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GROWTH BIOREGULATORY ROLE OF ROOT-APPLIED THIOUREA: CHANGES IN GROWTH, TOXICITY SYMPTOMS AND PHOTOSYNTHETIC PIGMENTS OF MAIZE

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While seed or foliar application of thiourea (TU) has the potential to modulate plant growth, studies reporting such bioregulatory role(s) of root-applied TU are limited. Here we report the growth regulation by root-applied TU in nine maize (*Zea mays* L.) varieties grown using sand culture. Thirty day old plants were treated with 0–1.25 mM TU levels dissolved in nutrient solution, and the data recorded 20 days later. The parameters studied were symptoms of TU toxicity, growth and photosynthetic pigment contents. Results revealed that 0.25 mM TU promoted the growth of all varieties; 0.50 mM did so in some, while 0.75–1.25 mM TU was inhibitory to all varieties. Higher TU levels (1.00–1.25 mM) produced more intensive toxicity symptoms such as yellowing of leaves, and browning and constriction of roots but with significant varietal differences; Pak-Afgoi showed the lowest while EV-20 had the highest incidence of symptoms. Lowest TU level (0.25 mM) increased the photosynthetic pigments in all the varieties, while higher levels (1.00–1.25) reduced chlorophyll (Chl) *b* and carotenoids (Car) contents and increased Chl:Car ratio more legibly in the sensitive varieties. There were no correlations of toxicity symptoms with growth and leaf pigments contents at 0.25 mM TU but these correlations were negative for shoot weight, number and area of leaves and Chl *b*, Car and Chl:Car ratio of leaves at 1.25 mM (toxic) level of TU. Likewise, root dry weight of maize varieties was negatively correlated with root constriction and root browning. Results suggest that higher levels of TU had pronounced influence on the root functions leading to reduced dry weight. From the current results we believe that TU has a definitive growth bioregulatory activity as it improved photosynthetic pigments contents at lower level (0.25 mM) but deteriorated them at higher levels (0.75–1.25 mM). Substantial varietal differences suggested that TU effects are genetically-related in maize. Possible physiological basis of the current observations are discussed.
Keywords: Carotenoids, chlorophyll *b*, growth, injury symptoms, varietal differences

INTRODUCTION

Exogenous application of various synthetic organic compounds can modify plant growth due to their biological properties (Tang *et al.*, 2009). Thiourea has three functional groups; *mino, imino* and *thiol* (http://www.trivenichemical. com/thiourea-1.html). By virtue of these groups, TU has great implications in changing plant growth both under normal and stressful conditions (Srivastava *et al.*, 2009; Anjum *et al.*, 2011). Being water-soluble and readily absorbable in living tissues, exogenous application of TU at appropriate concentrations is suggested to have multiple roles both under optimal and sub-optimal conditions (Jagetiya and Kaur, 2006; Anjum *et al.*, 2008; Mani *et al.* 2013).

Studies have shown that seed treatment of TU increased K^+ uptake and caused reduction in abscisic acid biosynthesis in chickpea (*Cicer arietinum*) (Aldasoro *et al.*, 1981). When applied as seed presoaking treatment, TU enhanced the mobilization of K^+ from endosperm and promoted the accumulation of malate due to carboxylation of phosphoenol pyruvate and increased CO_2 fixation in developing embryos of chickpea (Hernández-Nistal *et al.*, 1983). Foliar spray and seed priming with TU promoted the growth of cluster bean (*Cyamopsis tetragonoloba*), as evident from increase in plant height, leaf area, dry matter production and seed yield (Garg *et al.*, 2006). Without TU application, soybean (*Glycine max*) displayed poor photosynthetic capacity, dry mass and seed yield, while foliar spray with 2000 ppm of TU restored the normal metabolic processes with an increase in chlorophyll (Chl) contents, shoot dry weight and 100 seed weight mainly by delaying senescence (Jagetiya and Kaur, 2006). Photosynthetic pigments capture solar energy for its preservation as chemical energy in the form of reducing powers. Therefore, changes in their quantities are important to final dry matter and economic yield (Sabo *et al.*, 2002; Zhu *et al.*, 2011). Applied TU positively interacted with N and P in improving net photosynthesis, Chl, starch, proteins contents, nitrate reductase activity, dry matter yield and harvest index in cluster bean (Burman *et al.*, 2007).

Maize (*Zea mays* L.) is major amongst important cereal crops all over the world. It has great value as food, feed and fodder crop, and can ensure the food security because of its high nutritional value (Dowswell *et al.*, 1996). Thus, there is an urgent need to improve maize productivity. Being highly cross-pollinated, maize provides better chances of studying genetic basis of one specific or a group of treatments. The external use of TU may provide an attractive opportunity to determine varietal responses and roles of TU in improving maize yields.

Few studies report the influence of root applied TU on the growth and physiology of plant, but the results are variable with respect dose applied and plant species. For instance, treatment of potato roots with 5 kg/ha TU not only increased the root penetration into the soil but also improved phosphorus uptake, while higher levels were toxic (Swaminathan, 1980). Sahu and Singh (1995) established that in wheat, root-applied TU (10 kg/ha) at 30 days after flowering produced no changes in the dry matter partitioning in leaves, stem and ear but its application at maturity increased the grain yield characteristics. These studies indicated that modulation of plant growth is strongly dependent on the plant growth stage and dose of TU applied.

As evident from the above, TU has multiple roles in modulating growth and physiological functions of plants but such studies are altogether lacking in case of maize. We predict that TU has a growth bioregulatory activity and a marked impact on the maize growth. The objectives of this study were to find out the possible growth regulation by TU, optimum TU concentration and intra-specific response of maize to root-applied TU based on changes in growth, visual symptomatic and photosynthetic pigments.

MATERIALS AND METHODS

The experiments were conducted in spring season (March–April) of 2009 and 2010. Seeds of nine maize (*Zea mays* L.) varieties (Agaiti-2002, Pak-Afgoi, Sadaf, SWL-2002, EV-20, EV-77, EV-78, EV-79 and EV-5098) were obtained from Maize and Millets Research Institute (MMRI), Yousafwala, Sahiwal, Pakistan. The work was conducted to determine comparative efficacy and optimization of most appropriate TU levels in improving maize growth. Ten seeds of all varieties were sown in each of the plastic pots (dimensions 30 cm high, 15 cm from bottom and 20 cm neck diameter) containing 10 kg of water-washed sand. A hole was made in the bottom to replenish the solution. After germination, four healthy and uniform sized plants were retained in each pot. Plants were applied with 500 mL of half-strength Hoagland nutrient solution (Epstein and Bloom, 2005) to each pot after every seven days in order to avoid nutrient depletion. The experiments were conducted using completely randomized design with three replications. The ambient temperature (^{o}C), day length (h) and RH (%) during 2009 were 18.5±2.4, 14±0.5 and 55±5, respectively while during 2010 these conditions were 18.3±2.6, 13.8±0.5 and 53±6.

Selection of treatments was made based on the information from previous studies involving the exogenous application of TU. For this purpose, two preliminary experiments were performed using high range (2–10 mM) and low range (0.25–1.50 mM) concentrations of TU (Perveen, 2012). No plant survival was found in the high range, and these rates appeared to be growth inhibitory. However, lower levels (0.25–1.50 mM) of TU in low range improved growth; higher levels were inhibitory, while 1.50 mM TU was lethal to most of the varieties (data not shown). Thus, 0–1.25 mM TU levels were selected for the current experiments. In both years, half of 30 days old plants of each variety were applied with 0–1.25 mM TU levels using analytical grade TU (Merck, Germany) by dissolving in above-referred nutrient solution, while the control plants received nutrient solution only. While applying treatments, it was ensured that TU-containing solution was completely replaced with the existing one. Treated plants were grown for 20 more days before harvesting.

At harvest time, the intact plants were used for measuring shoot length, number of green leaves and leaf area per plant [as maximum leaf length × maximum leaf width × 0.68 (correction factor)] (Carleton and Foote 1965). For symptomatic observations, the leaves were visually scored for yellowing. After uprooting the plants, the shoots were separated from roots; roots briefly washed to remove sand and then blotted dried. Constriction and browning were visually scored by keeping the roots under convex lens fixed at one position and their length was measured subsequently. Both the parts were kept in separate paper bags and dried for seven days in an oven at 70^{o}C for dry weight determination. Shoot:root ratio was determined on dry weight basis to explore dry matter partitioning under TU treatments.

To measure the photosynthetic pigments, 0.5 g of fresh leaf tissue was extracted in 80% acetone and filtered. Absorbance of the filtrate were recorded at 645, 663 and 480 nm for Chl *a*, *b* and carotenoids (Car), respectively using spectrophotometer (U-2001, Hitachi, Japan). Actual amounts of Chl *a*, *b* and Car was computed using the formula given by Yoshida *et al.* (1976) and Davis (1976), respectively. Chl:Car ratio was determined to observe the TU-induced changes in these pigments.

Statistical analysis of the data was done using MSTAT-C computer program. The data were analyzed for the significance of variance sources (varieties, TU treatments and their interactions) using computer software MSTAT-C. The correlations amongst the plant growth, visual symptoms and photosynthetic pigments were computed based on percent increase or decrease over control at the lowest (0.25 mM) and the highest (1.25 mM) TU levels. For drawing correlations, the varieties were scored and the varieties with the lowest symptom were awarded the highest score.

RESULTS

***Growth characteristics*:** Shoot length increased from 4 to 22% in different varieties at 0.25 mM TU level except EV-20, which showed 22% decline. Shoot length decreased in all varieties at 0.75 mM and subsequent levels and was minimum at 1.25 mM TU level. The variety SWL-2002 followed by EV-20 exhibited the highest reduction (45 and 40%, respectively), while Sadaf displayed the least (23%) reduction under 1.25 mM TU application (Fig. 1a). Root length was greater than controls in EV-78 (24%) followed by Sadaf and Pak-Afgoi (10 and 7%, respectively) but lesser than control in EV-79 (8%) at 0.25 mM TU. Root length decreased at subsequent TU levels, and a highest reduction was observed at 1.25 mM in all varieties. At 1.25 mM TU, the root length was the least affected (23%) in Pak-Afgoi and the most affected (50%) in EV-20 (Fig. 1b).

Shoot dry weight was equal to control or greater than control in all varieties except EV-20, which showed 15% decline in shoot dry weight at 0.25 mM TU level. Shoot dry weight declined in all varieties at higher TU levels. A highest declined (61%) was observed in EV-20 and the lowest (26% each) in EV-77 and Pak-Afgoi (Fig. 1c). Although 0.25 mM TU level enhanced root dry weight in all the varieties, at higher TU levels it was markedly reduced. The maximal decline (68%) in root dry weight was evident in EV-20 and a minimal one (26%) in Pak-Afgoi (Fig. 1d). Shoot:root ratio was the highest in EV-78 followed by Pak-Afgoi and the lowest in EV-5098 under control conditions. At 0.25 mM TU, this ratio was maximally decreased in EV-20 (20%) but increased (1.60%) in EV-5098. However, at 1.25 mM TU, shoot:root dry weight ratio increased (by 0.31%) in Pak-Afgoi but decreased in all other varieties; EV-77 showing a maximum (51%) reduction (Fig. 1e).

Leaf area per plant was variable in all the varieties under control conditions, which was enhanced at 0.25 mM TU level in all the varieties except SWL-2002 (reduced by 13%) with a maximum increase (12%) in Agaiti-2002. However, at 1.25 mM TU level, the leaf area although declined in all the varieties, a minimum reduction was noticed in Pak-Afgoi (27%) and a maximum one in EV-79 (52%) and EV-20 (50%) over respective controls (Fig. 1f). Number of leaves per plant was variable under control condition. At 0.25 mM TU level, this number increased in Pak-Afgoi followed by Sadaf, while EV-78 and EV-5098 had equal to control, and rest of the varieties showed a reduction in this number; a greatest reduction was noted (36%) in EV-20 (Fig. 1g). Number of roots per plant was also variable under control condition, which increased in all varieties except in Sadaf, EV-5098 and EV-20 at 0.25 mM TU level. This number declined at higher TU levels with greatest decline recorded at 1.25 mM; EV-20 and SWL-2002 showing a highest (68 and 65%) and Pak-Afgoi a lowest (22%) decline (Fig. 1h).

***Visual toxicity symptoms*:** Although minor varietal differences were noted at 0 and 0.25 mM TU levels (data not shown), maize varieties showed large differences at higher (0.75–1.25 mM) TU levels in overall leaf yellowing, constriction and browning of roots. Pak-Afgoi followed by Agaiti-2002 manifested the lowest, while EV-20 followed by EV-79 and SWL-2002 the highest leaf yellowing. Lowest root constriction was observed in Pak-Afgoi followed by SWL-2002 while it was the greatest in EV-20 and EV-78. Root browning was the least in Pak-Afgoi and EV-5098, whilst the most in Sadaf and EV-20 (Table 1).

***Photosynthetic pigments*:** Under control conditions there was no difference in the Chl *a* content of the varieties. At 0.25 mM TU Chl *a* increased in Pak-Afgoi (14%), followed by EV-77 (9%), EV-5098 (8%) and EV-78 (1%), while decreased in other varieties. However, 1.25 mM TU although reduced Chl *a* in all the varieties, a maximum reduction was recorded in SWL-2002, EV-20 and EV-78 but a minimum one in Pak-Afgoi and EV-5098 (Fig. 2a). All varieties showed similar values of chlorophyll *b* under control conditions. Treatment with 0.25 mM TU increased Chl *b* in EV-5098 (21%), EV-79 and EV-77 (8%) and Pak-Afgoi (2%) but decreased in other varieties, being the lowest in Sadaf (20%). However, at 1.25 mM TU level, all the varieties showed a decline in Chl *b*, which reduced the most (50%) in EV-20 and the least (9%) in Pak-Afgoi (Fig. 2b). Root-applied TU at 0.25 mM was effective in improving Car contents in most of the varieties. However, 1.25 mM TU was damaging to Car in all varieties but with substantial differences among them. In this case, Sadaf and EV-20 indicated the greatest (52%) while Pak-Afgoi the lowest (9%) reduction in Car at the highest TU level (Fig. 2c). Although Chl:Car ratio decreased in all varieties except EV-79 (increased by 0.3%), a maximum decrease was noted in Agaiti-2002 and Pak-Afgoi (15 and 12%, respectively) at 0.25 mM TU. However, this ratio increased highly in EV-20 (41%) while decreased lowly (8%) in Pak-Afgoi at 1.25 mM TU level (Fig. 2d).

***Correlations of symptomatic and quantitative attributes*:** Showing no correlations at 0.25 mM, increase in TU-induced yellowing of leaves was negatively correlated with increased shoot dry weight, number of leaves and leaf area per plant. Among the photosynthetic pigments, increased leaf yellowing was not correlated with Chl *a*, while negatively related to increased Chl *b*, Car and Chl:Car ratio at 1.25 mM TU. Shoot dry weight showed positive correlation with Chl *a*, *b* and Car but not with Chl:Car ratio (Table 2). At 0.25 mM TU root constriction and browning had no correlation with number and dry weight of root per plant. However, at 1.25 mM TU high root dry weight, not the number of roots per plant was positively related to low constriction and browning of roots (Table 2).

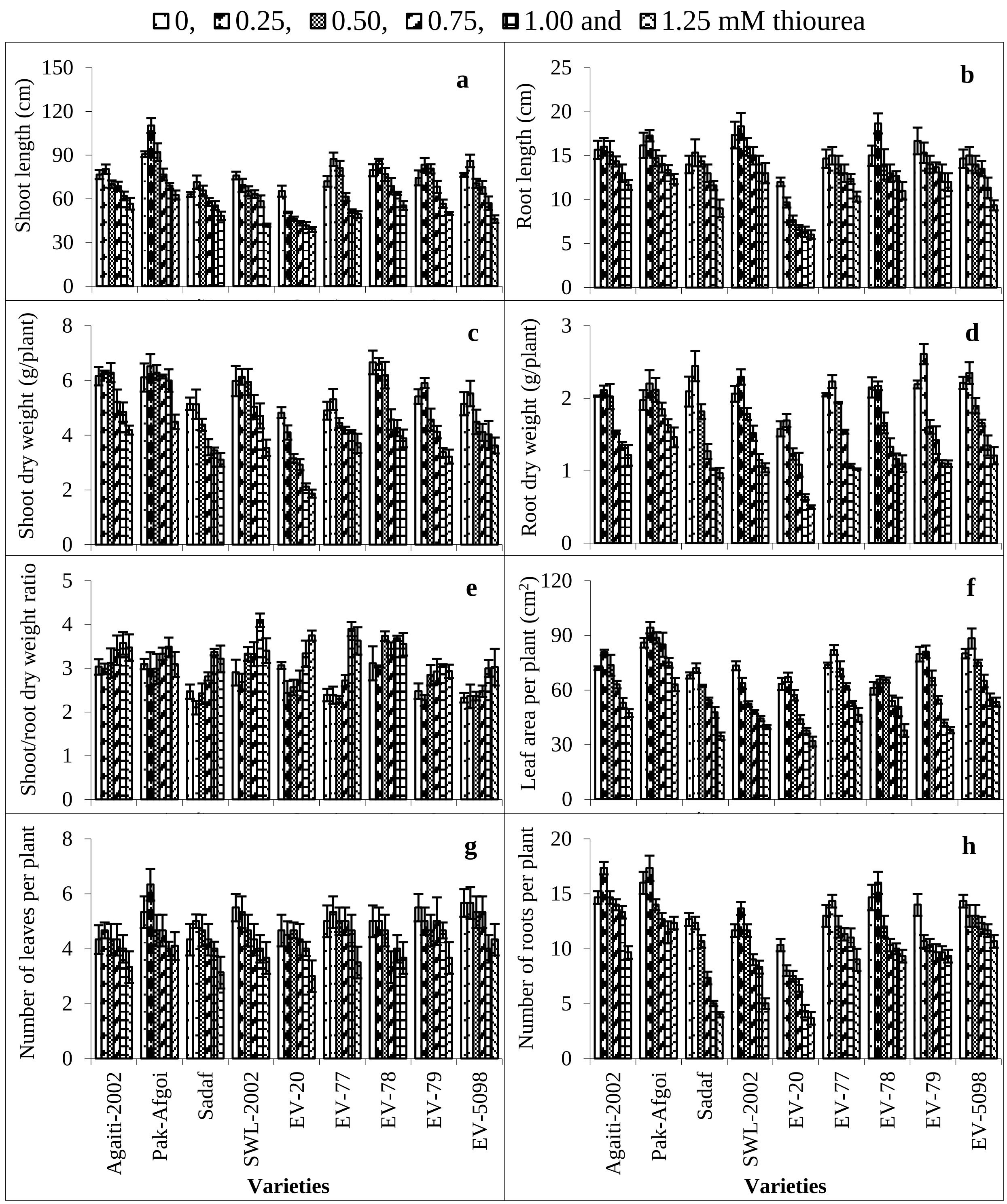

Figure 1a-h. Changes in some growth attributes of maize varieties with the soil application of TU

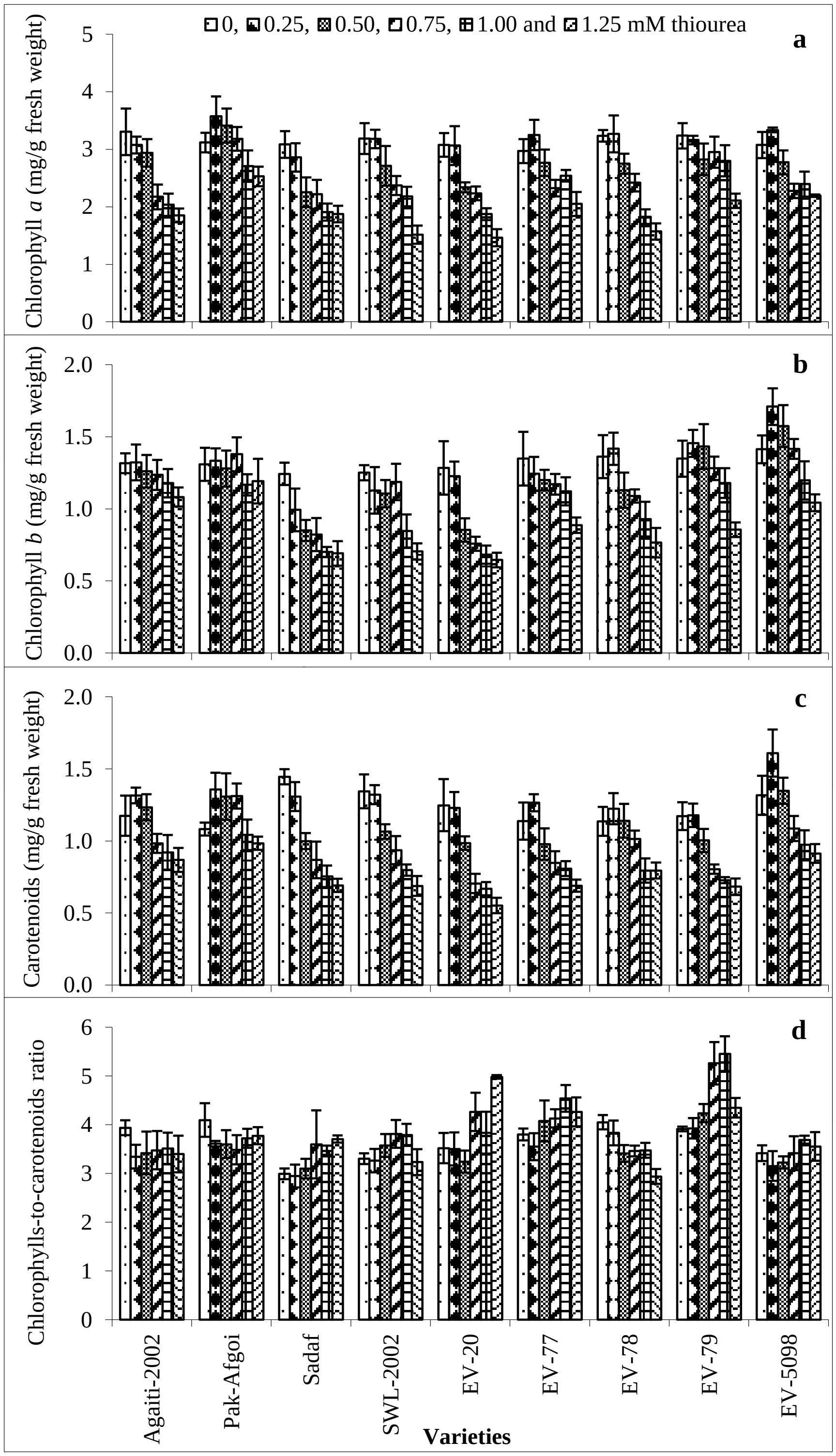

Figure 2a-d. Leaf photosynthetic pigment concentrations of maize varieties as influenced by soil application of increased levels of thiourea

Table 1. Visual symptoms and degree of TU effect on shoot and root of maize varieties. The toxicity symptoms were noted at the highest level (1.25 mM) of TU applied. The downwards arrows indicate increased degree of TU toxicity

Degree of thiourea toxicity	Leaf yellowing	Root constriction	Root browning
Low	Pak-Afgoi	Pak-Afgoi	Pak-Afgoi
↓	Agaiti-2002	SWL-2002	EV-5098
↓	EV-5098	Agaiti-2002	EV-77
↓	EV-77	EV-78	EV-79
↓	EV-78	EV-79	Agaiti-2002
↓	Sadaf	EV-77	EV-78
↓	SWL-2002	Sadaf	SWL-2002
↓	EV-79	EV-5098	EV-20
High	EV-20	EV-20	Sadaf

The downwards arrows indicate increased toxicity on the maize varieties

Table 2. Correlation coefficient (*r*) of growth attributes and photosynthetic pigments with visual toxicity symptoms of maize varieties at 0.25 and 1.25 mM TU level

Visual toxicity symptoms	Quantitative parameters	0.25 mM TU	1.25 mM TU
Leaf yellowing	Shoot dry weight	0.069 ns	-0.826**
	Number of leaves	0.524 ns	-0.952**
	Leaf area	0.129 ns	-0.886**
	Chlorophyll *a*	0.078 ns	-0.572ns
	Chlorophyll *b*	0.014 ns	-0.768*
	Carotenoids	0.210 ns	-0.823**
	Chlorophyll:carotenoids ratio	0.276 ns	-0.668*
Shoot dry weight	Chlorophyll *a*	0.382 ns	0.741*
	Chlorophyll *b*	0.334 ns	0.757*
	Carotenoids	0.470 ns	0.698**
	Chlorophyll:carotenoids ratio	-0.318 ns	0.527ns
Root constriction	Number of roots	0.318 ns	-0.188ns
	Root dry weight	-0.479 ns	-0.684*
Root browning	Number of roots	0.142 ns	-0.505ns
	Root dry weight	0.366 ns	-0.719*

Significant at ** $P < 0.01$, * $P < 0.05$ and ns non-significant

DISCUSSION

The available literature suggests that TU can effectively promote plant growth when applied as seed treatment or as foliar spray under optimal (Garg *et al.*, 2006; Jagetiya and Kaur, 2006) and suboptimal conditions (Anjum *et al.*, 2008; Srivastava *et al.*, 2009). However, root application of TU has not been the subject of intensive studies. In the present study, no maize survival was observed at 1.50 mM TU, 0.75–1.25 mM TU was inhibitory while its low range 0.25 and 0.50 mM promoted growth of all varieties. Of the two parts, root was affected more than shoot, as evident from increased shoot:root dry weight ratio in the TU sensitive varieties (Fig. 1a–f). It has been reported that terrestrial plants are generally more sensitive to higher levels of TU (WHO, 2003), but the exact physiological mechanism of TU toxicity to plants is not known yet. Nevertheless, root being directly exposed to soil-applied TU may pose itself as a weakened sink in utilizing assimilates for cellular growth (Herbers and Sonnewald, 1999; Ge *et al.*, 2006).

The TU-induced growth inhibition of maize varieties was due to overall yellowing of leaves and constriction and browning of roots (Table 1). Both symptomatic and quantitative data revealed that overall growth of Pak-Afgoi was the least affected (13%) followed by Agaiti-2002 (29%), EV-5098 (32%) and EV-78 (34%). Varieties EV-77, SWL-2002 and Sadaf indicated moderate inhibition (40, 55 and 55%, respectively) while EV-20 was the most affected (85%). Such reductions are observed as a result of aberrant metabolism due to applied TU (Gunther and Pestemer, 1990). Yellowing results due to loss of leaf pigments, especially the Chl *b* (Pratab and Sharma, 2010; Shah *et al.*, 2011). Although exogenous application of PGRs in appropriate concentrations has been reported to promote photosynthetic pigments (Sabo *et al.*, 2002; Burman *et al.*, 2007; Al-Whaibi *et al.*, 2012), supra-optimal levels are inhibitory (Kavina *et al.*, 2011). In our study, low concentration (0.25–50 mM) of root applied TU improved the photosynthetic pigments contents in most of the maize varieties, whilst higher levels (0.75–1.25 mM) were inhibitory, although marked varietal differences were discernible. Pratab and Sharma (2010) reported that Chl *b* is much prone to adverse conditions. On this analogy, greater contents of Chl *b*, compared to Chl *a*, especially in the tolerant varieties (Fig. 2a–b) suggest it to be an important strategy of maize to cope with TU toxicity.

Increase in Car is yet another important attribute of stress tolerance in plants due to having roles in light harvesting at photosystems and scavengers of reactive oxygen species via xanthophyll cycle in chloroplast (Rmiki *et al.*, 1999; Havaux *et al.*, 2007). In the current study, 0.25 mM TU level appeared to have improved the Car in most of the varieties (Fig. 2c). At higher TU levels (0.75–1.25 mM), TU tolerant varieties (Pak-Afgoi and EV-5098) indicated greater contents of Car and thus declined Chl:Car ratio as compared to sensitive varieties (Fig. 2d). This revealed that TU toxicity was offset by greater concentration of Car in the leaves most likely due to their antioxidative properties (Havaux *et al.*, 2007).

The abovementioned changes were validated by drawing correlations of visual TU-induced symptoms and some quantitative attributes of shoot and root in maize varieties. Negative correlations coefficients (*r*) between leaf yellowing and shoot dry weight, Chl b, Car and Chl:Car ratio revealed that higher levels of root applied TU were much more damaging to sensitive varieties, which appeared to be index of sensitivity of maize to TU. From the positive correlations of shoot dry weight with photosynthetic pigment contents (Table 2) it appears that the maintenance of leaf pigment concentrations in requisite concentrations is important to light reactions of photosynthesis, which generate reducing powers for use in the dark reactions of photosynthesis. Constriction and browning of roots exhibited strong inverse relationship with root dry mass but not its number (Table 2). Available literature suggests that loss of chlorophylls and carotenoids contents is due to deficiency of nutrients (Tejada-Zarco *et al.*, 2004). This suggests that higher levels of TU probably suppressed the water and minerals absorption by roots, thereby causing their deficiency within the plants and yellowing of leaves. However, no such effects were evident at lower (0.25–0.50 mM) TU levels. These findings further supported the notion that root-applied TU is a bioregulator of growth.

A critical analysis of results revealed that growth stimulating role of TU at low levels can be attributed to its multiple roles. It may act either as a nutritional supplement due to having nitrogen and sulfur or as biostimulator of cell growth; a role typical of plant PGRs. The former role of TU appears to be less likely at such low concentrations, while its latter role is more plausible. The stimulation of growth with soil applied TU might be due to its property of inhibiting urease and reduction in the volatilization of NH_3, which is otherwise toxic to the roots, and enhanced the urea use efficiency (Bayrakli, 1990). Srivastava *et al.* (2009), while using low levels of TU (6.5 mM) as seed treatment, reported that it signaled the expression of numerous genes in *Brassica juncea*, most of which were declared as markers of salinity tolerance. In the present case, as evident from symptomatic and quantitative plant attributes at low levels of TU, we infer that at low levels, TU facilitated nutrient acquisition and transport. Nonetheless, in-depth studies are imperative to figure out such a role of TU.

There are great differences in the amount of TU applied to seed, aboveground parts or roots. For seed and foliar use, relatively higher levels (5–10 mM) of TU are to be applied (Garg *et al.*, 2006; Burman *et al.*, 2007; Anjum *et al.*, 2008, 2011) while, as reported here, these levels were lethal when applied to roots. The present study revealed that instead of seed or foliar spray, root application of TU at low levels (0.25–0.50 mM), in addition to being very cost-effective, carries great physiological significance for maize and possibly other plant species.

In conclusion, although maize showed substantial varietal differences, Pak-Afgoi and EV-5098 responded better to root-applied TU, and are suitable for farmer's field. Greater contents of Chl *b* and Car and an improved Chl:Car ratio in the TU tolerant varieties indicated that TU tolerance in maize is genetically related. Although action of TU, envisaged here, points to its role as a candidate plant growth regulator, further studies are needed to explore biological basis of these findings. Nonetheless, use of TU at the lower levels (0.25–0.50 mM) is economical, and likely to have great physiological implications in plant biology.

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