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EFFECTS OF COPPER AND CHROMIUM AND HIGH TEMPERATURE ON GROWTH, PROLINE AND PROTEIN CONTENT IN WHEAT SEEDLINGS

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Abstract

Effects of interactions between high temperature and chromium (Cr(VI)) and copper (Cu) on wheat (*Triticum aestivum* L. cv. Dagdas 94) seedlings were investigated. High concentrations of Cr and Cu at 40°C decreased the root and shoot length and dry weight. The total chlorophyll content was decreased at 30 µM Cr + 40°C. At the high Cr and Cu concentrations, carotenoid content was increased compared to that of control groups. Heavy metal treatment increased proline content but decreased that of soluble protein. In addition, chromium showed greater toxic effects on growth and biochemical parameters than that of Cu.

Introduction

Numerous abiotic stress factors such as high temperature, drought, salinity, chemical toxicity, and oxidative stress severely threaten agriculture throughout the world (Wang *et al*. 2003). Abiotic stresses may lead to a yield loss exceeding 50% in plants, and are the main cause of agricultural crop losses world-wide (Bray *et al*. 2000). Increased heavy metal pollution of the soil is another important abiotic stress factor that threatens human health and the fertility of agricultural land. Metals such as lead (Pb), cadmium (Cd), chromium (Cr), and copper (Cu) may inhibit certain essential physiological activities (Foy *et al*. 1978). Pandey and Sharma (2003) showed that Cr treatment decreased the Fe concentration in cabbage leaves. High levels of Cu are also known to inhibit metabolic activities such as germination, root, leaf and stem growth and photosynthesis, biomass, and the pigment content (Foy *et al*. 1978, Pandley and Sharma 2003, Shanker *et al.* 2005, Mallick *et al.* 2010).

Organic compounds such as proline, glycine betaine and soluble carbohydrate accumulate to produce an osmotic effect, which can regulate the osmotic intensity of cellular fluids under stress conditions. Plant protein synthesis is also associated with stress tolerance mechanisms under various abiotic stress conditions, such as exposure to high temperature and heavy metals (Walters *et al.*1996, Rauser 1990).

In the present investigation, the effects of Cr and Cu on wheat seedlings and different temperatures on root and shoot length, dry weight, pigment, free proline and soluble protein contents are reported.

Materials and Methods

Wheat seeds (*T. aestivum* cv. Dagdas 94) were obtained from Adana Cukurova Agriculture Faculty. Seeds were germinated in Petri dishes under sterile conditions at a temperature of 24 ± 2°C for 48 hrs, before being transferred to pots containing a mixture of sand and perlite (1 : 1, v : v), which were irrigated with distilled water. The pots were kept in climate cabinet conditions for five days to allow the seedlings to grow with 24 hrs ventilation. At the end of this period, the

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seedlings were transferred into full strength Hoagland solution (Arnon and Hoagland 1950) and grown for a further 5 days in the same climate cabinet conditions (60% relative humidity; 24 ± 2°C; 12 : 12 hrs, day : night). The nutrient solution was replaced after a further 5 days.

The experiment was conducted at two different temperatures. In the first experiment 10-day-old plants were grown separately in nutrient solutions containing chromium ($K_2Cr_2O_7$) or copper ($CuSO_4 .5H_2O$) at a temperature of 24/16°C (day/night). Cr or Cu was dissolved in the Hoagland solution at concentrations of 10 and 30 µM. Control groups were grown with only Hoagland solution. In the second experiment, the same applications were repeated until the fourth day without any change in the initial conditions. At the end of the fourth day of heavy metal application, the cabinet temperature was increased from 24/16°C to 40/30°C (day/night), and the plants were exposed to this high temperature stress for 24 hrs. The seedlings were harvested after the fifth day of the heavy metal treatment. Each experiment was repeated three times.

The 15-day-old seedlings, which received temperature and heavy metal treatments, were harvested and the lengths of the root and shoot were measured. The samples were dried in an oven at 110°C for 24 hrs and dry root and shoot weights were recorded.

Chlorophyll was extracted by homogenizing fresh leaf material with 10 ml of 80% acetone and filtered. Chlorophyll, total chlorophyll, the chlorophyll a/b ratio, and the carotenoid contents were determined according to the method of Arnon (1949). These values were then corrected according to the method of Porra (2002).

Free proline was determinated in fresh material according to the Bates *et al*. (1973). Soluble protein was extracted from the fresh material (Jordan *et al.* 1992) and its level was determined after Lowry *et al.* (1951).

Analysis of variance (ANOVA) was used to compare the weights obtained at the end of the experiment. If a statistical difference was found in the groups compared by ANOVA, a multiple comparison method for different groups was used, i.e., Duncan's test.

Results and Discussion

The application of 10 or 30 µM Cr and 30 µM Cu at high temperature produced a lower root dry weight and root length in wheat seedlings compared to that of the control. In contrast to Cr, Cu had no effect at 24°C (Table 1). Shanker *et al.* (2005) demonstrated that inhibition of root cell growth reduced plant growth by impairing mineral and water transport to the plant. Mallick *et al.* (2010) found that Cr inhibited the root length more than the shoot of *Zea mays* seedlings because it accumulated in the roots.

The total chlorophyll content was not significantly affected by heavy metal treatment, whereas a high temperature - Cr interaction decreased the total chlorophyll content (Fig. 1). High temperature treatment with 10/30 µM Cr or 10 µM Cu increased the chlorophyll a/b ratio in wheat seedlings (Fig. 2). The reduced chlorophyll content with heavy metal stress was probably the result of heavy metals blocking the enzymes involved in the chlorophyll biosynthesis pathway. They indicated that plants raised under high Cu concentration, chlorophyll quantitiy decreased parallel to the decrease of iron (Fe).

The chlorophyll a/b ratio was significantly affected by Cu application (Fig. 2). Vernay *et al.* (2007) showed that the chlorophyll *a/b* ratio decreased significantly as a result of 100 µM Cr treatment. This is because the Cr treatment affected chlorophyll *a* more than chlorophyll *b*. Cr (VI) (0.10, 0.15, and 0.25 mM) treatments of *T. aestivum* seedlings led to a lower amount of chlorophyll *a* and *b* (Subrahmanyam 2008).

Table 1. Effects of Cr and Cu applications and heavy metal- temperature interactions on root and shoot length, root and shoot dry weight in wheat seedlings.

Heavy metal conc. (µM)	Root length (cm/plant)		Shoot length (cm/plant)		Root dry weight (mg/plant)		Shoot dry weight (mg/plant)	
	Night/day temperature (°C)							
	16/24	30/40	16/24	30/40	16/24	30/40	16/24	30/40
Control	15.01 ± 0.36a	14.07 ± 0.19 a	20.1 ± 0.12 a	16.90 ± 0.16 a	25.21 ± 0.7 a	28.78 ± 1.5 a	55.36 ± 0.9 a	53.35 ± 0.4 a
Cr 10	12.63 ± 1.01ab	9.64 ± 0.19 b	16.16 ± 1.0 b*	14.27 ± 0.11 b	21.8 ± 3.2 a	24.48 ± 0.6 b	48.8 ± 2.2 b*	47.3 ± 1.8 bc
Cr 30	9.97 ± 0.43 b*	9.07 ± 0.20 b*	14.87 ± 0.32 b*	11.98 ± 0.87c*	14.1 ± 1.1 b*	20.13 ± 1.1 c	43.6 ± 0.7 c*	42.4 ± 1.4 c*
Cu 10	14.39 ± 0.25 a	12.77 ± 0.45 c	16.39 ± 0.51 b*	16.28 ± 0.40 a	24.48 ± 0.9 a	25.31 ± 0.3 ab	42.9 ± 0.1 c*	49.7 ± 0.2 ab
Cu 30	14.03 ± 0.28 a	9.840 ± 0.21 b	15.89 ± 0.09 b*	14.63 ± 0.50 b	21.23 ± 0.2 a	16.27 ± 1.1 c*	42.6 ± 0.3 c*	46.3 ± 1.0 bc

The values have been given as arithmetic mean ± standard error (SH) (n = 9).

The letters a, b, c, express the difference at a level $p < 0.05$ in a group. * $p < 0.01$ is important at this level.

Carotenoid content was increased following the application of both heavy metals at a high temperature. However, 10 and 30 µM Cu treatments at 24°C decreased the carotenoid content of wheat seedlings (Fig. 3).

Lipids are essential for photosynthetic activity under conditions of environmental stress and the breakdown of lipids by high light and/or temperature may reduce photosynthesis (Mishra and Singhal 1992).

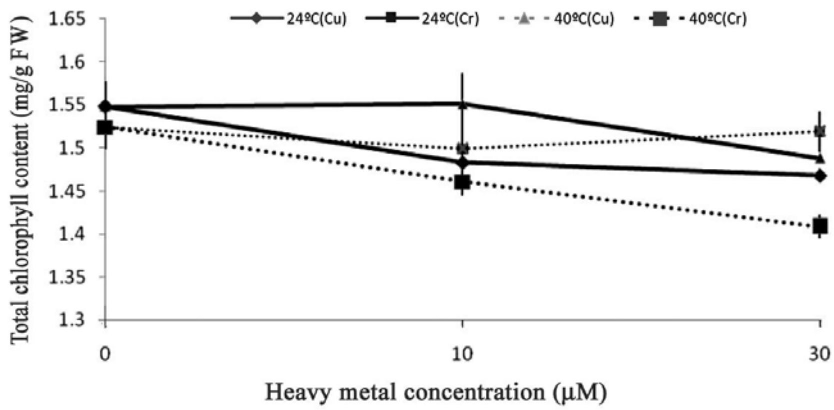

Fig. 1. The effect Cr and Cu and heavy metal-temperature interaction on the total chlorophyll content in wheat seedlings (n = 9).

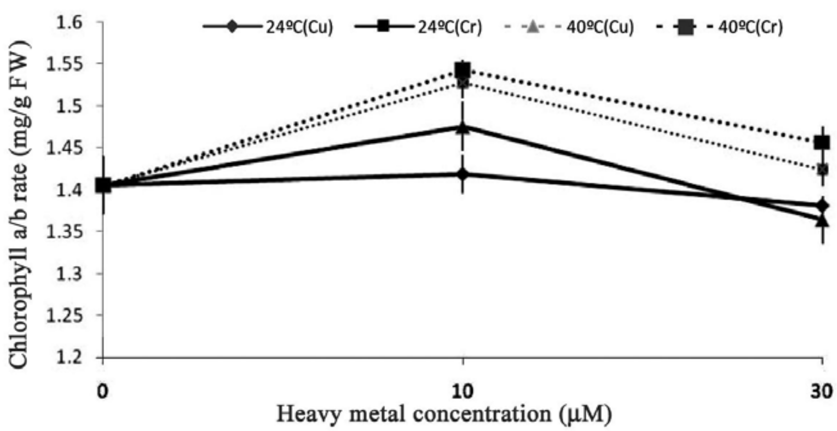

Fig. 2. The effect of Cr and Cu and heavy metal-temperature interaction on chlorophyll *a/b* rate in wheat seedlings (n = 9).

The free proline content of wheat seedlings was not significantly affected by Cu applications at 24°C, although Cu had a significant effect at high temperature. Chromium stimulated proline accumulation following low and high temperature treatments (Fig. 4). Substantial increase was reported in the proline content of leaves of wheat with increasing heavy metal concentrations (Panda *et al.* 2003).

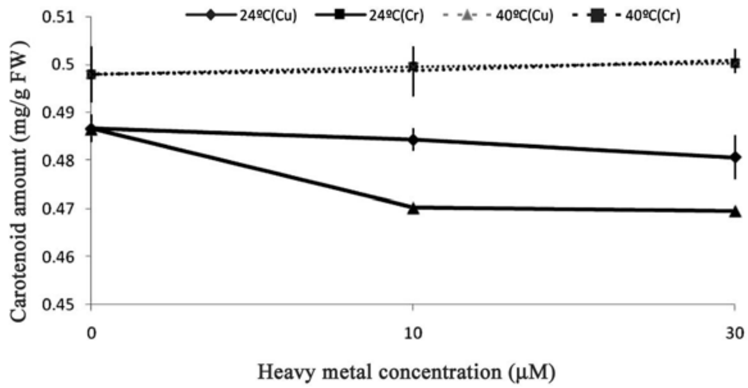

Fig. 3. The effect of Cr and Cu and heavy metal-temperature interaction on carotenoid content in wheat seedlings (n = 9).

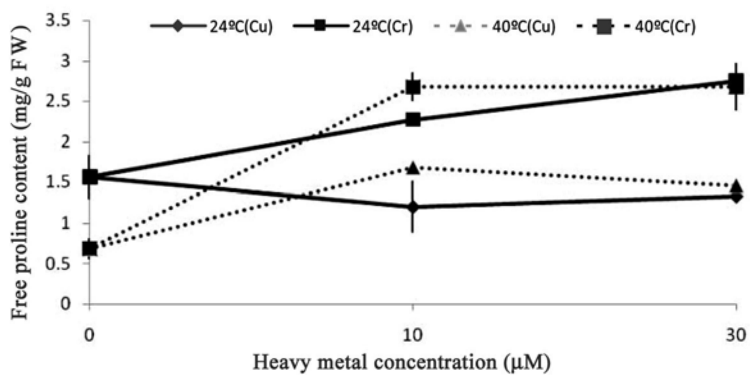

Fig. 4. The effect of Cr and Cu and heavy metal-temperature interaction on free proline content in wheat seedling (n=9).

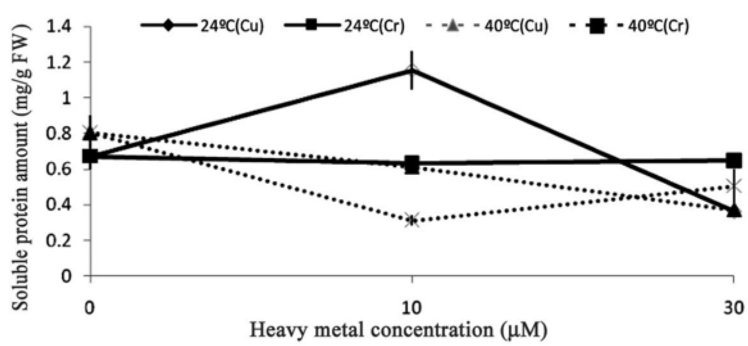

Fig. 5. The effect of Cr and Cu and heavy metal-temperature application on soluble protein content in wheat seedlings (n = 9).

10 µM Cu at 24°C increased the soluble protein content, but it decreased that at 30 µM Cu concentration. Cr at 24°C did not cause a significant change in the soluble protein content. The application of both heavy metals at high temperature led to a lower soluble protein content (Fig. 5). The significant reduction in the soluble protein concentration with the 30 µM Cu treatment at

24 and 40°C may be because protein was not synthesised under stress conditions and/or existing proteins were broken down into amino acids (Levitt 1972). The synthesis of new proteins by plants during stress conditions may be a defence mechanism that compensates for the breakdown of existing proteins into amino acids (Levitt 1972).

It is suggested that conventional and molecular improvements should be combined to develop cross-tolerances, such as temperature-heavy metal stress tolerance in agricultural plants.

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