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Influences of Thidiazuron Concentrations and Exposure Duration on Axillary Shoot Proliferation of Faba Bean [*Vicia faba* (L.)] Genotypes

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ABSTRACT

Background: Faba bean is one of the most important feed grain legume crops in the world. However, this crop is a recalcitrant species for *in vitro* regeneration.

Methods: In this study, *in vitro* shoot proliferation of ten faba bean genotypes, that are classified according to their seed size into small, medium and large, were investigated in response to different light regimes and thidiazuron (TDZ) treatments.

Result: Incubation under different light regimes, explant type as well as genotypes influenced seedling growth and axillary shoot multiplication of faba bean. The explants with single cotyledon and incubated under continuous darkness for 6 days resulted higher number of shoot than nodal explants. Medium size seeded genotype (ILB4347) produced the highest shoot and root length and shoot proliferation as compared with large (Luz) or small (Triple White) seeded genotypes. The effects of TDZ concentrations (2-10 μM/L) and exposure duration (10 and 20 days) on axillary shoot multiplication were investigated for the ten faba bean using cotyledonary explants. The overall mean of TDZ time exposure showed that treatment of 20 days exposure had higher number of shoot compare to 10 days exposure treatment with 3.77 shoot per explant. On the basis of genotype used, the three most responsive genotype in tissue culture were medium sized seeds genotypes (ILB4347, Hassawi 2 and Misr 3 with 4.76, 4.40 and 4.15 shoot per explant, respectively). ILB4347 were the most responsive genotype in tissue culture. Treatment of 8 μM TDZ after 20 days followed by 2 weeks on MS medium without PGRs generated the highest proliferation with 6.63 shoot per explant. These findings contribute towards effective *in vitro* propagation of faba bean.

Key words: Direct organogenesis, Fabaceae, *In vitro,* Micropropagation.

INTRODUCTION

Faba bean (*Vicia faba* L.; Fabaceae) is an excellent source of protein and is an important legume crop for human food and animal feed (Paul and Gupta, 2021). As a leguminous species, faba bean also plays a significant role in the organic fixation of nitrogen from the atmosphere (Adak and Kibritci, 2016; Jelenić *et al*., 2000). Despite the significance of this crop, there has been little progress in its improvement because of its high out-crossing rate (as high as 50%). In addition, this crop shows yield instability and, in general, a low level of genetic diversity which restricted its genetic improvement. Faba bean is a recalcitrant species for *in vitro* regeneration (Khalafalla and Hattori, 2000). It exhibits poor regeneration from callus and tissue cultures are often inhibited by the release of phenolic compounds from the tissues. For these reasons, it is difficult to conduct *in vitro* studies on this crop (Böttinger *et al*., 2001). Faba bean is one of the least-studied legumes and only a few reports describe *in vitro* culture. Previous tissue culture studies used individual seedlings (Busse, 1986), micro-cuttings and auxiliary buds (Selva *et al*., 1989), cotyledonary nodes (Khalafalla and Hattori, 1999) and protoplasts (Tegeder *et al.,* 1995) as explants. Anwar *et al*., (2011) used cotyledon explants with half embryonic axis in faba bean cultivars for indirect regeneration system (Adventitious shoot proliferation). These studies correlated regeneration efficiency to the culture conditions, such as the effect of hormones, the composition of the medium, or other physical conditions. Moreover, genotypes have shown to impact the faba bean regeneration. Ahmed *et al.* (2020) evaluated the growth, morphological changes and production of phenolics in the *in vitro* plantlets of five Egyptian faba bean cultivars. The authors revealed that cultivars 'Nubaria 2' and 'Skha 3' had the highest growth parameters and regeneration frequency (85.3% and 78.6%), respectively.

Thidiazuron (TDZ) is a powerful and potent synthetic growth regulator that has a wide array of effects on plant growth and development. However, prolonged exposure to

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TDZ can have negative effects on growing cultures and transferring the cultures to a medium without TDZ can alleviate these effects and promote further shoot proliferation and multiplication (Dewir *et al*., 2018). TDZ has been found to have both positive and negative effects on the growth and development of faba bean *in vitro*. TDZ has been shown to be effective in inducing adventitious bud formation and promoting shoot proliferation in faba bean explants (Anwar *et al*., 2011). However, high concentrations of TDZ can be toxic to plantlets (Khalafalla and Hattori, 1999). Additionally, the use of TDZ in faba bean tissue culture has been associated with morphological, physiological and cytogenetic abnormalities (Tegeder *et al*., 1995). Despite these drawbacks, TDZ has been successfully used in the regeneration and genetic improvement of faba bean through micropropagation and transformation protocols.

Overall, TDZ can be a valuable tool for the *in vitro* manipulation of faba bean, but its application should be carefully optimized to avoid negative effects (Dewir *et al*., 2018). An indirect somatic embryogenesis regeneration system of faba bean was reported by Griga *et al*. (1987). Reproducible regeneration was reported for faba bean using single cotyledon explants with half embryonal axis and cotyledonary node (Khalafalla and Hattori, 2000; Anwar *et al*., 2011) indicating that TDZ allows for successful micropropagation of faba bean, which is essential for future genetic improvement of faba bean. However, these studies also reported that the response of faba bean genotype differ among faba bean genotypes. Therefore, the objectives of this study were to screen the responsive faba bean genotypes of different sizes to different TDZ concentrations and exposure durations for their axillary shoot proliferation *in vitro*.

MATERIALS AND METHODS

Plant materials, surface sterilization and culture establishment

This study was conducted during the year 2020 at the plant tissue culture laboratory, College of Food and Agricultural Sciences, King Saud University. Ten genotypes of faba bean (*Vicia faba* L.) were obtained from ICARDA, Sudan, Spain, Egypt and Saudi Arabia. The genotypes name, pedigree, origin, seed type and agronomic features are listed in Fig 1A and Table 1. The selection criteria for those genotypes are based on origin and seed type (large, medium and small), to represent the Saudi landraces, Middle East, North Africa and Europe. Seeds were washed with tab water, then surface sterilized with 70% (*v/v*) ethanol for 30 seconds, followed by soaking in 20% (*v/v*) commercial Clorox™ (5.2% sodium hypochlorite) solution for 15 min. The seeds were washed five times with sterile distilled water under aseptic conditions prior to soaking overnight. The imbibed seeds were de-coated and cultured for 6 days into Petri dishes that contained 25 mL MS (Murashige and Skoog, 1962) medium without PGRs for their germination under dark conditions (Fig 1B). Prior to autoclaving at 121°C for 20 minutes, the pH of all the medium variants was adjusted to 5.7. Additionally, the medium was gelled with 0.7% (w/v) Sigma agar-agar. All the cultures were incubated at 25±2°C under a 16 h photoperiod provided by cool-white fluorescent tubes. The intensity of the lights was set at 35 µmol m^{-2} s^{-1} photosynthetic photon flux (PPF). The length of shoots and

Table 1: List of faba bean (*Vicia faba*) genotypes and their agronomic characteristics.

Genotype	Pedigree	Origin	Plant height	No. of branches per plant	No. of seeds per plant	100-seed weight (g)	Seed weight per plant (g)
Large size seed :>110 g/100 seeds*							
Luz	-	Spain	47.40	3.60	25.80	112.13	28.76
Medium size seed : 60-110 g/100 seeds*							
Hassawi 1	Landraces	KSA	42.75	3.80	45.00	62.17	22.88
Hassawi 2	Landraces	KSA	46.50	3.20	31.60	72.74	23.34
Hassawi 3	Landraces	KSA	56.00	3.20	30.60	77.28	20.60
ILB4347	ILB 4347	ICARDA	53.60	3.80	27.00	86.31	24.54
Misr 3	Line 667×(Cairo 241×Giza 461)	Egypt	58.60	4.60	54.40	78.14	34.73
Sakha 1	85/283/620×88/724/716	Egypt	53.40	4.80	56.80	93.01	52.64
Sakha 2	Reina Blanka×461/845/83	Egypt	54.40	4.80	42.00	82.31	37.67
Small size seed:<60 g/100 seeds*							
Gazira 2	Landraces	Sudan	49.33	2.30	44.33	40.46	17.40

(Data collected from genotypes grown at King Saud University Experimental Farm).

KSA: Kingdom of Saudi Arabia, ICARDA: International Center for Agricultural Research in Dry Areas. *Classification based on Cubero and Nadal (2015).

roots was recorded for Luz (large seeded); ILB4347 (medium seeded) and Triple white (small seeded). All measurements were obtained from 10 randomly selected explants.

Effect of explant type (cotyledonary node with and without single cotyledon) and light regime on shoot multiplication and growth of faba bean

De-coated seeds (Fig 2A,B) of three genotypes (Luz, ILB 4347 and Triple White) were cultured on basal MS medium supplemented with 30 g/L sucrose and incubated under three different light regimes (T1: 6 days in dark, T2: 3 days in dark followed by 3 days in light and T3: 6 days in light) and two different explants (cotyledonary nodes explant consisting of 5 mm hypocotyl and epicotyl tissues with or without single cotyledon) as described in Fig 2 C,D. The explants were then cultured onto MS medium without PGRs for two weeks. The illumination was set at 16:8 photoperiod provided by cool white fluorescent tubes and light intensity of 100-150 µmol m^{-2} s^{-1} PPFD. The shoot growth and shoot multiplication responses were assessed following 2 weeks culture period whereby, number of axillary shoots developed per explant and shoot length were recorded. All measurements were obtained from 10 randomly selected explants.

Effect of thidiazuron concentration and exposure duration on axillary shoot multiplication of faba bean genotypes

For axillary shoot multiplication, cotyledonary node with single cotyledon explants of the ten genotypes (Table 1) were cultured in Magenta GA-7 culture vessels (77×77×97 mm; Sigma Chemical Co., St. Louis, Missouri, USA) that contained MS medium supplemented with 30 g L^{-1} of sucrose and five different concertation of thidiazuron (TDZ; 2, 4, 6, 8 and 10 µM) and two different duration of exposure to TDZ at 10 or 20 days. The regenerated axillary shoots from explants were sub-cultured onto MS medium for 2 weeks without PGRs for their elongation. All cultures were incubated at 25±2°C under cool-white fluorescent tubes that provided a 16:8 h photoperiod and light intensity of 100-150 µmol m^{-2} s^{-1} PPFD. All measurements were obtained from 10 randomly selected explants.

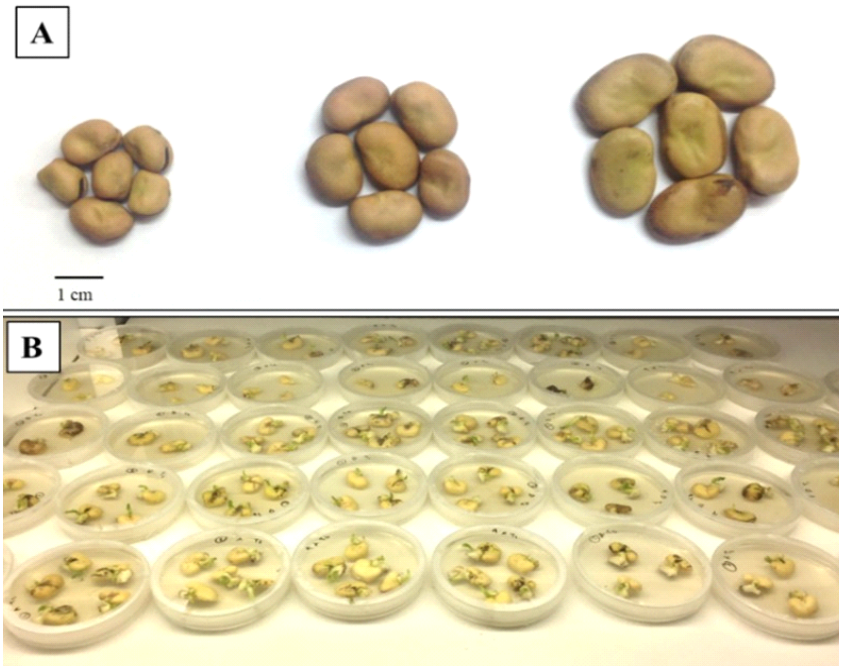
Experimental design and data analysis

All experiments were conducted in a completely randomized design. The impact of the treatments was evaluated using Tukey's multiple range test in SAS (version 9.4; SAS Institute, Inc., Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Effect of light regime and explant type on axillary shoot multiplication and growth of faba bean seedling

The seedling growth (shoot and root length) of three faba bean genotypes under three different light regimes are presented in Table 2 and Fig 3. The overall mean of shoot and root length showed that T1 treatment gave higher shoot and root length followed by T2 and T3 . It is noted that medium size seeded genotype ILB4347 showed the highest shoot and root length mean with 3.57 cm and 5.11 cm, respectively, as compared with large and small seeded



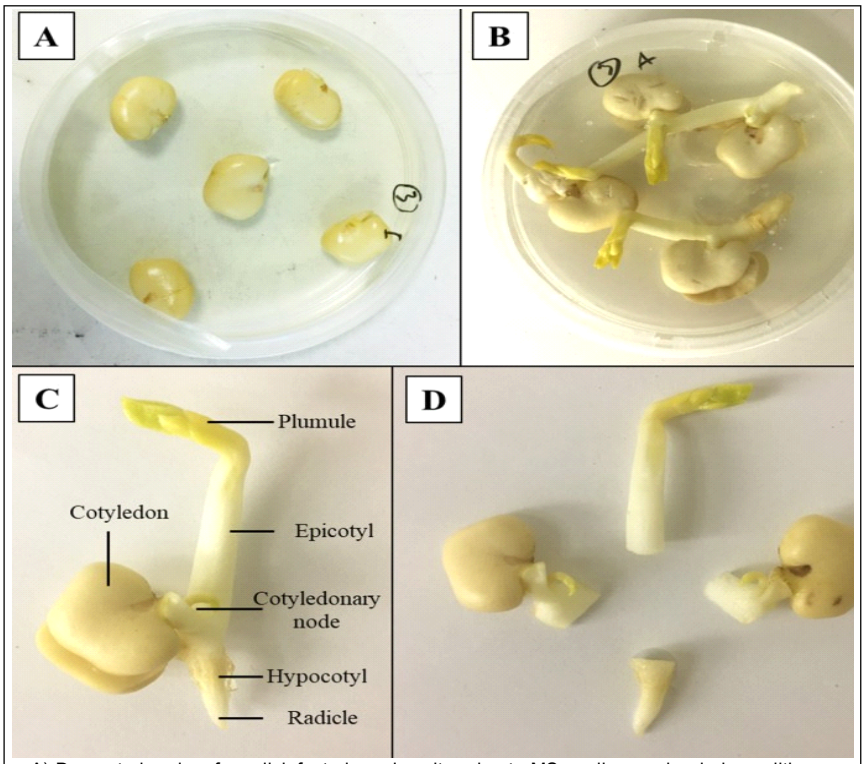
A) Seed size classifies as small, medium and large, B) Disinfected seeds of different sizes cultured onto MS medium under dark conditions.

Fig 1: Establishment of *in vitro* culture of faba bean (*Vicia faba*).

genotypes. After that, the seedlings were used as source of explants by excising seedlings into half containing 5 mm hypocotyl and epicotyl tissues with or without single cotyledon. The two different explants were culture on MS basal media for 2 weeks. The average of number of shoot generated by each treatment is presented in Table 3. The explants with single cotyledon gave higher number of shoot than explant without single cotyledon with an average of 1.13 shoot (Fig 3B). On the basis of genotype used, ILB4347 on explant with single cotyledon generated the highest number of axillary shoot followed by Luz and Triple White. This result was positively correlated (r=0.657) with seed size (100-seed weight). The overall results showed that explant with single cotyledon treated by T1 treatment gave the highest axillary shoot with 1.22 shoot. Although, the value is not significantly different from the other light incubation treatments, but T1 treatment produced more axillary shoot regardless of explant source. Therefore, explant with single cotyledon and T1 treatment were used in the further experiment. Shoot length of each treatment was also measured and the results were presented in Table 4. The explants containing single cotyledon had longer shoot compared explants without cotyledon with 11.05 cm compared to 5.89.

Effect of TDZ concentrations and time of exposure (TE) on axillary shoot multiplication of faba bean genotypes

Effect of TDZ concentrations and time of exposure on shoot multiplication of ten faba bean genotypes is presented in Table 5. The overall mean of time exposure of TDZ showed that treatment of 20 days exposure had higher number of shoot compare to treatment 10 days exposure with 3.77



A) De-coated and surface disinfected seeds cultured onto MS medium under dark conditions, B) germinated seedlings *in vitro*, C and D) Different explants used for *in vitro* shoot multiplication.

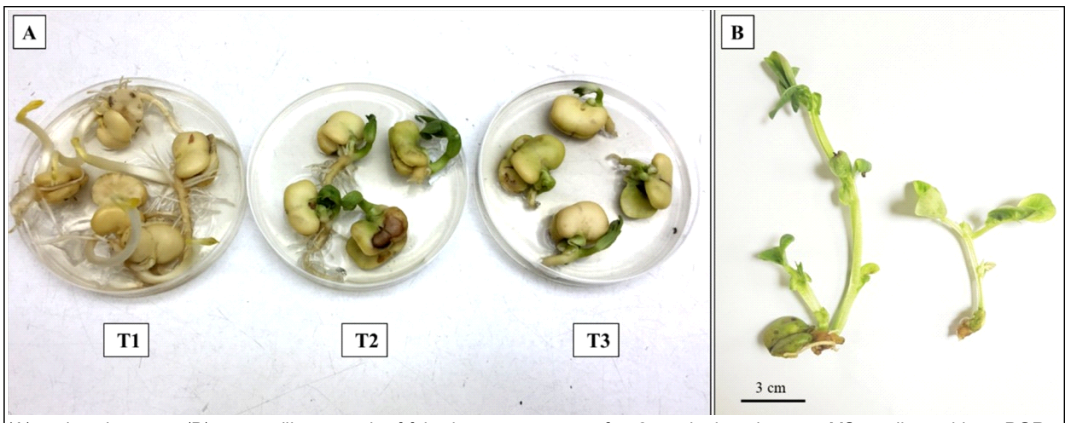
Fig 2: Establishment of in vitro culture of faba bean (*Vicia faba*).

Table 2: Effect of light incubation regime on seedling growth of faba bean genotypes after 6 days in culture.

Genotype	Shoot length (cm)				Root length (cm)			
	T1	T2	T3	Mean	T1	T2	T3	Mean
Luz (Large seeded)	3.77	1.58	1.20	2.18 ^{ns}	3.57	0.95	0.90	1.81 ^{ns}
ILB4347 (Medium seeded)	6.33	3.05	1.33	3.57 ^{ns}	11.23	3.05	1.05	5.11 ^{ns}
Triple white (Small seeded)	5.60	3.43	1.65	3.56 ^{ns}	1.20	3.27	1.00	1.82 ^{ns}
Mean	5.23 a	2.69 b	1.39 b		5.33 ns	2.42 ns	0.98 ns	

T1: 6 days in dark, T2: 3 days in dark followed by 3 days in light and T3: 6 days in light. different letters denote statistical difference ($P \leq 0.05$) according to Tukey's multiple range test, ns= Not significantly difference.

shoot/explant. Treatment of 10 µM TDZ after 20 days in culture generated the highest number of shoot/explant with 4.81 shoot/explant. On the basis of genotype used, the three most responsive genotype in tissue culture were ILB4347, Hassawi 2 and Misr 3 with 4.76, 4.40 and 4.15 shoot/explant, respectively. The clumps of multiple shoots were then cultured on MS without PGRs for shoot elongation (Fig 4A, 4B). Interestingly, the number of shoot/explant increased after 2 weeks in culture (Fig 4C) indicating remaining effect of TDZ in the explant. The shoot multiplication of ten faba bean genotypes after 2 weeks culture on MS free PGRs is presented in Table 5. The number of shoot/explant of genotype ILB4347 and Hassawi 2 increased to 6.26 and 6.10, respectively. Treatment of 8 µM TDZ after 20 days followed by 2 weeks on MS free PGRs generated the highest number of shoot/explant with 6.63 shoot/explant. Based on this screening, it was concluded that ILB4347 were the most responsive



(A) and explant type (B) on seedling growth of faba bean genotypes after 2 weeks in culture on MS medium without PGRs. T1: 6 days in dark, T2: 3 days in dark followed by 3 days in light and T3: 6 days in light.

Fig 3: Influence of light incubation regime.

Table 3: Effect of light incubation regime and explant type on number of axillary shoots of faba bean genotypes after 2 weeks in culture on MS medium without PGRs.

Genotype	Number of axillary shoots							
	Cotyledon explant				Node explant			
	T1	T2	T3	Mean	T1	T2	T3	Mean
Luz (large seeded)	1.33	1.00	1.33	1.22ab	1.33	0.50	1.00	0.94 ns
ILB4347 (medium seeded)	1.75	1.50	1.25	1.50b	1.25	0.75	0.25	0.75 ns
Triple white (small seeded)	1.00	1.00	0.50	0.83a	1.00	0.67	0.50	0.72 ns
Mean	1.36ns	1.17ns	1.03ns	1.18*	1.19ns	0.64ns	0.58ns	0.81*

T1: 6 days in dark, T2: 3 days in dark followed by 3 days in light and T3: 6 days in light. ns: not significantly difference, *: significant difference at $P \leq 0.05$ between mean value of explant source, different letters denote statistical difference ($P \leq 0.05$) according to Tukey's multiple range test.

Table 4: Effect of light incubation regime and explant source on shoot length of faba bean genotypes after 2 weeks in culture on MS medium without PGRs.

Genotype	Shoot length (cm)							
	Cotyledon explant				Node explant			
	T1	T2	T3	Mean	T1	T2	T3	Mean
Luz (large seeded)	12.53	14.33	14.13	13.66 b	9.73	3.80	4.33	5.95ns
ILB4347 (medium seeded)	12.05	13.75	11.75	12.52 ab	6.00	7.20	9.50	7.57ns
Triple white (small seeded)	5.10	11.67	4.80	7.19 a	2.40	5.00	4.60	4.00ns
Mean	9.89ns	13.25ns	10.23ns	11.12*	6.04ns	5.33ns	6.14ns	5.84*

T1: 6 days in dark, T2: 3 days in dark followed by 3 days in light and T3: 6 days in light. ns: not significantly difference, *: significant difference at $P \leq 0.05$ between mean value of explant source, different letters denote statistical difference ($P \leq 0.05$) according to Tukey's multiple range test.

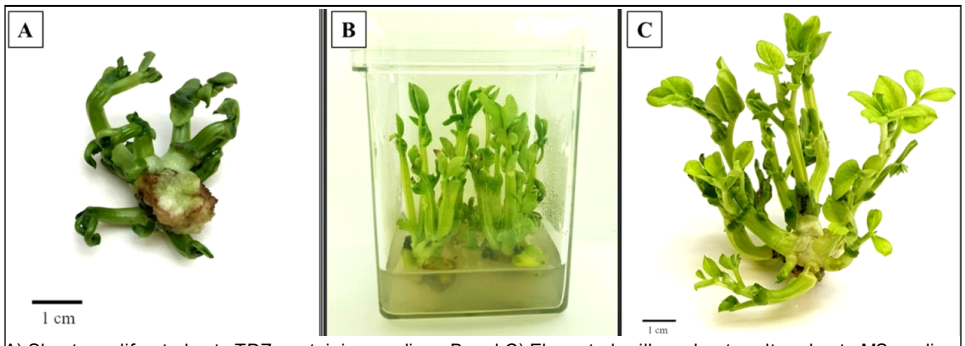
genotype in tissue culture and treatment 8 µM TDZ after 20 days followed by 2 weeks on MS free PGRs was the best treatment for shoot induction media.

Unlike other cytokinins, TDZ demonstrates resistance to endogenous *cytokinin oxidase*, resulting in its considerable stability in tissue culture (Mok *et al*., 1982). The metabolic rate of TDZ is notably sluggish, whereas zeatin is fully metabolized by plant tissues mere hours after its application (Mok and Mok, 1985). TDZ effectively suppresses the activity of cytokinin oxidase (Horgan *et al*., 1988; Hare *et al*., 1994), thereby leading to the accumulation of purine cytokinins in plant tissues. TDZ can therefore be utilized exclusively to facilitate distinct morphogenic and regeneration pathways. The appropriate and optimal concentration of TDZ is species-specific. TDZ enhances the synthesis of adenine-type cytokinins, modulates endogenous hormones and exhibits both auxin and cytokinin-like activities. Moreover, it promotes stress genes

Table 5: Effect of TDZ concentrations and exposure duration on axillary shoot multiplication using cotyledon explants of ten faba bean (*Vicia faba*) genotypes followed by 2 weeks culture on MS medium without PGRs.

Genotype	TE1 (10 days)						TE2 (20 days)					
	2 µM TDZ	4 µM TDZ	6 µM TDZ	8 µM TDZ	10 µM TDZ	Mean	2 µM TDZ	4 µM TDZ	6 µM TDZ	8 µM TDZ	10 µM TDZ	Mean
Luz	2.00	2.50	2.67	4.00	4.67	3.17 ns	3.50	2.50	4.50	3.33	3.67	3.50 ns
Hassawi 1	2.25	3.00	2.00	3.00	2.67	2.58 ns	2.00	3.00	3.50	4.50	2.67	3.13 ns
Hassawi 2	2.00	2.25	1.33	2.40	2.00	2.00 ns	4.00	3.00	3.50	5.50	6.00	4.40 ns
Hassawi 3	2.50	2.50	3.00	3.67	3.00	2.93 ns	2.67	3.00	2.25	3.33	4.00	3.05 ns
ILB4347	2.50	2.50	3.00	3.50	2.00	2.70 ns	2.50	3.00	4.30	9.00	5.00	4.76 ns
Misr 3	1.00	3.25	3.00	2.67	2.00	2.38 ns	3.00	4.00	4.25	4.50	5.00	4.15 ns
Sakha 1	2.50	4.00	3.00	3.50	3.00	3.20 ns	3.00	3.50	2.00	3.75	6.00	3.65 ns
Sakha 2	1.75	1.75	1.50	2.67	3.00	2.13 ns	1.75	2.50	2.67	5.67	7.00	3.92 ns
Gazira 2	2.50	3.25	2.50	3.75	3.40	3.08 ns	3.50	2.75	3.00	4.25	4.00	3.50 ns
Triple white	3.00	2.50	3.25	3.25	2.67	2.93 ns	2.60	3.25	3.50	4.00	4.75	3.62 ns
Mean	2.20 a	2.75ab	2.53 ab	3.24b	2.84 ab	2.71**	2.85 a	3.05 a	3.35a	4.78b	4.81b	3.77**
Subculture on MS medium without PGRs (2 weeks)												
Luz	2.25	2.50	3.00	7.30	5.00	4.01 ns	3.50	2.67	5.00	5.67	7.00	4.77 ns
Hassawi 1	2.67	3.00	3.00	3.50	3.33	3.10 ns	3.00	5.00	4.00	5.67	5.30	4.59 ns
Hassawi 2	3.00	5.00	4.50	5.00	6.00	4.70 ns	4.00	5.00	5.50	6.00	10.00	6.10 ns
Hassawi 3	2.50	3.00	4.00	5.50	4.00	3.80 ns	3.00	3.50	4.67	9.00	5.00	5.03 ns
ILB4347	3.50	4.00	3.00	4.00	7.33	4.37 ns	4.00	4.50	5.50	11.00	6.30	6.26 ns
Misr 3	3.00	3.50	3.25	3.50	4.00	3.45 ns	3.00	4.00	9.00	6.67	5.00	5.53 ns
Sakha 1	1.50	3.25	7.00	3.67	4.33	3.95 ns	3.00	4.00	4.25	4.50	6.00	4.35 ns
Sakha 2	3.00	3.75	5.75	5.25	5.00	4.55 ns	4.50	4.00	5.00	6.00	7.00	5.30 ns
Gazira 2	4.25	3.67	4.00	4.75	4.25	4.18 ns	3.00	3.50	3.80	6.75	4.50	4.31 ns
Triple white	4.00	5.00	5.00	5.50	6.33	5.17 ns	4.50	5.00	4.00	5.00	8.00	5.30 ns
Mean	2.97a	3.67ab	4.25ab	4.80b	4.96 b	4.13**	3.55a	4.12a	5.07ab	6.63b	6.41b	5.16 **

**: Significant difference at $p \leq 0.01$ between mean value of TE, different letters denote statistical difference ($P \leq 0.05$) using Tukey test.



A) Shoots proliferated onto TDZ-containing medium, B and C) Elongated axillary shoots cultured onto MS medium without PGRs.

Fig 4: Axillary shoot multiplication of faba bean (*Vicia faba*).

and leads to the production of ethylene and stress signaling molecules. TDZ can thus be used solely to achieve different morphogenic and regeneration pathways (Dewir *et al*., 2018).

The present study screened the most responsive faba bean genotypes in order to develop an efficient and effective protocol for shoot multiplication of faba bean *in vitro*. The current study tested two different explants which were cotyledonary node containing 5 mm hypocotyl and epicotyl tissues with or without single cotyledon. These explants were originated from seed which were germinated on three different light treatment. The investigations found that cotyledonary node with single cotyledon that incubated on 6 days in dark had the highest number of shoot. Therefore, this explant was used for screening the most responsive faba bean genotypes. The ten faba bean genotypes were screened under different TDZ concentrations and time of exposure to TDZ. The overall mean showed that genotypes of ILB4347 and Hassawi 2 were the most responsive genotypes in tissue culture. Based on this experiment, treatment of 8 μM TDZ for 20 days in culture obtained the highest number of shoot after subsequent culture in MS free PGRs for 2 weeks. This treatment also resulted the highest percentage of callus regeneration. Our results obtained 11 shoot per explant after 34 days in culture as compared with 5.9 shoot per explant after 45 days observed by Abdelwahd *et al*. (2008). Previous study by Anwar *et al*. (2011) demonstrated that TDZ (6 μM) in combination with 2-iP (10 μM) and kinetin (4 μM) was optimal for expansion of the meristematic zone followed by adventitious bud/shoot induction.

The effectiveness of TDZ in the *in vitro* propagation of various plant species within the Fabaceae family has been well established. Parveen and Shahzad (2010) detailed the in vitro propagation of *Cassia sophera* using cotyledonary node explants excised from axenic seedlings aged 21 days. All explants cultured on MS medium supplemented with different TDZ concentrations (0.1-10 μM) exhibited multiple shoot formation, with 2.5 mM concentration proving optimal for producing a maximum of 6.7 shoots per explant. Similarly, multiple shoots of *Pterocarpus marsupium* were induced from cotyledonary nodes of 18-day-old axenic seedlings on MS medium supplemented with 0.1-10 μM TDZ. Notably, a concentration of 0.4 μM TDZ resulted in the highest shoot regeneration frequency (90%) and number of shoots per explant (15.2) (Husain *et al*., 2007). In the case of *Senna alata*, a high number of nodes and shoots (8.3 and 12.6, respectively) were obtained by utilizing MS medium containing 1 mg/L TDZ and 0.1 mg/L NAA (Lara *et al*., 2022).

Significant advancements have been made in the field of tissue culture for recalcitrant plant species through the application of TDZ. The utilization of TDZ at lower concentrations within the range of nanomolar to a few micromolar has demonstrated the capacity to stimulate axillary shoot production, whereas elevated concentrations promote the development of callus and the propagation of adventitious shoots as noted by Huetteman and Preece (1993), Debnath (2018) and Vinoth and Ravindhran (2018). Various plant species exhibit a greater receptiveness to a wider range of TDZ concentrations and subculture cycles, as evidenced by the abundance of successful tissue culture protocols employing TDZ, wherein these plant species display normal growth. Nevertheless, the implementation of TDZ at low concentrations, pulse treatment and short period exposure durations are effective strategies to circumvent TDZ-induced abnormalities. In the case of *Bactris gasipaes*, a pulse treatment with a concentration of 0.36 μM TDZ for a duration of 14 days effectively mitigated the adverse effects brought about by prolonged exposure to TDZ (Graner *et al*., 2013).

CONCLUSION

The present study investigated the optimal formulation for axillary shoot multiplication in different faba bean genotypes. Light regime, genotype and TDZ concentration and duration of exposure significantly influenced axillary shoot multiplication of faba bean. TDZ at 8 μM concentration and exposure duration of 20 days resulted the highest the highest proliferation with 6.63 shoot per explant. Moreover, different faba bean genotypes displayed varied responses. The three most responsive genotype in tissue culture were medium sized seeds genotypes (ILB4347, Hassawi 2 and Misr 3 with 4.76, 4.40 and 4.15 shoot per explant, respectively.

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Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Abdelwahd, R., Hakam, N., Labhilili, M. and Udupa, S.M. (2008). Use of an adsorbent and antioxidants to reduce the effects of leached phenolics in *in vitro* plantlet regeneration of faba bean. African Journal of Biotechnology 7: 997-1002.

Adak, M.S. and Kibritci, M. (2016). Effect of nitrogen and phosphorus levels on nodulation and yield components in faba bean (*Vicia faba* L.). Legume Research-An International Journal. 39(6): 991-994. doi: 10.18805/lr.v0iOF.3773.

Ahmed, A.H.H., Desouky A.F., Reda A.S.A, Ibrahim H.M.M, Sttzel, H. and Hanafy, M.S. (2020). Impact of chitosan on shoot regeneration from faba bean embryo axes through its effect on phenolic compounds and endogenous hormones. Plant Archives. 1: 2269-2279.

Anwar, F., Alghamdi, S.S., Ammar, M.H. and Siddique, K.H.M. (2011). An efficient *in vitro* regeneration protocol for faba bean (*Vicia faba* L.). Journal of Medicinal Plant Research. 28: 6460-6467.

Böttinger, P., Steinmetz, A., Schieder, O. and Pickardt, T. (2001). *Agrobacterium*-mediated transformation of *Vicia faba*. Molecular Breeding 8: 243-254.

Busse, G. (1986). *In vitro* cultivation of *Vicia faba* L. and induction of morphogenesis. Biologisches Zentralblatt. 105: 97-104.

Cubero, J.I. and Nadal, S. (2005). Faba bean (*Vicia faba* L.). In: Genetic Resources, Chromosome Engineering and Crop Improvement. [Singh, R.J. and Jauher, P.P. (eds)], CRC Press, Florida. pp. 163-186.

Debnath, S.C. (2018). Thidiazuron in Micropropagation of Small Fruits. In: Thidiazuron: From Urea Derivative to Plant Growth Regulator. [Ahmad, N. and Faisal, M. (eds)], Springer, Singapore. pp. 139-158.

Dewir, Y.H., Nurmansyah, Naidoo, Y. and Teixeira da Silva, J.A. (2018). Thidiazuron-induced abnormalities in plant tissue cultures. Plant Cell Reports. 37: 1451-1470.

Graner, E.M., Oberschelp, G.P., Brondani, G.E., Batagin-Piotto, K.D., de Almeida, C.V. and de Almeida, M. (2013). TDZ pulsing evaluation on the *in vitro* morphogenesis of peach palm. Physiology and Molecular Biolology of Plants. 19: 283-288

Griga, M., Kubalakova, M. and Tejklova, E. (1987). Somatic embryogenesis in *Vicia faba* L. Plant Cell Tissue and Organ Culture. 9: 167-171.

Hare, P.D., Staden, J. and Van Staden, J., (1994). Inhibitory effect of TDZ on the activity of cytokinin oxidase isolated form soybean callus. Plant Cell Physiol. 35: 1121-1125.

Horgan, R., Burch, L.R. and Palni, L.M.S. (1988). Cytokinin Oxidase and the Degradative Metabolism of Cytokinins, Plant Growth Substances, In: Plant Growth Substances, [Pharis, R.P. and Rood, S.B., (Eds.)], Springer-Verlag, Berlin. pp. 282-290.

Huetteman, C.A. and Preece, J.E. (1993). Thidiazuron: A potent cytokinin for woody plant tissue culture. Plant Cell Tissue and Organ Culture 33: 105-119.

Husain, M.K., Anis, M. and Shahzad, A. (2007). *In vitro* propagation of Indian Kino (*Pterocarpus marsupium* Roxb.) using thidiazuron. *In vitro* Cellular and Developmental Biology-Plant. 43: 59-64.

Jelenić, S., Mitrikeski, P.T., Papeš, D. and Jelaska, S. (2000). *Agrobacterium*-mediated transformation of broad bean *Vicia faba* L. Food Technol. Biotechnol. 38: 167-172.

Khalafalla, M.M. and Hattori, K. (1999). A combination of thidiazuron and benzyladenine promotes multiple shoot production from cotyledonary node explants of faba bean (*Vicia faba* L.). Plant Growth Regulation. 27: 145-148.

Khalafalla, M.M. and Hattori, K., (2000). Differential *in vitro* direct shoot regeneration responses in embryo axis and shoot tip explants of faba bean. Breeding Science. 2: 117-122.

Lara, E.Y.C., Imakawa, A.M., Da Silva, D. and Sampaio, P.D.T.B. (2022). *In vitro* regeneration of *Senna alata* (L.) Robx. (Fabaceae): An ancient medicinal plant. Advances in Forestry Science. 3: 1831-1838.

Mok, M.C. and Mok, D.W.S. (1985). The metabolism of [^{14}C]-thidiazuron in callus tissues of *Phaseolus lunatus*. Physiologia Plantarum. 65: 427-432.

Mok, M.C., Mok, D.W.S. and Armstrong, D.J. (1982): Cytokinin activity of N-phenyl-N-1, 2, 3-thidiiazol-5ylurea (TDZ). Phytochemistry 21: 1509-1511.

Murashige, T. and Skoog, F. (1962): A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum. 15: 473-495.

Parveen, S. and Shahzad, A. (2010). TDZ-induced high frequency shoot regeneration in *Cassia sophera* Linn. *via* cotyledonary node explants. Physiology and Molecular Biology of Plants. 16: 201-206.

Paul, S.K. and Gupta, D.R. (2021). Faba bean (*Vicia faba* L.), a promising grain legume crop of Bangladesh: A review. Agricultural Reviews. 3: 292-299. doi: 10.18805/ag.R-203.

Selva, E., Stouffs, B. and Briquet, M. (1989). *In vitro* propagation of *Vicia faba* L. by micro-cutting and multiple shoot induction. Plant Cell Tissue and Organ Culture. 18: 167-179.

Tegeder, M., Gebhardt, D., Schieder, O. and Pickardt, T. (1995). Thidiazuron-induced plant regeneration from protoplasts of *Vicia faba* cv. Mythos. Plant Cell Reports. 15: 164-169.

Vinoth, A. and Ravindhran, R. (2018). *In vitro* Morphogenesis of Woody Plants using Thidiazuron. In: Thidiazuron: From Urea Derivative to Plant Growth Regulator. [Ahmad, N. and Faisal, M. (Eds)] Springer, Singapore. pp. 211-230.