\text{TI}_{\text{shm}}$  at both concentrations.

The intraspecific variation, as shown by the coefficient of variation, was higher at 10  $\mu\text{M}$ . It was also greater for the reduction of root and shoot mass, compared to their length (Table 1).

The less susceptibility of the shoot growth compared to the root growth to Cu toxicity in solution is reported also in barley (Stiborová *et al.* 1986), rice (Gupta and

Mukherji 1977), oat (Dinev 1998), maize (Jiang *et al.* 2001). This is probably a consequence of the preferential accumulation of Cu ions in the roots and their low mobility inside the plant (Tukendorf and Baszynski 1985, Fargašová 2001). The most tolerant appears to be *Ae. triuncialis* (genome formula UC), followed by *Ae. geniculata* (UM); *Ae. cylindrica* (CD) and *H. villosa* (V) are more sensitive. This suggested that U genome of *Ae. triuncialis* and *Ae. geniculata* might carry tolerance genes. So far, genes, controlling the excess Cu tolerance, have been reported in *Thinopyrum bessarabicum*, *Agropyron repens* and *Secale cereale* (Manyowa and Miller 1991). The *Aegilops* species, which are the most closely related to the common wheat, have been shown to carry genes for Al- and B-toxicity tolerance (Manyowa and Miller 1991).

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