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Influence Different Concentrations of Thidiazurone (TDZ) on in Vitro Micropropagation of Two Cultivars of Ficus Carica L.

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Abstract

A study was done to test the effect of different factors on the multiplication and growth of fig plants in vitro. They were explants excised from two different cultivars wazziri and mission cultivar locally named (Bathinjani) and inoculated on two types of media murashiage and skooge 1962 (MS) and woody plant media (WPM) results were recorded after 6 weeks, where MS media gave a highest shoot number, leaf number and shoot length (1.50, 3.10, and 2.57) respectively, Bathinjani cultivar gave maximum means of shoots and leaves number (1.5 and 2.73)respectively, Bathinjani proliferated on MS media gave highest value of same characteristic mention above (1.67, 3.20 and 2.97) respectively, explants of the best cultivar were transferred into the most efficient medium. An explant 1cm length of the Bathinjani cultivar was cultured in MS medium containing TDZ at levels (0, 0.5, 1, 1.5, 2, 2.5 and 3) mg.-l to study its effect on shoot multiplication and growth the result showed the highest shoot number and its length was 7.20 shoot, 2.33cm respectively at 2mg.l -1 with a significant increase compared with rest treatments followed by concentration at 1.5 mg.l-1 also gave significant increasing of shoot number 5.22 so the concentration at 2.5 achieved high value of shoot length was 2.20cm, then plantlet subculture on MS media of rooting stage which contain half strength MS with adding Auxines IAA and IBA at (0, 0.25, 0.5 and 1) mg.l-1 for both, the data appeared IBA and IAA at 0.5 mg.l-1 gave highest value of root number and rooted percentage reached to (2.77, 2.38) and (99.33, 96.08) % respectively compared with 0.0 and 1 mg.l-1 gave lowest value for same characteristics.

Keywords:

Thidiazurone, in vitro, micropropagation, cultivars, ficus carica L.

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Introduction

Fig plants (Ficus carica L.) back to the Moraceae family are deciduous trees also classified as semi-tropical trees often characterised by having more than one stem (Abdulkareem et al., 2022). Its fruits can be eaten fresh or dried, or they may be used in food industries such as jam (Shi et al., 2024). It contains large amounts of calcium, potassium, iron, phosphorus, and vitamins such as VB1, B2, and B5 (Sarkhosh et al., 2022; Acarsoy Bilgin et al., 2020). The traditional propagation method of fig by cutting it needs more labour, cost and time in addition it may not guarantee the produces healthy plant disease in addition to adhering to the season when propagating. (Chauhan & Kanwar, 2012; Kanwar et al., 2010) while in vitro propagation by using tissue culture solved all the mentioned obstacles through using many different ways like direct method and indirect organogenesis methods such as somatic embryogenesis. (Yildiz, 2012) indicated that using leaf and internode segments removed by indirect organogenesis from in vitro led to germinated seedlings 30 days old, (Prabhuling et al., 2018) demonstrated an increased success rate of indirect organogenesis from segments (Kurmi et al., 2011) of fig mature plant (Parab et al., 2021) found the success of Ficus carica var. Black Jack propagation in a way of indirect organogenesis from apical buds with different types of plant growth regulators to the media (Fakhrian et al., 2022). The success or failure and growth of the explants depend on the nature of the type of medium and its components, in addition to the concentrations of added hormones. researches and many references have improved that the medium type is determined factor for explant differentiation depends especially in establishment stage of tissue culture Therefore, the appropriate media is key to success in plant tissue culture, moreover nutrient of media content , where one kind of media most important in plant tissue culture technique is Murashige and Skoog medium (MS) which consist of a group of macro and micro elements it has prominent role in the growth of the plant parts and are formulated energy source (sucrose) and inorganic salts, as well as alcohols, vitamins, growth regulators, amino acids, and medium hardeners such as (agar) (Al-sumaidai, 2017), And there are another kind of mediums have achieved results in this field, experiments and studies have appeared the success of WPM in the growth and multiplication of woody plants and trees in tissue culture, which made up of micro and macro elements , vitamins, amino acids, It differs from MS medium in concentrations and some of its components (Parab et al., 2021) found growing of Fig Black Jack in WPM containing BAP gave highest number of multiple shoots (Abdulkareem et al., 2022; Al-Jubori et al., 2023; Mozafari et al., 2016)indicated that MS medium achieved positive results in the growth and regeneration of excised parts compared to WPM medium. (Kinfe et al., 2017) proved that cultivation on MS medium containing 0.5 mg. l-1 BAP had a positive effect on shoot initiation while (Lloyd & McCown, 1980) found that WPM was more proper and successful for culturing of Vitis thanbergii compared to MS medium. (Desai et al., 2018) found WPM added to it 1mg.l (BAP+IBA) gave a maximum value of shoots induced and the highest root length and number. (Kajiet al., 2013) showed that the WPM medium is more influential and gave the highest number of shoots and leaves, compared to the MS medium. Plant growth regulators have an important role in the various stages of laboratory reproduction, as research has confirmed that cytokinin is of great importance in the propagation stage by increasing the features of vegetative growth (Folta et al., 2006), multiplying shoots, and increasing the number of leaves of the cultivated part. where Thidiazuron(TDZ) is one of the most effective cytokinins commonly used whether it is used as a herbicide or Plant growth regulator, It has a stimulating effect on the multiplication of axillary buds in woody plant species (Rajabi et al., 2018), It also has a positive performance in initiating the dormant of lateral buds and stimulating the emergence of branches, it is one of the growth regulators has direct influence Where it works on stimulating the accumulation of internal cytokinens and

thus stimulates the emergence of lateral buds at low levels Whereas, high levels are inhibitors. the studies emphases that TDZ has been used to stimulate shoot multiplication in fig tissue culture (Toron et al., 2024). Research by (Ghorbani & Hatami, 2016) demonstrated that TDZ was effective in inducing shoot proliferation in fig (Ficus carica L.) explants. also, TDZ has an activity to induce the formation of multiple shoots from single explants. result by (Sarkhosh et al., 2022; Majid, 2018) showed that TDZ significantly increased the shot number per explant for fig in vitro. TDZ has been employed in the induction of somatic embryogenesis in fig. The result of the study by (Rezaei et al., 2014) showed the role of TDZ in somatic embryogenesis and plantlet regeneration in fig in vitro. So (Abdulkareem, 2023; Bani et al., 2017; Parab et al., 2021) found there was an increase in the number of shoots for explant which grew on media containing TDZ at low concentrations while the number decreased at high concentrations of TDZ. (Parab et al., 2021) Found positive facts about vegetative growth with Added TDZ at lower levels. Auxins IBA and IAA have an important role in the rooting stage, commonly used to induce callus formation in the early stages and stimulate the emergence of roots from explants (Ahmed & Maher, 2022) It stimulates cell division and elongation in the cambium layer, promoting the formation of root primordial, IBA is particularly effective in promoting root initiation and elongation in woody plants. the research has proved that adding auxin IBA or IAA to the media half strength gave positive results for roots number and length, whereas the use of the Auxins IAA, NAA and IBA to media gave a significant result of roots growth compared to media without any auxin (Parab et al., 2021) found adding IAA at 8 mM to media gave highest roots number and rotted plantlet percentage of Ficus carica var Blackjack. (Heisler & Byrne, 2020; Al-Obaidy & Khierallah, 2017) proved that the success of root induction by adding IAA in WPM media can give positive correlation between IAA and root induction (Pal et al., 2014) have shown maximum rooting percentage with a higher number of root number of invitro pomegranate. (Kanwar et al., 2010) proved auxins type of appeared great effect in rooting of explants. (Patil et al., 2011) reported that high levels of IBA stimulated root formation with increasing root length and numbers. (Osama, 2022) illustrated adding 0.5 mg. l−1 IBA with half MS medium gave significant results for rooting of 'Malase Yazdi' and the highest root number and its length. The research aims to find out the best cultivar and test the most appropriate medium for the growth of planted parts and know the optimal concentration of cytokinein in the multiplication stage, in addition to studying the effect of concentrations and its kinds of Auxin is most efficient for rooting percentage (Barhoumi et al., 2024).

Material and Methods

Transplants of ficus carica (Sriskanda et al., 2021) of two cultivars mission c.v locally named (Bathinjani) a relatively large black fruit and the other cultivar Waziri its fruit yellow medium size, (2) years old were chosen for in vitro regeneration, healthy apical buds excised from the mother plant, at the first stage explant were washed with tap water for 30 minutes and then surface sterilization with sodium hypochlorite 2.0% with adding few drops of tween 20 for 10 minutes after that rinsed three times with sterilization water, the basal media of initiation stage were two type of media murashiage and skooge, 1962 (Murashige & Skoog, 1962) (MS) and woody plant media (WPM) (Lloyd & McCown, 1981), sucrose at 3%, glutamine 200 mg.l-1, myoinositol 100 mg.l-1 with hormones 0.5mg.l-1 NAA(Naphthaleneacetic acid) and 3mg.l-1 2iP (isopentyl adenine) . Then pH was adjusted to 5.7 before solidified with agar 7 g.l-1 the medium was dispensed in a glass jar of 40 ml each covered with propylene caps then auto-cleaved at 121 c for 15 min. After culturing apical buds were incubated at 25c with 16 hours of light using fluorescence lambs and 8 hours dark. In the multiplication stage explant of the cultivar which gives the highest efficiency from the previous stage subcultured on the best media contains cytokinin TDZ (Thidiazuron) at concentrations (0, 0.5, 1, 1.5, 2, 2.5 and 3) mg.l-1 the multiplication measurements were recorded after 6 weeks from subculture

date, then plantlet transformed to media of rooting stage which contain half strength MS with adding Auxines IAA and IBA at (0, 0.25, 0.5 and 1) mg.l-1 for both, the data of this stage were taken after 12 weeks. The experiment was designed as a factorial by using (CRD) a completely randomizing design with 10 replicates.

Results and Discussion

Table 1. Influence media type on some growth characteristics of Bathinjani and Wazziri cultivars

	Shoot number			Shoot length(cm)			Leaf.no.shoot^{-1}		
MEDIA_TYPE	**BN**	**WA**	**mean**	**BN**	**WA**	**mean**	**BN**	**WA**	**mean**
M.S	1.67	1.33	1.50	2.97	2.17	2.57	3.20	3.00	3.10
WPM	1.33	1.33	1.33	2.43	2.70	2.56	2.25	2.22	2.23
MEAN	1.5	1.33		2.43	2.7		2.73	2.61	
LSD	Cult 1.01. med. 1.01 cult.med 1.43			Cult 0.45. med. 3.18 cult.med 3.19			Cult 0.99 med. 0.7 cult.med 0.7		

Effect of Media Type on Growth Cultivars

The results of Table (1) showed a clear influence of two media kinds MS and WPM on two cultivars of fig Bathinjani and wazziri where MS media achieved the highest means (1.5) of shoot numbers compared with WPM gave the lowest number 1.33 without significant difference. In the same way, the Bathinjani cultivar gave increasing in shoot number of 1.5 compared to wazziri 1.33. Concerning the interaction (cultivars and media type) Bathinjani proliferated on MS media and got the highest means of shoots number 1.67. Compared with the other treatments gave lowest value 1.33. The results of the same table showed that MS gave the highest shoot length of 2.57cm with a significant difference compared with WPM gave the lowest length of 2.56 cm. On the same side length of the shoot increased with the Wazziri cultivar at 2.7 cm while the Bathinjani cultivar gave the lowest value of 2.43 cm. Concerning the interaction between media type and cultivars the results of the table cleared that the interaction treatment Bathinjani proliferated in MS medium gave the highest shoot length of 2.97cm while the wazziri cultivar proliferated in MS medium gave the lowest shoot number of 2.17. Concerning the effect of media type on leaf number MS gave the highest number of leaves 3.1 leaf. shoot-1 while WPM gave the lowest number 2.23 leaf. shoot-1, concerning the effect of cultivar Bathinjani cultivar gave the highest leaf number 2.73 leaves. shoot-1 while the other cultivar gave the lowest value 2.61 leaf. shoot-1. The interaction between the cultivar and the result of the same table appeared Bathinjani proliferated in MS media giving the highest leaf number 3.2 leaves. shoot-1 compared with the wazir cultivar in WPM gave the less number was 2.22 leaves. shoot-1. From the table results We find Bathinjani cultivar has been more efficient in effect on some characteristics, which might explain the increased capability of organogenesis linked genotype of the cultivar compared with the Wazziri cultivar This is confirmed in study results used two cultivars of Strawberry Calypso and Sveva so proven sveva cultivar better than Calypso in increasing leaf and shoot length. And it agrees with (Alwash & Jassim, 2014; (Desai et al., 2018; Passey et al., 2003; Landi & Mezzetti, 2006) Despite the availability of several types of nutrient media, MS is considered one of the most successful media and gives positive results in plant tissue culture. The results Give an increase to the leaf and shoot number and length of shoot These results agree with (Mozafari et al., 2016) they got that MS medium was the best compared with another medium in the regeneration and increased some growth characteristic, and (Kinfe et al., 2017; Shahatha et al., 2012) found Superiority of explant grown in MS medium supplement 0.5 mg. l-1 BAP compared to other media.

Table 2. Effect of adding many levels of Thidizuron (TDZ) in MS media on proliferation characteristic of Bathinjani cultivar

TDZ mg.l^{-1}	Shoot number	shoot length (cm)
0	2.40	1.10
0.5	3.60	1.36
1	4.40	1.86
1.5	5.22	1.93
2	7.20	2.33
2.5	4.52	2.20
3	2.45	1.95
LSD	**1.034**	**0.335**

Effect of Thidizuron (TDZ) adding in MS media on shoots growth

The Data in Table (2) illustrated significant differences between TDZ levels on the number and length of shoots. Were highest shoot number was 7.20 shoot at 2mg.l -1 compared with other treatments after it concentrates 1.5 mg. l-1 also a significant increase of shoot number 5.22 while 0mg. l-1 gave the lowest value of 2.40. The outcome of the same table demonstrates differences between TDZ concentrations in the effect on the shoot length were 2mg.l-1 got a max value of 2.33 cm compared with the other treatments, in the same way, there is increasing in the shoot length (1.93,1.95, 2.20) cm for the treatment (1.5,3, 2.5) mg. l-1 respectively in comparison with the lowest length of shoot 1.1 at 0mg.l-1. It is noticeable from the results theirs an increase in both characteristics with increasing TDZ concentration up to 2 mg and then occurs inverse relationship where there is a decrease in value with an increase the concentration. It is clear from the results that there is a high in the number of shoots and their lengths with increasing TDS concentrations until concentration 2mg.-1 gave the highest values. Then, with increasing concentration, the values for those characteristics mentioned above decreased. This is confirmed by the results (Annon & Abdulrasool,2020) emphases that high levels of TDZ lead to a lower shoot number and shoot length and agree with (Parab et al., 2021) found the number of shoots per explant increased with low levels of TDZ, the opposite happens at maximum concentrations.

Table 3. Effect of Auxins IAA and IBA on root number

Root number					
IBA mg. L^{-1}	**IAA mg· L^{-1}**				**mean**
	0	**0.25**	**0.5**	**1**	
0	1.10	1.20	1.36	1.86	1.38
0.25	2.12	2.22	2.34	2.83	2.38
0.5	2.32	2.30	4.48	1.98	2.77
1	1.10	1.26	1.33	1.27	1.24
LSD $_{0.05}$	0.463				0.232
mean	1.66	1.75	2.38	1.99	
LSD $_{0.05}$	**0.232**				

Effect of Auxins IAA with IBA on the Number of Roots

Data of table.3 indicates the influence of Auxins IAA and IBA on the root numbers We found that the concentration of IBA at (0.5mg.l-1) had the maximum mean of root numbers reached 2.77 root/explant with a positive difference from rest levels, following it the treatment 0.25mg.l-1 gave a high root number of 2.38

root/explant compared with the lowest value of 1.24 root/explant for the concentration 1mg.l-1. The same results were clear that a significant effect among concentrations of IAA was the treatment 0.5mg.l-1 gave the highest root number 2.38 root/explant followed by its levels at mg.l-1 gave 1.99 root/explant while control treatment at 0 mg.l-1 gave a minimum number of 1.66, The interaction IBA and IAA the results of the table.3 appeared the treatment 0.5 IBA+ 0.5 IAA gave the highest value of 4.48 with a difference of the other levels compared with the lowest value 1.10 root/explant for both the interaction treatment (0.0 +0.0), (1 IBA+ 0 IAA),

Table. 4 Influence of two Auxins type IAA and IBA on Rooted percentage

Rooted percentage (%)					
IAA	**IBA**				**mean**
	0	**0.25**	**0.5**	**1**	
0	75.00	90.00	92.00	94.00	87.75
0.25	96.00	93.00	99.33	99.67	97.00
0.5	99.67	99.67	100.00	98.00	99.33
1	93.00	90.00	93.00	80.00	89.00
LSD $_{0.05}$	4.801				2.401
mean	90.92	93.17	96.08	92.92	
LSD $_{0.05}$	**2.401**				

Effect of IAA and IBA on Rooted Percentage

The result in Table (4) shows the effect of IAA and IBA on the percentage of rooted plantlets (%), that there are significant differences among IBA concentrations where (0.5mg.l-1) got the highest percentage reached 99.33 % while 0.0 mg.l-1 gave minimum percentage 87.75 % in the same way Data was clear that there were significance differences among IAA concentrations were 0.5mg.l-1 gave a maximum value was 96.08% and the percentage decreased with increasing IAA concentration at 1mg.l-1 gave 92.92% while 0mg.l-1 gave a minimum percentage of 90.92% concerning of interaction between IBA and IAA and their effect on the rooted plantlets (%) from the same table was obvious the media contained IBA 0.5+IAA0.5) mg.l-1 achieved max percentage of 100% on the contrary the lowest value was 75% for (0 IBA+0IBA).

The results clear Auxines (IBA and IAA) at 0. 5mg.l-1 gave the best results of the number and percentage of roots compared with control and high concentration 1mg.l-1 were the value decreased. the result agrees with (Parab et al., 2021) found WPM supplemented with IAA increased root percentage formation and root number of the root, (AL-Hussini et al., 2007; Parab et al., 2021) got there is a relationship between IAA and root formation where the use of IAA led to the success of root induction, (Ružić et al., 2012; Yildiz, 2012) obvious use IBA as prime auxine increased number of roots. (Al-Jubori & Al-Amery, 2022; Osama, 2022) found half MS medium contained 0.5 mg. l−1 IBA was more effective for in vitro rooting giving the highest number of roots and root length.

Conclusions

MS media was superior to woody plant media (WPM), in increasing shoot number, leaf number and shoot length (1.50, 3.10, and 2.57) respectively However, when compared to Bathinjani with wazziri for the same features, concerning multiplication stage 2mg.L-1 yielded the greatest shoot length (5.76) and shoot number (3.11cm) per explant, the highest root growth was attained with IBA (Indole-3-butyric acid) and IAA (Indole-3-acetic acid) at 0.5.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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