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Research Paper

# Kinnow mandarin plants grafted on tetraploid rootstocks are more tolerant to Cr-toxicity than those grafted on its diploids one

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ABSTRACT

This study compared the effects of chromium toxicity on the biomass, photosynthesis, antioxidants, reactive oxygen species, and nutrient concentration in Kinnow mandarin (*Citrus nobilis* Lour x *Citrus deliciosa* Ten) plants grafted on diploids (2x) and tetraploids (4x) of *Poncirus trifoliata* [L.], *Citrus reshni,* and *Citrus limonia* Osbeck. Plants were grown under controlled conditions and irrigated with complete nutrient solution supplemented with chromium (0.75 mM). After 120-d of growth under chromium stress, plant biomass, total chlorophyll concentrations, photosynthetic activity, stomatal conductance, transpiration rate, water use efficiency, $H_2O_2$ concentrations, rate of $O_2^-$ generation, lipid peroxidation, antioxidant activities (superoxide dismutase, peroxidase, catalase) and mineral nutrient concentration (Ca, Mg, K, P and Cr) were determined. Chromium inhibited plant growth, and decreased chlorophyll concentration, photosynthetic activity, stomatal conductance, transpiration rate, water use efficiency, and activities of antioxidant enzymes, but the rate of lipid peroxidation and formation of reactive oxygen species were increased. Chromium stress also caused alterations in nutrients concentration in roots and leaves. Nevertheless, it was observed that Kinnow mandarin plants grafted on the tetraploid rootstocks showed high tolerance to chromium toxicity as reported by maintaining greater biomass accumulation and less reduction in the attributes commented before. In addition, plants with 4x rootstocks had higher Ca, Mg, K and P concentration in their roots and leaves in comparison to 2x rootstocks. Plants grafted on 4x rootstock presented higher quantity of chromium in roots than leaves, suggesting that the chromium tolerance of plants with tetraploid rootstocks may be attributed to chromium sequestration to roots with lower transfer to leaves; consequently avoiding oxidative damage to green pigments and the photosynthetic apparatus.

## 1. Introduction

Kinnow mandarin (KM; *Citrus reticulata* Blanco) is a hybrid between King mandarin and Willoleaf mandarin, and accounts for more than 70% of all citrus fruits in Pakistan (Khan et al., 2010). Pakistan has highly favourable environmental conditions for successful Kinnow cultivation, but in recent years, yields have been reduced. Various biotic and abiotic agents are responsible for this decline in productivity and fruit quality. Various environmental stresses like salinity, heat, drought, and heavy metal toxicity are playing a major role in limiting the productivity and deterioration of fruit quality (Balal et al., 2012). Among all these abiotic stresses, heavy metal toxicity appears to be highly detrimental to plants (Kalaivanan and Ganeshamurthy, 2016).

Heavy metals like arsenic, aluminum, boron, chromium, cadmium, lead and zinc may induce very deleterious effects on plant growth and productivity (Mustafa and Komatsu, 2016). The level of heavy metal toxicity is increasing worldwide, due to the excessive and imbalanced use of agrochemicals, addition of toxic effluents from industries into below and above ground water sources, and mineral dissociation. All these factors are responsible for abrupt increase in deleterious elements in the food chain (Adrees et al., 2015). It is also documented that heavy metal toxicity is surpassing the toxicity generated by radioactive wastes

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(Mustafa and Komatsu, 2016). Only few heavy metals, especially copper, zinc, manganese, and iron are essentially required by plants at low concentrations for the formation of many essential proteins and regulation of enzymatic activities. However, the others particularly arsenic, chromium, cadmium, lead, and mercury are highly detrimental to crop plants (Ovecka and Takac, 2014).

Regarding the heavy metal toxicity in crops, chromium (Cr) is emerging as a serious environmental threat in many developing countries. Leather, mining, chromic acid manufacturing, and battery industries are main contributors in this regard (Afshan et al., 2015). The leather industry alone has the highest contribution by adding up to the 40% Cr going into the environment (Kocurek et al., 2015). Chromium is a nonessential element to plants; hence, they do not possess specific mechanisms for its uptake. Therefore, the uptake of this heavy metal is through carriers used for the uptake of essential metals for plant metabolism. Plants can absorb Cr in Cr (VI) and Cr (III) forms, and moved in the plant xylem, or accumulated in the root cell (Shanker et al., 2005).

Chromium causes various morpho-physiological, biochemical and anatomical disturbance in plants (Lopez-Luna et al., 2016). Chromium generates very drastic effects on seed germination and excessive amounts of Cr in growing medium can inhibit germination up to 60% (Jain et al., 2000) by suppressing the enzymatic activities of amylase and protease (Sethy and Ghosh, 2013). Plants growing under Cr-toxicity also showed reduction in their growth and development due to disturbances in cell division and elongation in roots and leaves by interacting negatively with various metabolic activities (Tiwari et al., 2013). Cr-induced toxicity resulted in malfunctioning and biosynthesis inhibiting of chloroplasts, alterations in electron transport and oxidative stress (Reale et al., 2016; Da-Costa et al., 2016; Gill et al., 2015) leading to reduced photosynthetic activity. High ratios of Cr within plant tissues also disturb the plant water relations causing reductions in turgor pressure and plasmolysis of epidermal as well as cortical cells; consequently plants wilting occurred (Vazques et al., 1987). Chromium also drastically influences plants potential to uptake various beneficial nutrients like K, Mg, Mn, P, Fe and N from soil (Khan, 2001; Shanker et al., 2005). All these factors lead to reduce photosynthetic activity, reduced growth, development, production, and fruit quality of horticultural crops.

Various approaches have been used to alleviate the metal-induced toxic effects on commercially important agronomic as well as horticultural crops (Keller et al., 2015). Production of metal tolerant genotypes by various breeding and biotechnological tools is one of these strategies. Another strategy is the alleviation of metal-caused toxicity by exogenous application of various growth hormones and regulators (Piotrowska-Niczyporuk et al., 2012; Farooq et al., 2016). Grafting is also considered as very effective technique to reduce the growth and yield limiting effects of abiotic stresses specially the salinity and heavy metal toxicity in horticultural crops (Nawaz et al., 2016; Penella et al., 2016). The genetic makeup of rootstock induces resistance against edaphic and climatic factors in scion by enhancing the nutrient uptake, movement and nutrient use efficiencies (Habran et al., 2016). In the last decade, several reports indicate that biotic and abiotic stress-tolerance in citrus is highly linked with ploidy level. Thus, the use of tetraploid rootstocks in citrus have increased its tolerance to salt stress (Saleh et al., 2008; Ruiz et al., 2016), boron toxicity (Ruiz et al., 2016), and drought condition (Allario et al., 2013). These authors have reported that tetraploid citrus have lower rates of plant transpiration, present thicker greener leaves and shorter thicker roots, and have bigger stomata and lower stomatal density. In addition, compared to diploid, tetraploid citrus has higher expression level of stress related genes and higher content of stress related metabolites which could be beneficial for its stress tolerance (Tan et al., 2015).

Since rootstock conferred resistance to grafted citrus trees (Syvertsen and Garcia-Sanchez, 2014), and tetraploid rootstocks can be more tolerant to abiotic stress than diploids, the effects of high Cr concentration in the nutrient solution in Kinnow mandarin grafted on both diploid and tetraploid rootstocks were investigated in the present study. The objective of this experiment was to test the relative tolerance between tetraploid and diploid plants in three different citrus rootstocks, and providing insight into the causes of the different behaviour in Cr tolerance between both ploidy genotypes in Kinnow mandarin. The research finding of this study will be highly useful for plant breeders and genetic engineers working on metal tolerance in plants.

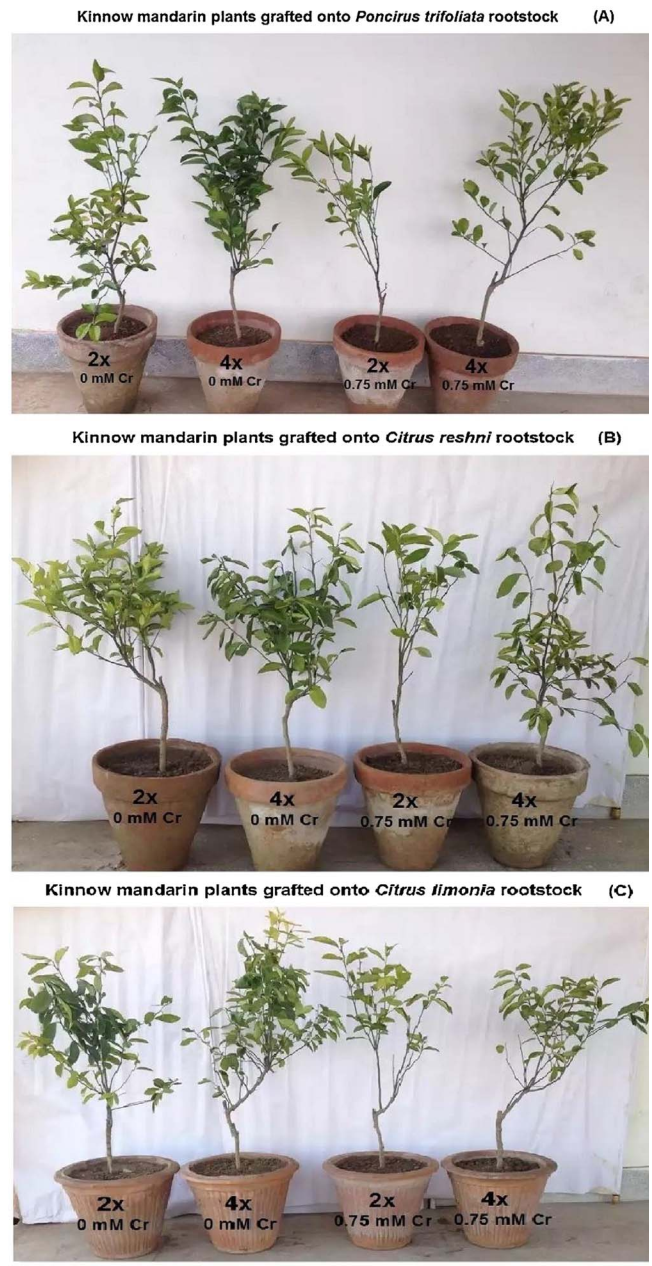


**Fig. 1.** Kinnow mandarin plants grafted on diploid (2x) and tetraploid (4x) plants of Poncirus trifoliata, Citrus reshni and Citrus limonia under normal (0 mM Cr) and chromium-stressed (0.75 mM Cr) conditions.

## 2. Materials and methods

### 2.1. Plant materials and growth conditions

Twenty-four-month old diploid (2x) and tetraploid (4x) plants of three citrus rootstocks species, *Poncirus trifoliata* [L.] (PT), *Citrus reshni* (CH) and *Citrus limonia* Osbeck (CL) were used in this investigation. The