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THE EFFECTS OF THIDIAZURON ON CALLUS DEVELOPMENT AND ORGANOGENESIS FROM MATURE EMBRYOS OF SELECTED TURKISH BREAD AND DURUM WHEAT VARIETIES

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ABSTRACT. The effects of cytokinin-like Thidiazuron growth regulator on the regeneration responses of callus cultures of Turkish bread *Triticum aestivum* L. cv. (Başak 95, Gerek 79 and Bezostaja 1) and durum *Triticum durum* Desf. cv. (Kunduru, Çakmak 79 and Kırmızı 5132) wheat varieties have been investigated in this study.

High callus induction frequencies are found to be independent of bread and durum wheat varieties whereas the callus weight is found to be variety-dependent. For bread wheat, Başak 95 and for the durum wheat, Kunduru is found to be the best performers.

TDZ treatments are found to be negatively affecting the regeneration capacity of all the tested bread wheat varieties whereas for the durum wheat variety of Kunduru positive effect is observed.

Since the culture efficiency is a derivation from the regeneration capacity, this parameter yielded very similar results as in the case of regeneration capacity for both bread and durum wheat varieties.

In bread wheat varieties, the TDZ treatments increased the number of regenerated plants more than 2-fold when compared with the control and likewise very similar results were obtained from durum wheat varieties.

Unfortunately, following their transfer to soil, plants that were treated with various concentrations of TDZ displayed reduced vigor probably due to underdeveloped roots. In addition, majority of these plants did not sufficiently develop above the ground parts when compared with the control plants.

The simplicity and rapid development of shoots using mature embryos could potentially be used for regenerating superior plants following gene transfer studies in the future.

INTRODUCTION

Wheat species is grown across a wide range of environments around the world. For the production of transgenic wheat plants, one of the most important prerequisites is to develop a tissue/cell culture regeneration system.

Within the last 20 years numerous synthetic compounds were tested for their suitability in plant tissue/cell culture systems. Although majority of these compounds were not initially intended for such purposes, a few of them later discovered to be displaying phytohormone-like activities.

One such compound is Thidiazuron (TDZ; **N-phenyl-N'-1,2,3-thiadiazol-5ylurea**), a substituted phenylurea compound. TDZ was first synthesized and marketed as an efficient cotton defoliant under the trade name Dropp (Arndt *et al*., 1976).Then TDZ has emerged as a highly efficacious bioregulant at much lower concentrations in tissue cultures of a diverse array of species with different physiological consequences (Wang *et al*., 1986; Babiker *et al*., 1992; Lin *et al*., 1994; Veneglat and Sawhney, 1994, *etc*).

Although majority of these studies are related with the effects of TDZ on dicotyledonous plant species, very limited information is available regarding the effects of TDZ on monocotyledonous species (Shan et al., 2000).

In the present study, we have investigated the effects of Thidiazuron on the callus development and organogenesis, using mature embryos of selected Turkish bread and durum wheat varieties.

MATERIAL AND METHODS

Plant Material. Turkish bread wheats, *Triticum aestivum* L. cv. Gerek 79, Bezostaja 1, Başak 95 and durum wheats *Triticum durum* Desf. cv. Kunduru, Çakmak 79, Kırmızı 5132 varieties were used as sources of mature embryos.

Surface Sterilization of Seeds and Culture Conditions. Mature wheat seeds were surface-sterilized according to a procedure developed in our laboratory previously (Özgen *et al*., 1998). Callus induction medium, consisting of MS basal salts (Murashige & Skoog, 1969) supplemented with 30 g/l Sucrose + 2 mg/l 2,4-D were adjusted to pH 5.8 and autoclaved for 20 min. at 121 ^{0}C and 1.1 kg/cm2 pressure. Shoot induction medium, consisting of MS basal salts supplemented with 30 g/l Sucrose and various concentrations of TDZ (0, 0,25, 0,50, 0,75 and 1 mg/l) were also sterilized as described above. The root induction medium is basically a hormone-free basal MS medium supplemented with only 30 g/l Sucrose.

Seeds were incubated at 33 ^{0}C for 2 hours in sterilized distilled water for imbibition. Then, mature embryos (c.a. 15 embryos/Petri plate in triplicates) were

aseptically removed and placed, scutellum up, in petri plates containing callus induction medium. They were incubated at 25±1 ^{0}C for 15 days in darkness. The callus tissues (c.a. 15 callus/Petri plate in triplicates) that are formed were transferred to TDZ-containing MS medium for shoot regeneration for 5 weeks at 25±1 ^{0}C in a 16 h light (2000 lux) 8 h dark photoperiod. The shoot regenerated plants (c.a. 15 shoots/Petri plate in triplicates) were transferred into hormone-free rooting medium in baby jars for 5 weeks at 25±1 ^{0}C in a 16 h light 8 h dark photoperiod. Finally, the regenerated whole plants were transferred to pots containing garden soil.

Data and Analysis:

- Weight of Callus is measured by weighing the individual calli.

- Regeneration Capacity = (# of regenerants / # of embryo with callus) X 100
- Number of Plants Regenerated and Transferred to Soil is recorded by counting the number of plants obtained.
- All sets of data were analyzed by using One-Way and Two-Way Analysis of Variance of MINITAB Release 13 Statistical Software Package.

RESULTS & DISCUSSION.

1. Callus induction and Callus Weight

The callus induction frequency of both Turkish bread and durum wheat varieties were observed to be independent of varietal influence (non-significantly different) and extremely high callus induction frequencies were obtained in both species.

However, a definitive varietal influence was observed on callus weights. Statistical analysis indicated that the bread wheat Gerek 79 variety and durum wheat Çakmak 79 variety performed poorly whereas the best performers were found to be Başak 95 (bread) and Kunduru (durum) (*Table 1*) (*Figure 1*).

The observed varietal effect on callus weight is not surprising since it is well known from the results obtained in our laboratory previously (Birsin & Özgen, 2004) and elsewhere (Arzani & Mirodjagh 1999) that genotype plays an important role on wheat development.

2. Regeneration Capacity and Culture Efficiency

A general negative effect of TDZ on the regeneration capacity of bread wheat varieties at high TDZ concentrations (0.75 and 1 mg/L) was observed from overall means and confirmed by statistical analysis (*Table 2*).

However, for the durum wheat varieties TDZ was found to be affecting the regeneration capacity positively for the Kunduru variety (*Table 2*) (*Figure 2*).Within the tested TDZ concentrations, 1 mg/L concentration was found to be affecting the regeneration capacity negatively as seen from the overall means the statistical analyses also confirmed this finding.

3. Number of plants regenerated and transferred to soil

The majority of the plants that were treated with TDZ developed weak roots when compared with the control plants. Regardless of their root strength, plants that were regenerated and transferred to soil were counted and the data were analyzed statistically. The results revealed a strong influence of TDZ on the number of plants

regenerated for both bread and durum wheat varieties. Although, there is a non-significant varietal influence on the number of regenerated plants, concentration of TDZ applications are found to be affecting the number of plants regenerated and transferred to soil significantly. While extremes (hormone free and 1,0 mg/l) caused low level recovery of plants that were regenerated and transferred to soil, mid-ranges (0,25 0,50 and 0,75 mg/l) caused up to 2- fold increases which were also confirmed to be statistically significant for both bread and durum wheat varieties (*Table 3*).

Following their transfer to soil, plants that were treated with various concentrations of TDZ displayed reduced vigor and majority of these plants did not sufficiently develop above the ground parts when compared with the control plants (*Figure 3*). Plants that were treated with TDZ did not developed well-established roots prior to their transfer to soil. The reason for this behavior is probably due to a side effect of TDZ on root morphogenesis (Meyer and van Staden, 1988). As pointed by Lu (1993) for dicotyledonous plants, these unwanted effects of TDZ might be reduced if TDZ exposure is kept less than 8 weeks. Likewise, the same line of argument should be extended to monocotyledonous plant, in this case, especially for bread and durum varieties.

CONCLUSION

1. In this study, we have used mature embryos of wheat varieties as explant material and successfully demonstrated the recovery of callus followed by plant regeneration in response to TDZ treatments. The use of mature embryos for wheat regeneration eliminates the need for immature explant material, and growth of donor plants. Thus, the simplicity and rapid production of shoots from the mature embryo culture could favor its use over the alternative explant sources.
2. While the callus induction frequency was found to be independent of Turkish bread and durum wheat species and varieties within species, the callus weight is found to be dependent on varieties and the best responded varieties are Başak 95 (bread) and Kunduru (durum).
3. While TDZ is found to be slightly reducing the regeneration capacity of bread wheat varieties, a positive effect is observed for durum wheat varieties especially for Kunduru.
4. Within the tested range of TDZ concentrations, 0.75 and 1.0 mg/l were found to have detrimental effects on regenerations events for bread wheat varieties whereas for the durum wheat varieties 1.0 mg/l concentration created a similar result.
5. Although for both wheat species, TDZ is found to be promoting the number of plants regenerated and transferred to soil (up to 2-fold), due to its possible side effects on the root morphogenesis these plants did not sufficiently developed as compared with the controls.

FUTURE PROSPECTS

This study centered on the possibility of using TDZ on wheat tissue culture systems and particular emphasis is given on the regeneration of bread and durum

wheat plants from callus cultures. Therefore for future studies, the following suggestions would be useful:

- TDZ treatments should not exceed 1.0 mg/l and if possible should be kept below 0.75 mg/l.
- For regeneration studies TDZ exposure should not exceed 8 weeks.
- For increasing the rooting of TDZ-treated regenerants, low concentrations of natural auxins like IBA should be tested in order to counter the accumulated-effects of TDZ.

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Table 1. Weight of callus of Turkish bread and durum wheat varieties.

		Weight of
Bread wheat varieties	**Başak**	
	Bezost	0.95 ±
	Gerek 79	0.78 ± 0.0403
Durum wheat varieties	**Kund**	0.94 ±
	Cakm	0.80 ±
	Kırmızı 5132	0.89 ± 0.0301

* - *Significantly different at P<0.01.*

Table 2. Regeneration capacity of callus and culture efficiency of Turkish bread and durum wheat varieties.

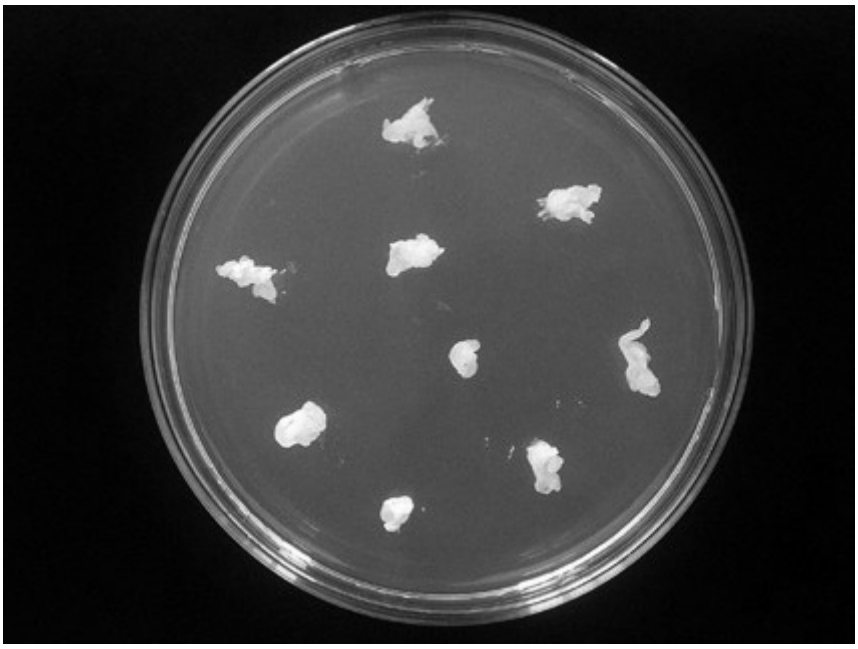
** *-Significantly different at P<0.01. Mean ± St.Err.*

TDZ (mg/l)	Bread wheat varieties			Overall Means	Durum wheat varieties			Overall Means
	Ba ak 95	**Gerek 79**	**Bezostaja 1**		**Kunduru**	**3akmak 79**	**K rm z 5132**	
	No of plants regenerated	No of plants regenerated	No of plants regenerated		No of plants regenerated	No of plants regenerated	No of plants regenerated	
0.0 (Control)	6.66 ± 0.33	6.00 ± 1.15	8.00 ± 1.53	***6.88 ± 0.58***	**5.66 ± 0.33**	**6.00 ± 1.15**	**5.66 ± 0.88**	***5.77 ± 0.11***
0.25	12.66 ± 1.45	10.00 ± 1.00	12.33 ± 0.88	***11.66 ± 0.83*****	**6.33 ± 0.33**	**11.33 ± 1.33**	**10.66 ± 0.33**	***9.44 ± 1.56*****
0.50	11.66 ± 0.88	12.00 ± 0.57	13.33 ± 0.33	***12.33 ± 0.50*****	**11.66 ± 0.66**	**12.33 ± 0.33**	**9.66 ± 0.66**	***11.21 ± 0.80*****
0.75	11.33 ± 0.33	13.66 ± 0.33	8.00 ± 2.52	***10.99 ± 1.64*****	**11.33 ± 1.20**	**12.00 ± 0.57**	**7.33 ± 0.88**	***10.22 ± 1.45*****
1.0	9.33 ± 0.333	7.33 ± 1.86	10.33 ± 1.45	***8.99 ± 0.88***	**8.33 ± 0.66**	**3.33 ± 0.33**	**6.33 ± 0.66**	***5.99 ± 1.45***

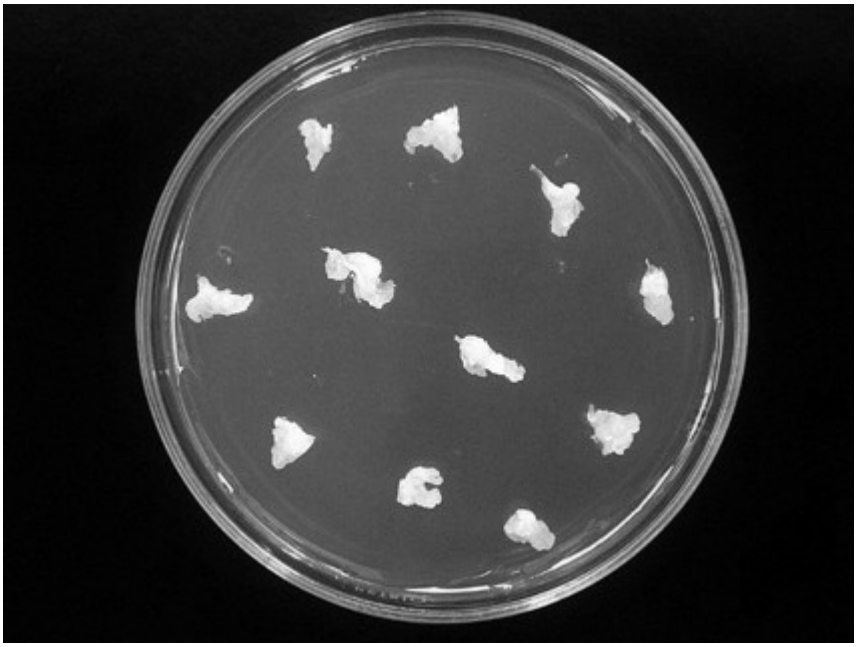
TDZ (mg/l)	Bread wheat varieties			Overall Means	Durum wheat varieties			Overall Means
	Ba ak 95	**Gerek 79**	**Bezostaja 1**		**Kunduru**	**3akmak 79**	**K rm z 5132**	
	Regeneration capacity of callus (%)	Regeneration capacity of callus (%)	Regeneration capacity of callus (%)		Regeneration capacity of callus (%)	Regeneration capacity of callus (%)	Regeneration capacity of callus (%)	
0.0 (Control)	95.55 ± 2.22	100 ± 0.00	93.33 ± 6.67	***96.29 ± 1.96***	91.10 ± 4.45	86.66 ± 6.67	88.88 ± 2.22	***88.88 ± 1.28***
0.25	95.55 ± 4.45	79.99 ± 6.67	93.33 ± 6.67	***89.62 ± 4.85***	87.77 ± 2.94	93.33 ± 3.85	93.33 ± 3.85	***91.47 ± 1.8***
0.50	86.02 ± 7.30	100 ± 0.00	97.77 ± 2.22	***94.59 ± 4.33***	100 ± 0.00	91.11 ± 5.88	88.88 ± 2.22	***93.33 ± 3.39***
0.75	88.88 ± 2.22	79.99 ± 6.67	77.77 ± 4.44	***82.21 ± 3.39*****	93.33 ± 3.85	100 ± 0.00	97.77 ± 2.22	***97.03 ± 1.96***
1.0	86.50 ± 3.71	46.66 ± 6.67	86.66 ± 3.85	***73.27 ± 13.3*****	95.23 ± 4.76	35.53 ± 2.23	53.3 ± 3.87	***61.35 ± 17.6*****
Overall Means	90.50 ± 2.12	81.32 ± 9.75	89.77 ± 3.49		93.48 ± 2.05**	81.32 ± 11.6	84.43 ± 7.96	

Table 3. The number of plants regenerated of Turkish bread and durum varieties.

** *- Significantly different at P<0.01. Mean ± St.Err.*.



A



B

Fig. 1. *Representative photographs of Çakmak 79 (Panel A) and Kunduru (Panel B) callus induction events on 15th days of inoculation.*

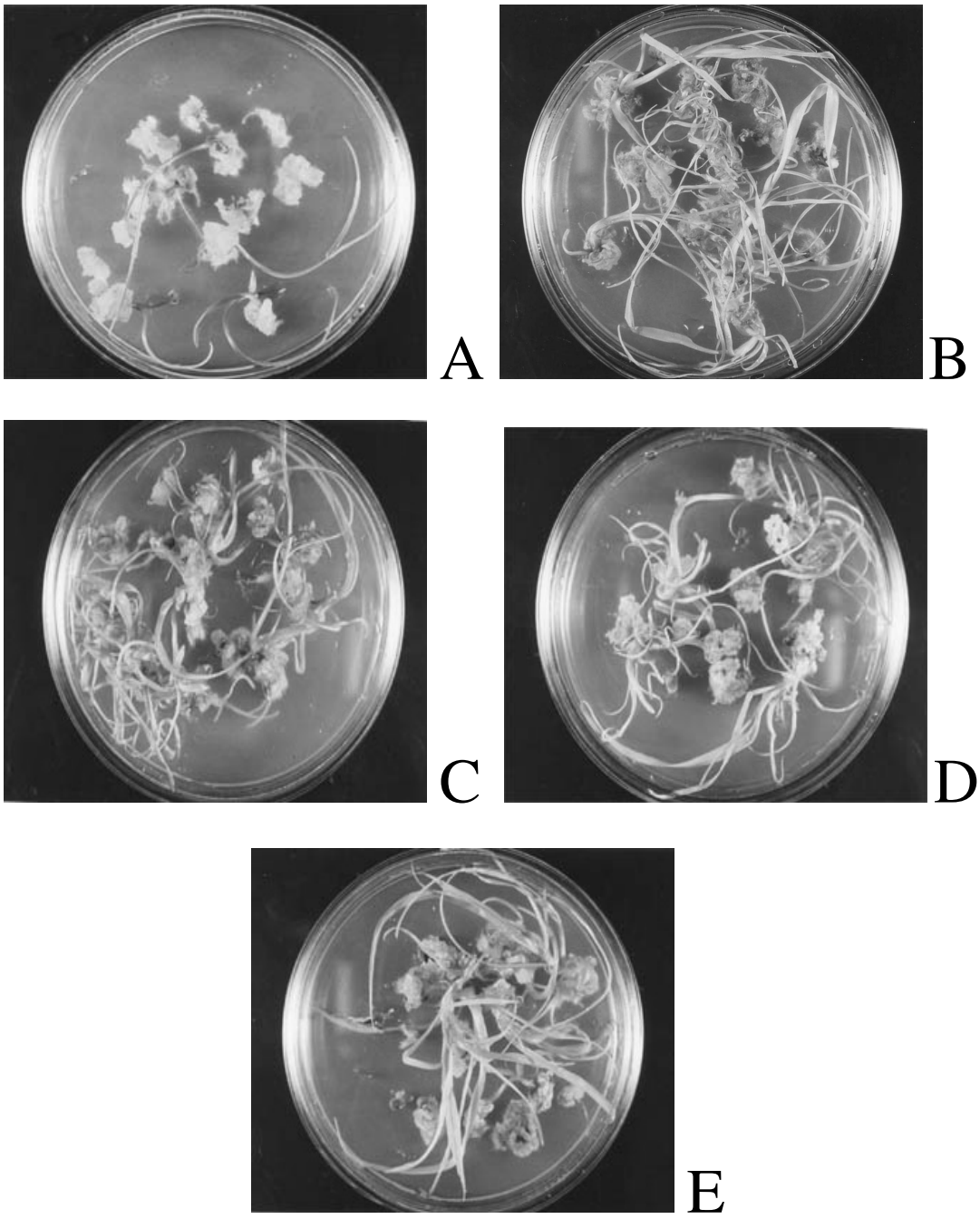


Fig. 2. *Representative photographs of Kunduru callus regeneration events on 5th weeks of inoculation. Panel A: Control, B: 0.25 mg/l TDZ, C: 0.50 mg/l TDZ, D: 0.75 mg/l TDZ and E: 1.0 mg/l TDZ.*

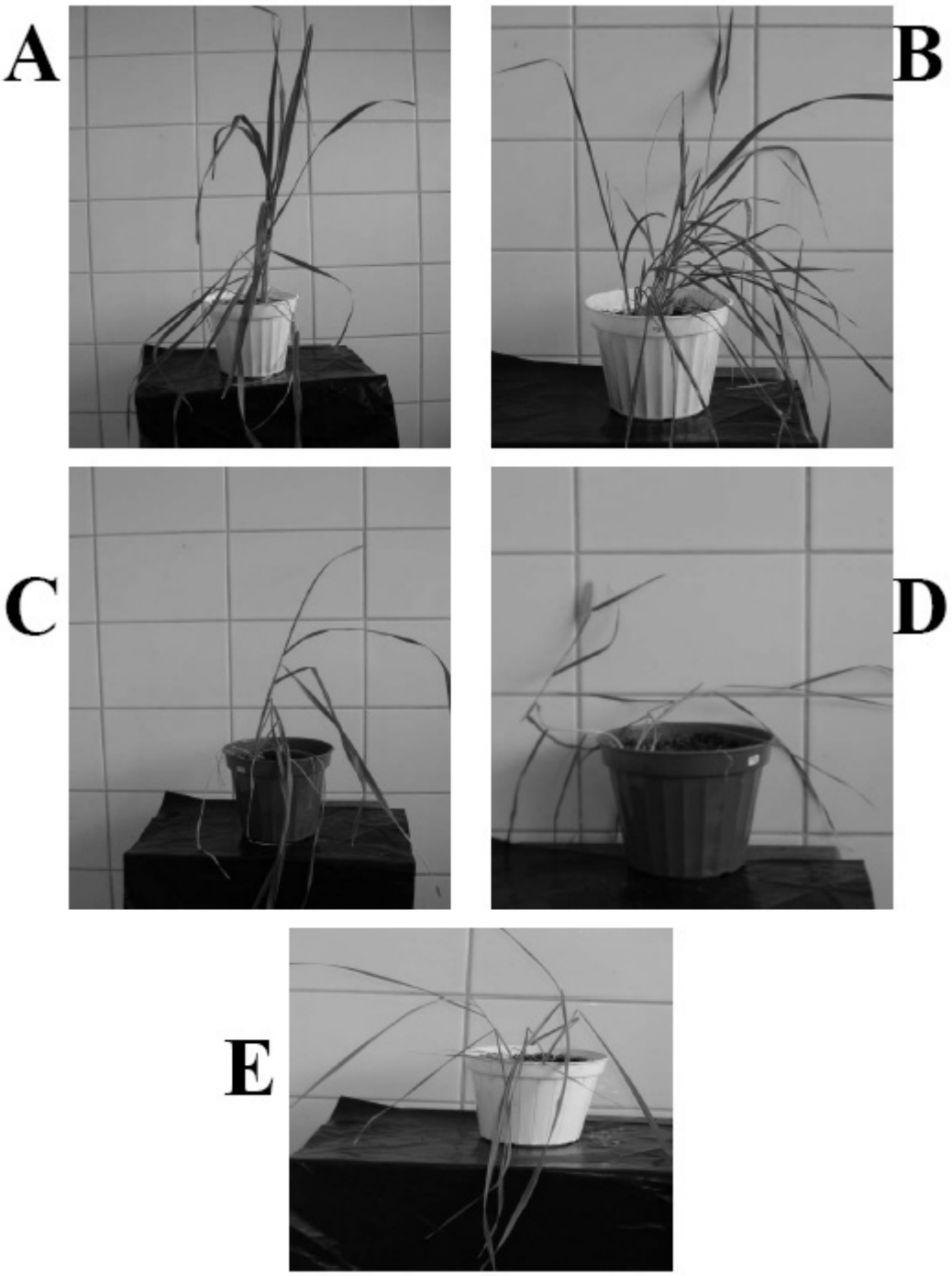


Fig. 3. *Representative photographs of Bezostaja 1 regenerants that were transferred to soil after rooting. Panel A: Control, B: 0.25 mg/l TDZ, C: 0.50 mg/l TDZ, D: 0.75 mg/l TDZ and E: 1.0 mg/l TDZ.*