EFFECTS OF CADMIUM ON GROWTH AND THE UPTAKE OF CADMIUM AND MICRONUTRIENTS BY TOBACCO (NICOTIANA TABACUM L.) CULTIVARS

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Abstract. Tobacco (Nicotiana tabacum L.) can accumulate higher concentrations of cadmium (Cd). However, genotypic differences of tobacco cultivars in Cd uptake and their responses to Cd toxicity have not been thoroughly investigated. This study was conducted to determine the resistance of four tobacco cultivars to Cd toxicity under greenhouse conditions. Four doses of Cd (0, 5, 10 and 20 mg kg\textsuperscript{-1} soil) were used to test the resistance of tobacco cultivars. Increasing the Cd applications significantly (P < 0.01) decreased the shoot dry matter yields of genotypes, while shoot Cd concentration and content significantly (P < 0.01) increased. The highest decrease in dry matter under the highest Cd (Cd20) treatment was in Özbaş (58.5% reduction), Birlik/124 (52.4% reduction) and Canik190/5 (51.1% reduction) cultivars, while the lowest decrease was in Xanthi/81 (44.6% reduction) cultivar. The amount of Cd removed from soil was significantly different among the tobacco cultivars. The highest Cd uptake was obtained in Cd20, while the lowest was in Xanthi/81 (395.2 µg Cd plant\textsuperscript{-1}) and Canik190/5 (232.6 µg Cd plant\textsuperscript{-1}) cultivars, respectively. The results revealed that Canik190/5 cultivar removes lower Cd compared to the other cultivars, whereas Cd uptake of Xanthi/81 cultivar is higher, thus is considered more resistant to Cd toxicity than other cultivars.

Keywords: Nicotiana tabacum L., heavy metal, pollution, soil, yield

Introduction

Cadmium (Cd), having a toxic effect on all living organisms, is one of the most dangerous heavy metal pollutants in the ecosystem (Mishra et al., 2019). The major sources of Cd in soils are geological parent material and inputs from human activities. Extraneous sources of cadmium are phosphorus fertilizers (54 to 58%), atmospheric storage (39 to 41%) and sewage sludge and farm manure applications (2 to 5%) (Cheng et al., 2014). The Cd uptake ratio of plants is high, due to its high-water solubility and mobility in soil compared to the other metals. Therefore, Cd is considered to be the most dangerous heavy metal accumulated in soils. The Cd causes many physiological changes due to changes in nitrogen and carbohydrate metabolism in plants. Cadmium inactivates the enzymes in -SH groups of proteins, closes stomata, causes water loss by transpiration and deterioration of chlorophyll biosynthesis (Sharma et al., 2019). The severity of heavy metal stress of plants varies depending on the environmental factors and the growth stage of plants (Stolt et al., 2003). The Cd resistance, uptake and transport of Cd in plant significantly vary among plant species. Grant et al. (1998) indicated that Cd concentrations of wheat, pea, maize and tobacco grown in the same solution culture were considerably different, and wheat contained lower Cd
concentration than the others. The magnitude of Cd accumulation and location of Cd in plants are major determinants of the plant resistance to Cd toxicity (Obata and Umebayashi, 1993; Quezada-Hinojosa et al., 2015). The responses of plant species and even between cultivars of a particular species to Cd toxicity considerably differ due to the prominent genetic variability among the different cultivars. The results reported by Erdem et al. (2012, 2017) for tobacco, Socha et al. (2015) for soybean, Wang et al. (2016) for maize, Zhang et al. (2002) for wheat and Zorrig et al. (2019) for lettuce clearly point out the effects of genetic variation in Cd uptake of plants. Several metal-tolerance mechanisms were introduced to explain the responses minimizing the adverse effects of heavy metal exposure. The Cd immobilization at the cell wall is the first prevention mechanism to prevent from excess Cd effect (Rizwan et al., 2016). Preventing the penetration of Cd into the cytosol, complexation of Cd with peptides or proteins to form phytochelatins and metallothioneins, compartmentalizing of Cd within vacuoles, and improving the antioxidative defense systems are major causes defined to explain the Cd detoxification in plants (Kirkham, 2006). Tobacco is one of the most common substances consumed for pleasure in the world. However, smoke of tobacco products is the most prevalent means of Cd reception of human, especially for smokers (Willers et al., 2005). Tobacco is known as an efficient Cd accumulator (Lugon-Moulin et al., 2004). Therefore, studies on investigating the effects of different Cd concentrations on Cd uptake of the commonly grown tobacco cultivars are needed. Determining the genetic variation of tobacco cultivars in Cd accumulation and uptake mechanisms are also important for the plant breeders. Research, particularly, focusing on metal uptake of international and local commercial tobacco cultivars used for tobacco products is missing.

This study was carried out to investigate the effects of different Cd concentrations on the growth of various tobacco cultivars, to determine the responses of tobacco cultivars in terms of Cd concentration and uptake, and to comprehend the interactions between Cd and Fe, Zn, Cu and Mn in tobacco plants.

Materials and methods

Materials

A pot experiment using Özbas, Canik190/5, Xanthi/81 and Birlik/124 tobacco cultivars was carried out at the greenhouse in Gaziosmanpasa University, Tokat, Turkey. Some characteristics of the genotypes used in the experiment are given in Table 1. The soil, used in the pot experiment was sandy loam, high in CaCO₃ content (11.8%), slightly alkaline in pH (8.01), low in organic matter content (0.89%) and Zn concentration (0.38 mg kg⁻¹), sufficient in Fe, Mn and Cu concentrations (6.65, 3.97 and 1.98 mg kg⁻¹), respectively), and low in Cd concentration (0.004 mg kg⁻¹).

Table 1. Some characteristics of tobacco genotypes used in the experiment (Peksüslü, 1998)

| Genotypes | Flowering   | Plant height (cm) | Number of leaves | Quality degree |
|-----------|-------------|-------------------|------------------|---------------|
| Özbaş     | Medium late | 60-85             | 35-40            | Medium        |
| Canik190/5| Early       | 75-95             | 32-35            | Medium        |
| Xanthi/81 | Medium late | 65-105            | 30-32            | High          |
| Birlik/124| Medium late | 85-125            | 40-44            | High          |
**Methods**

**Experiment**

Greenhouse experiment was carried out in Field Research Centre of Tokat Gaziosmanpasa University located at 40°20’02.19”N latitude and 36°28’30.11”E longitude and 623 m above sea level, Tokat Province in middle Black Sea region of Turkey. The pot experiment was laid in a randomized plot design with three replications. Tobacco seedlings in a 4-leaf stage were transferred to the plastic pots filled with 2.75 kg sandy loam soil. Air-dried soil, prior to filling the pots, was homogeneously mixed with a fertilizer solution containing 250 mg N kg\(^{-1}\) soil as \(\text{Ca(NO}_3\text{)}_2.4\text{H}_2\text{O}\), 100 mg P kg\(^{-1}\) soil as \(\text{KH}_2\text{PO}_4\), 2.0 mg Fe kg\(^{-1}\) soil as Fe-EDTA and 2.0 mg Zn kg\(^{-1}\) soil as \(\text{ZnSO}_4.7\text{H}_2\text{O}\). The treatments were control (Cd0), 5.0 (Cd5), 10 (Cd10) and 20 (Cd20) mg Cd kg\(^{-1}\), and each treatment was replicated three times. The Cd was applied as a solution containing 3(CdSO\(_4\))\(_2.8\text{H}_2\text{O}\). Pots were watered as needed using deionized water. At 42 days after the transplanting of seedlings, leaves were harvested when the Cd toxicity symptoms (decline in growth and chlorosis in leaves) were observed (Fig. 1). The leaves were dried in an oven at 70 °C till constant weight. Dry weights (DW) of leaves were expressed as g DW of plants. Dried plant materials were powdered and then digested in a microwave oven using 2 ml of 35% \(\text{H}_2\text{O}_2\) and 5 ml of 65% \(\text{HNO}_3\). The Cd, Fe, Zn, Cu, and Mn concentrations were determined by inductively coupled plasma atomic emission spectroscopy (Varian Vista) (Bataglia et al., 1978). The Cd content of plant material was calculated by multiplying the dry weight of shoot by the concentration of Cd. The measurements of the metal concentrations were cross-checked by using the reference leaf samples of the National Institute of Standards and Technology (Gaithersburg, MD, USA).

![Image](image_url)  
**Figure 1.** Effects of increasing doses of cadmium on tobacco genotypes
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Statistical analyses

Analyses of variance (ANOVA) were performed to test the Cd dose effects on element concentrations and content of four different tobacco cultivars and on dry matter yield. The means were compared with Duncan’s homogeneity test to determine whether the average concentrations and content of tobacco grown in the treated pots differed from those of tobacco grown in the control pots. The dose effect was considered significant when the P-value lower than 0.05. The statistical analyses were performed using the MSTAT-C statistical software.

Results and discussion

Effect of cadmium on shoot dry matter yield, Cd concentration and Cd content

The increasing Cd doses significantly (P < 0.01) reduced the shoot dry matter yield of four tobacco cultivars (Table 2). The highest decrease in dry matter yield with increasing Cd dose was observed in Özbaş, Birlik/124, and Canik190/5 cultivars respectively, and the lowest was in Xanthi/81 cultivar. The dry matter yield of Özbaş cultivar in control treatment (0 mg Cd kg⁻¹, Cd0) was 6.90 g plant⁻¹ which reduced to 2.87 g plant⁻¹ (58.5% reduction) with Cd20 application, whereas dry matter yield of Xanthi/81 cultivar was 7.63 g plant⁻¹ under Cd0 and decreased to 4.23 g of plant⁻¹ (44.6%) with Cd20 treatment (Table 2). The results of dry matter yield revealed that the Xanthi/81 is the least affected genotype from Cd toxicity. In a similar study conducted under greenhouse conditions testing five Cd doses (0, 100, 200 and 300 ppm) using 17 different tobacco cultivars, dry matter yields of cultivars significantly decreased with increasing Cd doses. However, the decrease in dry matter yield was significantly different among genotypes. The highest decrease in dry matter yield was reported in Connecticut (21% reduction), Drama (19% reduction), Myrodata Agryniou (18% reduction) and Katerini 53 (5% reduction) genotypes respectively, while the lowest decrease was obtained in Katerini 53 (5% reduction) and Argyroudis genotypes (Vasiliadou and Dordas, 2009).

Erdem et al. (2017) reported a significant decrease in dry matter yield of Xhanti 2A tobacco cultivar with increasing Cd doses (0, 10 and 20 mg Cd kg⁻¹). Dry matter yield in Cd20 (1.48 g of plant⁻¹) treatment was 47.5% lower compared to the control (2.82 g plant⁻¹). In another study conducted with maize, a significant decrease in shoot dry matter yield was reported with increasing Cd doses. The reduction in shoot dry matter yield was 11.9% with 10 mg Cd kg⁻¹ and 23.5% with 20 mg Cd kg⁻¹ treatment (Khurana and Jhanji, 2014). The decrease in dry matter yield with Cd treatment was attributed to the phytotoxic effect of Cd (Pereira et al., 2011). Cadmium toxicity negatively affects the photosynthesis of plants by inhibiting the activities of photosynthetic enzymes involved in Kalvin cycle and chlorophyll biosynthesis and consequently results in a decrease in dry matter yield (Sun et al., 2017).

Cadmium can be easily transferred to the green parts of plants following the taken up by roots. The mobility of Cd in soils is higher than other heavy metals (Mn, Zn, Mo and Se), thus can be easily taken up by many plant species (Moral et al., 2002). Therefore, a positive relationship was often reported between the increasing doses of Cd application and shoot Cd concentration and content. Shoot Cd concentrations and content of four tobacco cultivars were significantly (P < 0.01) increased with increasing Cd application to the soil (Table 2). The lowest shoot Cd concentration under Cd20 treatment was obtained for Canik190/5 (79.8 mg kg⁻¹) and the highest Cd concentration was for Özbaş (117.5 mg kg⁻¹) cultivars (Table 2).
Table 2. The effects of increasing Cd doses on dry matter yield, shoot Cd concentration and content of four different tobacco cultivars

| Cultivar        | Cd doses (mg kg⁻¹) | Shoot dry matter** (g plant⁻¹) | Decrease in shoot dry matter (%) | Shoot Cd concentration** (mg kg⁻¹) | Shoot Cd content** (µg plant⁻¹) |
|-----------------|-------------------|--------------------------------|---------------------------------|-----------------------------------|---------------------------------|
|                 | 0                 | 6.90ₐ                          | -                               | 0.39ₙ                               | 2.7₀ₙ                           |
| Özbaş           | 5                 | 4.97₇                          | 27.9                            | 47.₆₇                              | 237₃₇                           |
|                 | 10                | 4.₁₁₇                          | 40.5                            | 65.₀₇                              | 260.₀₇                          |
|                 | 20                | 2.₈₇₇                          | 5₈.₅                            | 11₇.₅₇                             | 337₃₇                           |
| Average         |                   | 4.7₁₇                          | -                               | 5₇.₄₇                              | 20₉₃₇₁₈₇                          |
| Canik190/5      | 0                 | 5.₉₉ₐ                          | -                               | 0.₃₄ₙ                               | 2.₀₅ₙ                           |
|                 | 5                 | 5.₅₁₇                          | 8.₀                             | 4₂.₉₇                              | 2₃₈₅₇                           |
|                 | 10                | 4.₀₃₇                          | 3₂.₇                            | 5₅.₄₇                              | 2₂₂₇₆                           |
|                 | 20                | 2.₉₃₇                          | 5₁.₁                            | 7₉.₇₆                              | 2₃₂₆₆                          |
| Average         |                   | 4.₆₂₇                          | -                               | 4₄.₆₇                              | 1₇₃₃₇₁₈                          |
| Xanthi/81       | 0                 | 7.₆₃₆                          | -                               | 0.₃₅ₙ                               | 2.₆₂₆                           |
|                 | 5                 | 5.₆₈₇                          | 2₅.₆                            | 4₁.₈₇                              | 2₃₉₁₆                           |
|                 | 10                | 4.₃₇₇                          | 4₂.₇                            | 7₁.₂₇                              | 3₁₀₆₆                           |
|                 | 20                | 4.₂₃₇                          | 4₄.₆                            | 9₃.₄₆                              | 3₉₅₂₆                           |
| Average         |                   | 5.₅₈₈                          | -                               | ₅₁.₇₆                              | 2₃₆₉₆                           |
| Birlik/124      | 0                 | ₅.₃₁₆                          | -                               | 0.₃₉ₙ                               | 2.₁₉₆                           |
|                 | 5                 | ₄.₇₁₇                          | 1₁.₂                            | ₅₂.₁₆                              | 2₄₃₂₆                           |
|                 | 10                | ₃.₆₉₇                          | 3₀.₅                            | ₈₄.₈₆                              | 3₁₂₉₆                           |
|                 | 20                | ₂.₅₃₇                          | ₅₂.₄                            | ₁₁₂.₄₆                             | 2₈₄₁₈                           |
| Average         |                   | ₄.₀₆₈                          | -                               | ₆₂.₄₆                              | ₂₁₀₆₉₆                          |

**p < 0.01, *p < 0.05, ns: non significant; small letters: differences in genotypes in terms of cadmium doses; capital letters: differences between genotypes

The Cd uptake and accumulation considerably differ between plant species and genotypes of the same species (Grant et al., 1998). The differences in Cd uptake and accumulation among plant species are considered as the main factors in determining plant tolerance to Cd toxicity (Obata and Umebayashi, 1993). The results indicated that the Cd uptake of plants significantly different between the cultivars used in the experiment. The highest Cd uptake (39₅.₂ µg plant⁻¹) at Cd20 dose was in Xanthi/81 cultivar and the lowest was in Canik190/5 (2₃₂.₆ µg plant⁻¹) (Table 2).

The results of shoot Cd concentration and content revealed that Cd uptake of Canik cultivar is lower than the other tobacco cultivars. On the other hand, the resistance level of Xanthi/81 cultivar to Cd toxicity is considered higher compared to the other three tobacco cultivars due to the lower decrease in dry matter yield and higher Cd uptake under the increased Cd application doses (Table 2; Fig. 2). The Cd resistance of Xanthi/81 cultivar could be related to the detoxification of heavy metals. Similar to the finding of the current study, many studies have indicated that most of the plant species are capable of tolerating high Cd concentrations in soil (Mench et al., 1989; Cieśliński et al., 1998; Rizwan et al., 2016). Plants can build up tissue tolerance at different levels when exposed to high Cd concentration. Khan et al. (1984) reported that Cd accumulates in the cell wall, whereas Vazquez et al. (1992) reported that Cd accumulates in the vacuole in a study conducted with bean plants.
Interaction of Cd with mineral nutrients (especially microelements) is an important effect of Cd toxicity in plants. The increasing Cd application dose to soil caused a decrease in the shoot Zn concentrations of 4 different tobacco varieties (Table 3). The decreases in shoot Zn concentration were insignificant for Canik190/5 and Birlik/124 cultivars, while it was significant (P < 0.01) for Özbay and Xanthi/81 cultivars. The Zn concentration of Xanthi/81 cultivar under Cd0 dose was 108.2 mg kg⁻¹, which decreased to 74.5, 68.7 and 66.8 mg kg⁻¹ at Cd5, Cd10, Cd20 doses, respectively. Vasiliadou and Dordas (2009) reported that increasing Cd application doses resulted in statistically significant reductions in shoot Zn concentrations (-0.542 *** of 17 different tobacco cultivars. The decrease in Zn concentrations under increasing Cd doses may be linked to the antagonistic relationship between Cd and Zn. Higher Cd uptake of plants grown under Zn deficiency can be attributed to the competition of Cd and Zn, which have similar chemical properties, for absorption points on the membrane (Cakmak et al., 2000) and, increase in the membrane permeability (Cakmak and Marschner, 1988).

The shoot Fe concentrations of 4 different tobacco cultivars decreased with increasing Cd doses. The decrease was statistically significant in Canik190/5 while it was insignificant in the other three cultivars (Table 3). The Fe concentration of Canik190/5 cultivar was 64.8 mg kg⁻¹ under control treatment, which decreased to 55.3, 54.8, and 57.4 mg kg⁻¹ with Cd5, Cd10, and Cd20 treatments, respectively. Significant increases and decreases occurred in shoot Mn concentrations of the cultivars with Cd application. However, shoot Cu concentrations of all cultivars, particularly in the Cd10 and Cd20 treatments, decreased with Cd application (Table 3).

For example, shoot Cu concentration in Cd0 dose was 6.71 mg kg⁻¹, which decreased to 6.56 in Cd5, while increased to 28.7 and 25.9 mg kg⁻¹ in Cd10 and Cd20 doses, respectively (Table 3). Erdem et al. (2012) reported that the increasing doses of Cd resulted in a statistically significant (P < 0.05) increase in shoot Cu concentration of tobacco plants. The results pointed out the importance of Cu absorption and/or translocation to the shoots, in contrast to the other essential micronutrients, under the...
high concentration of Cd in the root zone. In addition, shoot Cu concentrations of all tobacco cultivars used in the experiment were not affected by Cd5 treatments, whereas Cd10 and Cd20 treatments significantly affected the shoot Cu concentrations. Ramos et al. (2002) reported that increasing doses of Cd caused a decrease in shoot Fe, Zn and Cu concentrations of lettuce plants grown under the water culture conditions, while an increase in Mn concentration. The results indicated a general decrease in the Zn, Fe and Mn concentrations of tobacco plants with increasing the doses Cd application to soils. This situation reveals that the most important cause of the decrease in ion uptake of plants grown under Cd stress is the prevention of root growth and development due to damage of plant roots under Cd toxicity (Rizvan et al., 2016).

**Table 3. The effects of increasing Cd doses on shoot Zn, Fe, Mn and Cu concentrations of 4 different tobacco cultivars**

| Cultivar       | Cd doses (mg kg⁻¹) | Zn** (mg kg⁻¹) | Fe** (mg kg⁻¹) | Mn** (mg kg⁻¹) | Cu** (mg kg⁻¹) |
|----------------|--------------------|----------------|----------------|----------------|----------------|
|                | 0                  | 82.5a          | 71.8ab         | 36.9           | 5.98b          |
|                | 5                  | 65.9ab         | 58.8ns         | 41.0           | 6.26b          |
|                | 10                 | 60.5b          | 58.4ns         | 41.3           | 29.5a          |
|                | 20                 | 68.7ab         | 62.1ns         | 39.5           | 26.6a          |
| **Average**    |                    | 69.4AB         | 62.8AB         | 39.7           | 17.1           |
| Özbaş          | 0                  | 85.2ns         | 64.8           | 38.8           | 6.16b          |
|                | 5                  | 73.3ns         | 55.3b          | 37.2           | 4.54b          |
|                | 10                 | 62.8ns         | 54.8b          | 33.6           | 28.3a          |
|                | 20                 | 60.3ns         | 57.44b         | 34.5           | 27.7a          |
| **Average**    |                    | 70.4AB         | 58.1B          | 36.0           | 16.7           |
| Canik190/5     | 0                  | 108.2a         | 74.04a         | 38.8           | 4.92c          |
|                | 5                  | 74.5b          | 60.05a         | 34.2           | 3.92c          |
|                | 10                 | 68.7b          | 57.2w          | 39.1           | 28.7a          |
|                | 20                 | 66.8b          | 65.85ns        | 36.5           | 23.6b          |
| **Average**    |                    | 79.64a         | 64.24AB        | 37.1           | 15.30          |
| Xanthi/81      | 0                  | 66.9ms         | 78.32m         | 39.6           | 6.71b          |
|                | 5                  | 60.7ms         | 59.0m          | 38.2           | 6.56b          |
|                | 10                 | 52.9ms         | 71.7m          | 35.5           | 28.7m          |
|                | 20                 | 58.1ms         | 72.9ms         | 34.8           | 25.9a          |
| **Average**    |                    | 59.7b          | 70.558         | 37.0           | 17.0           |
| Birlik/124     | 0                  | ns             | ns             | ns             | ns             |
|                | 5                  | ns             | ns             | ns             | ns             |
|                | 10                 | ns             | ns             | ns             | ns             |
|                | 20                 | ns             | ns             | ns             | ns             |
| **Average**    |                    | ns             | ns             | ns             | ns             |

**p < 0.01, *p < 0.05, ns: non significant; small letters: differences in genotypes in terms of cadmium doses; capital letters: differences between genotypes**

**Conclusions**

Cadmium pollution in soils is a worldwide serious problem for sustainable agriculture and human health (Dumat et al., 2019). This study presents novel information about the effects of toxic Cd doses on the growth of tobacco cultivars, the amount of Cd uptake from the soil, and uptake of microelements. Shoot dry matter yields of tobacco cultivars significant (P < 0.01) reduced with the increasing doses of...
Cd application to soil. The greatest reduction has occurred in Özbaş, Birlik/124 and Canik190/5 cultivars, while the lowest was in Xanthi/81 cultivar. The Cd concentration and content of shoots significantly (P < 0.01) increased with the increase in Cd doses. The Xanthi/81 took up the highest Cd concentration from soil under the highest Cd treatment (Cd20), while Canik190/5 cultivar removed lower Cd from soil compared to the other cultivars. The Cd treatments resulted in a decrease in shoot Zn and Fe concentrations of tobacco cultivars, while increases and decreases occurred for Mn concentrations. The Cu concentration of shoots significantly increased in Cd10 and Cd20 treatments. The results revealed that Canik190/5 cultivar uptakes lower Cd from the soil compared to the other three tobacco cultivars. In contrast, Xanthi/81 cultivar uptakes higher Cd from the soil, thus considered more resistant to Cd toxicity than other tobacco cultivars. The data obtained will contribute to the basic knowledge needed for further research on the genetic and biochemical basis of cadmium uptake of tobacco genotypes.

Conflict of interests. The authors have not declared any conflict of interests.

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