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Thidiazuron (TDZ) Induced in Vitro Axillary Shoot Regeneration of Desi Chikpea (*Cicer arietinum* L.)

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Abstract
Desi Chcikpea (Cicer auritinum L.) is samll sized chickpea of blackish color with rough seed coat. Desi Chcikpea is very popular in Indian subcontinent due to its use as in making food and also as medicinal plant especially against Diabetes. Desi chickpea is also cultivated crop in SouthEastern Anatolian region of Turkey. shoot tip and cotyledonary node explants excised from in vitro germinated seeds were inoculated on MS medium fortified with different TDZ concentrations (0.25-3.0 mg/L). Multiple shoot formation without any callusing was evidented after four weeks of culture. Both explants and interactive effect of explants X TDZ concentration was insignificant. Whereas, TDZ concentrations yielded 93.33-100.00% shoot formation frequency. Contrarily, explants, TDZ concentration and interactive effect of explants X TDZ concentration was statistically significant. Shoot tip explant yielded 12.54 shoots compared to 11.46 shoots for cotyledonary node. The highest shoot counts of 17.90 were recorded for medium augmented with 0.25 mg/L TDZ. Similarly, maximum shoot counts from explants X TDZ concentration were also recorded on medium augmented with 0.25 mg/L TDZ for both explants. In vitro regenerated shoots were shorter in length and failed to root on the rooting medium enrched with 0.25-1.00 mg/l IBA. The results revealed that both explants can be used for multiple shoot formation of desi chickpea. However, it is recommended to use other explants and plant growth regulators to obatin normal shoots for rooting and acclimatization.

Keywords: Desi chickpea, in vitro, Shoot tip, Cotyledonary node

INTRODUCTION

Chcikpea (*Cicer auritinum* L.) is one of the most cultivated legume crop since ancient time [1] due to its high nutritional value. It ranks second among legumes in the modern World followed by common beans and cultivated in more than 50 countries [2].The major contributors of chickpea are India, Pakistan and Turkey [3]. Two types of cultivated chickpea known as Kabuli and Desi [4,5] are the most commonly grown chickpea [6]. In Turkey, Kabuli type chickpea is cultivated [7] like other Mediterranean countries [8]. However, desi type chickpea is also cultivated in some part of Turkey [3] especially in South Eastern Anatolian region. Desi Chcikpea is normally samller sized with rough seed coat [7]) of different colour like brown or balck [5]. However, South Asian countries are major producers of desi chickpea [8], where it is used for making dhal and flour [6]. The nutritional value of desi chickpea is almost similar to Kbuli type with slight variation in carbohydrates [9], protein contents and digestibility [10]. Desi Chickpea is considered to be a good medicinal plant due to its usage against diabetes [11] and minimizing the cardiovascular risk [12].

Application of in vitro plant tissue culture techniques enables the researchers to improve plant of economic importance. Kabuli Chcikpea is one one of the recalcitrant plant for plant tissue culture and several reports highlight the developmement of in vitro tissue culture techniques by using different plants/genotypes, explants, plant growth regulators, culture medium and culture conditions [13-19]. Contrarily, there is no report about in vitro shoot induction of Desi Chickpea. Keeping in view, this study was designed to investigate the in vitro response of two different explants exposed to different concentrations of TDZ.

MATERIALS AND METHODS

The seeds of desi chickpea were procured from Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir, Turkey. Seeds were exposed to commercial bleach containing 5% NaOCl for 5 min and stirred thrice with sterilized water for 5 min to remove the trashes of NaOCl. After sterilization process, seeds were inoculated on MS [20] medium for germination of seeds. Two different explant used for in vitro shoot formation were shoot tip and cotyledonary node. Both explants were simultaneously taken from 12-14 days old seedlings and cultured on MS medium fortified with different concentrations of TDZ ranged from 0.25-3.0 mg/L (Table 1). After 10 weeks of culture, in vitro shoots around 1.0 cm length were isolated aseptically followed by culture on MS medium augmented with 0.25-1.0 mg/l Indole-3-butyric acid (IBA).

The MS medium used in this study was made by using 0.44% MS, 3.0% sucrose and 0.65% agar was used for solidifying the medium. The pH of the medium was maintained to 5.8 after adding TDZ in the culture medium. The culture conditions for both in vitro shoot formation and rooting was in the growth room maintained at temperature of 24 ± 2 °C and equipped with White Light Emitting diodes (LEDs) for 16-h light photoperiod.

The experimental design was double factorial (explants and TDZ concentrations) in split plot arrangement ahving three replicates with 5 explants per replicate. After completion of experiment, data about shoot formation frequency (%) and shoot counts per explant were taken and tabulated followed by performing analysis of variance (ANOVA) using SPSS 20.00 for Windows. The post hoc tests were performed using Duncan's multiple range test to compare the differences among treatments. Data given in percentages were subjected to arcsine square root transformation [21] before statistical analysis.

RESULT AND DISCUSSION

Selection of proper explant alongwith type and concentration of plant growth regulators regulates the in vitro shoot formation especially for recalcitrant legumes plants [19, 22]. Chickpea is also supposed to be recalcitrant plant for in vitro plantlet regeneration due to facing problems during rooting or adaptation [17]. Shoot tip or cotyledonary nodes explants taken from in vitro germinated seedilings are very potent for inducing shoots due to presence of actively dividing meristems. The potential of these meristematic regions can be manipulated by applying exogenous PGRs and other culture conditions which in turn induced multiple shoots. Both explants have been used successfully for inducing shoots of other legumes [23] and Kabuli chickpea also. Shoot tip explants for inducing shoots of Kabuli chickpea have been reported by [18, 24-26]. Whereas, cotyledonary node explant has also been used for in vitro shoot regeneration of chcikpea [27-28]. On the other hand, TDZ is cytokinin type PGRs [29-30] and even low concenration of TDZ in the culture medium proved to be sufficient for multiple shoot induction [31-33]. Number of studies also revelaed the use of TDZ either alone or in combination with auxins for kabuli type chickpea [13-15, 19, 28, 34-35].

The present study reports the use of two most potent explants for multiple shoot induction of desi chickpea in repsonse to different concnetrations of TDZ. The response of both explants towards in vitro shoot regeneration behaviour was identical to TDZ concentrations. There was direct shoot induction from both explants within 2 weeks followed by multiple shoots within 4 weeks. However, there was no sign of callus induction from both explants until to the end of the experiment. The results are contrarilt to the previous report of [36] who achieved green calli in response to TDZ.

Both explanst responded well to TDZ concentrations and resulted in high shoot formation frequency. Shoot formation frequency of both explants were same and recorded as 98.89%. TDZ concentration almost resulted in 100% shoot formation except a single concentration of 0.50mg/L which yielded 93.33%. Parveen et al [18] used different concentrations of TDZ and achieved shoot formation frequency of 53-85%. Comparasion of explant x TDZ concenration also yielded the same response as 0.50mg/L TDZ induced 93.33% shoot formation and rest of all concentrations yielded 100% shoot formation (Table 1). Variable response of PGRs on shoot induction in chcikpea has been highlighted by Al-Tanbouz and Abu-Qauod [19].

Table 1. Comparative effects of explants and TDZ concentartion on in vitro shoot formation (%) of desi chickpea (Cicer arietinum L.)

Explant	TDZ (mg/L)	Shoot formation frequency (%)	Shoot counts
Shoot tip	-	98.89^{ns}	*12.54a
Cotyledonary node	-	98.89	11.46b
-	0.25	100.00a*	17.90a**
-	0.50	93.33b	8.77b
-	1.00	100.00a	10.00b
-	1.50	100.00a	15.60a
-	2.00	100.00a	9.13b
-	3.00	100.00a	10.60b
Shoot tip	0.25	100.00^{ns}	17.13ab**
	0.50	93.33	6.20d
	1.00	100.00	12.20abcd
	1.50	100.00	15.07ab
	2.00	100.00	11.27bcd
	3.00	100.00	13.40abc
Cotyledonary node	0.25	100.00	18.67a
	0.50	93.33	11.33bcd
	1.00	100.00	7.80cd
	1.50	100.00	16.13ab
	2.00	100.00	7.00cd
	3.00	100.00	7.80cd

**significant (P<0.01) using DMRT, *significant (P<0.05) using DMRT, ns:nonsignificant;

The results regarding impact of explants (p≥0.05), TDZ concentrations (p≥0.01) and explant x TDZ concenration (p≥0.01) on shoot counts is given in Table 2. Although, both explants produced shoots counts very close to each other, shoot tip explants generated relatively slighter more shoots (12.54) compared to cotyledonary node (11.46). The response of TDZ concentrations was highly variable as highest shoot counts (17.90) were recorded from medium with 0.25 mg/L TDZ. Yosefiara et al [14] also achieved maximum shoot counts from TDZ medium uased at the rate of 0.2 mg/L. They also reported decreased shoot counts with increased TDZ concentration. Similarly, low concentration of TDZ have been proven more efficent for inducing multiple shoots of chickpea [13,15,19]. In this study, similar pattern was also followed by TDZ concentrations but 1.50 mg/L TDZ induced shoot counts of 15.60. The difference was due to response of both explants which yielded more shoots. Whereas, both explants responded in variable way to rest of the each TDZ concentration. Contrarily, Parveen et al [18] reported highest shoot counts of chickpea using shoot itp explant cultured on medium containing 3.0 mg/L TDZ. The interactiv effect of explant x TDZ concenration highlighted it clearly as cotyledonary node generated more

shoots (11.33) compared to shoot tip (6.20) on medium with 0.50 mg/L TDZ. Contrarily, shoot tip explants was more responsive than cotyledonary node explants at higher concentrations of 2.0 and 3.0 mg/L TDZ (Table 2). The results suggest that shoot count is dependant on explant type and need specific TDZ concentration. Similarly, Kadri et al [36] reported variable response of each cultivar in response to TDZ concentration.

Although, TDZ induced multiple shoots from both explants, these shoots were very short (less than 1 cm in length) after 8 weeks of culture. Therefore, data regarding shoot length were not tabulated. Contrarily, longer shoots of chcikpea in response to TDZ concentration was reported by Anwar et al. [15] and Parveen et al [18]. The longer shoots (near to 1 cm in length) were cut off from explants for rooting and cultured on medium having 0.25-1.0 mg/L IBA. All shoots failed to induce rooting might be due to suppressive effect of TDZ in the culture medium [37] which produced abnormal shoots with shortler length and lighter in colour. Abnormal or deformed shoots in response to TDZ concentartion have been evidented by many reserachers in chickpea [14-15] and other legumes [38-40]. Rooting of in vitro induced shoots of Kabuli chickpea is considered to be relatively difficult with zero rooting [41] or less responsive to exogenous application of auxins [16, 42]. On the other hand, several reports successfully highlights the rooting followed by acclimatization of chickpea [15, 17].

Exploitation of explants for in vitro shoot regeneration is an important step for developing in vitro shoot regeneration protocol by exposing teh explants to different PGRs at different concentrations. In this study, both explants responded well to different TDZ conecnetrations and its suggest that these explants can be used in future for application of other biotechnological tools. However, there is need to do more research on developing normal shoots for rooting and acclimatization of desi chikpea**.**

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