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IN VİTRO DİRECT WHOLE PLANT REGENERATİON OF WATER LETTUCE (*Pistia stratiotes* L) USİNG THİDİAZURON

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The study presents the *in vitro* whole plant regeneration of water lettuce (*Pistia stratiotes* L.), an important medicinal aquatic plant that has also been used for phytoremediation studies. The plants were surface sterilzied with H_2O_2 and cultured on 0.65% agar soldified MS medium enriched with 0.05-0.60 mg/L Thidiazuron (TDZ) and placed under red:blue (3:1) light emitting diodes (LEDs) at 16 h photoperiod. The 100% callus induction and plantlets regeneration fequency was recorded after 12 weeks of culture. The 19.0-39.67 number of whole plantlets per explants were recorded on medium containing TDZ. Maximum of 39.67 plantlets were recorded on medium supplemented with 0.10 mg/L TDZ. Contrarily, minimum plantlets (19.0) were scored on medium enriched with 0.05 mg/L TDZ. Higher concentration of TDZ in the culture medium was also detrimental and resulted in low plantlets per explant. As direct plantlets regneration (plantlets with roots) was observe, therefore they were directly transferred to aquariums containing tap water with continuous provision of oxygen. Almost 100% plants survived in the aquariums and continued their growth by developing new leaves and increase in size. This study reports the successful whole plant regeneraation which can be used for employing biotechnological tools like secondary metabolites, genetic transformation or phytoremediation studies.

Keywords: *In vitro*, whole plant regeneration, Thidiazuron, water lettuce

INTRODUCTION

Water lettuce (*Pistia stratiotes* L.) belongs to Araceae family is an important stoloniferous floating plant of aquatic environment. The most common places where water lettuce plants can be easşly found are likes lakes, streams or stagnat water ponds. The plant shows wide distribution in tropical and subtropical regions of Africa, Asia, and America (Tripathi *et al.*, 2010). The plant is perennial monocotoledenous with relatively soft leaves that are arranged in rosette formation. Hot season is important for flowering whereas, fruit bearings ocur after rainfall (Chadha, 1998). It considered to be the most reproductive plant of fresh water environments due to its properties and uses. Alkaloids, glycosides, flavonoids, and steroids are the essentially important biological compounds it contains. The plant leaves are rich in vitamin A, B and C which are used to cure diseases like eczema, leprosy, ulcers, piles, and syphilis. Besides thta, curing of chronic skin diseases is also done by using leaf extracts alongwith coconut oil (Kirtikar and Basu, 2001). Furthermore, it has diuretic, antidiabetic, antidermatophytic, antifungal, and antimicrobial properties (Prem Kumar and Shyamsundar, 2005), antiseptic, antitubercular, and antidysentric activities. Use of water lettuce for phytoremediation of Cd (Maine and Duarte, 2001), nitrate and ammonium (Nelson and Smith, 1981), and other heavy metals (Mishra and Tripathi, 2008) highlights the importance of this fresh water plant. Water lettuce make heavy foliage in water which inhibits the algae growth and keep water bodies clean.

Water lettuce can be multiplicate by vegetative means in water bodies (Aasim *et al*., 2013) or by *in vitro* regeneration means (Zhang *et al.*, 2008; Aasim *et al.*, 2013). There is still need to develope efficient, reliable and reproduceable *in vitro* regeneration ptotocol fort his highly economic plant. Keeping in view, this study was designed to produce whole plants using thidiazuron under *in vitro* conditions. This regeneration protocol will be beneficial for the application of biotechnological tools like genetic transformation, secondary metabolites isolation or phytoremediation studies against heavy metals from water bodies.

MATERIALS AND METHODS

The plants of Water Lettuce were procured from Department of Fishries and Aquaculture, Faculty of Agriculture, Ankara University, Ankara, Turkey. These plants were kept in aquariums equipped with oxygen supply upto *in vitro* regeneration studies. Plants were washed with tap water for 5 min before sterilization. Surface sterilization was used by using H2O2 using protocol reported by Aasim *et al.* (2013). Therafter, leaves were removed from surface sterilized plants and shoot meristem explants were cultured on 0.44% MS

medium (Murashige and Skoog, 1962) enriched with 0.05-0.60 mg/L TDZ (Table 1), 3.0% sucrose and solidified with 0.65 percent agar in Magenta GA_7 vessels. 500 mg/L Duocid (Broad spectrum antibiotic) was aslso added to the culture medium for eradication of bacterial contamination.

Table 1:Effects of TDZ on *in vitro* whole plantlet regeneration of water lettuce (*Pistia stratiotes* L.)

TDZ (mg/L)	Frequency of callus Regeneration (%)	Frequency of Shoot Regeneration (%)	Plantlets per explant
0.05	100^{ns}	100^{ns}	19.00^{b}
0.10	100	100	39.67^{a}
0.20	100	100	37.33^{a}
0.40	100	100	19.67^{b}
0.60	100	100	20.00^{b}

Means followed by different small letters within columns are sigificantly different using DMRT test at P<0.005

The experiments were run in triplicate and each treatment contained 8 explants. The pH was adjusted to 5.8±0.1 after adding TDZ followed by autoclaving at 104 kPa atmospheric pressure and 120ºC for 21 min. All cultures were incubated under 16 h light photoperiod using red:blue (3:1) Light emitting diodes (LED) lighting system. After 12 weeks of culture, *in vitro* regenerated plantlets were isolated from explant and transferred to aquariums containing tap water and with continuous supply of oxygen.

Data about callus induction frequency (%), shoot regeneration frequency (%) and number of plantlets per explants were scored and subjected to statistical analysis. The experimetal data was analysed by using One Way ANOVA using SPSS 17 computer statistical software. Post hoc tests were done by using Duncans multiple range test. Data given in percentages were subjected to arcsine transformation (Snedecor and Cochran, 1967) before statistical analysis.

RESULTS AND DISCUSSION

The study presents the use of shoot meristem explant for direct whole plant regeneration of water lettuse using TDZ as plant growth regulator in the culture medium. Shoot meristem explant is very potent explant for *in vitro* shoot regeneration and used for for other aquatic plants like water hyssop (Karataş *et al.*, 2013a and 2016; Karataş and Aasim, 2014a), *Hygrophila polysperma* (Karataş *et al.*, 2013b), *Ceratophyllum demersum* (Karataş *et al.*, 2014a), *Rotala rotundifolia* (Karataş *et al*., 2014b), *Limnophia aromatica* (Karataş and Aasim, 2015a), and *Lysimachia nummularia* (Karataş and Aasim 2015b), *Hemianthus callitrichoides* (Barpete *et al.*, 2015). Similarly, Aasim *et al.* (2013) reported use of shoot meristem of water lettuce for *in vitro* propagation cultured on agar solidified or liquid medium supplemented with kinetin. In this study, we used TDZ, which is a cytokinin type plant growth regulator used for *in vitro* propagation of recalcitrant plants (Aasim *et al.*, 2009). Although, BA is the most preferably PGR for *in vitro* propagation of aquatic plants, there are certain reports which successfully presented the use of TDZ for aquatic plants like water lettuce (Zhang *et al.*, 2008), dwarf hygro (Karataş *et al.*, 2013b) Water hyssop (Karataş and Aasim, 2014).

Results revealed 100% callus induction (Table 1) on shoot meristem explant after 2-3 weeks of culture on agar solidified medium. Callus formation in other aquatic plants using TDZ has been reported earlier for *B. monnieri* (Praveen *et al.*, 2009), *Aponogeton madagascariensis* (Carter and Gunawerdena, 2011), *R.rotundifolia* (Çiftçioğlu, 2013) and *H. polysperma* (Karataş *et al.*, 2013). Although callus induction was not too much, but it later on turned into direct plantlets with roots (Fig 1a,b).

Figure 1:*In vitro* whole plant regeneration of water lettuce (*Pistia stratiotes* L) (a) multiple plantlets regeneration after 8 weeks (b) whole plant regeneration with roots

Similarly, Zhang *et al.* (2008) gained first calli followed by shoots of water lettuce on agar solidified medium. Contrarily, Aasim *et al.* (2013) failed to obtain shoots of water lettuce

using shoot meristem explant on agar solidifed medium supplemented with kinetin. Results revealed that 100 % shoot regeneration frequency (Table 1) was achieved using TDZ in the culture medium in line with Aasim *et al*. (2013) who also achieved 100% regeneration frequency of water lettuce using kinetin or BA in the liquid medium. Contrarily, Karataş *et al.* (2013b) reported 62.5-100% shoot regeneration frequency of dwarf hygro on medium containing 0.10-1.60 mg/L TDZ using leaf explant that might be due to different plant and explant used. Vijayakumar *et al.* (2010) reported 50-95% shoot regeneration frequency whereas, Karataş and Aasim (2014) reported 100% shoot regeneration frequecy using TDZ for *B. Monnieri.*

Results on plantlets per explants showed the clear bearings of TDZ that was ranged 19.0-39.67 (Table 1). Maximum number of 39.67 plantlets per explants were recorded on medium supplemented with 0.10 mg/L TDZ followed by 37.33 plantlets per explant on medium provided with 0.20 mg/L TDZ. These results are in line with Karataş and Aasim (2014) who also obtained maximum number of 28.2 shoots per explant of *B. monnieri* cultured on medium contained 0.20 mg/L TDZ. Yenice (2010) achieved an average of 50.74 plants per explants of *Lemna minor* on medium with 0.6 mg/L TDZ. Karataş *et al.* (2013b) achieved 9.40-12.68 shoots per explant of dwarf hygro cultured on TDZ enriched medium. Similarly, TDZ has been reported for *in vitro* multiplication of other aquatic plants like *Ipomoea aquatica* (Akaracharanya *et al.*, 2001), *Trapa japonica* Flerov (Hoque *et al.*, 2001), *Spartina alterniflora* (Wang *et al.*, 2003), *Lemna gibba* var. Hurfeish and *Spirodela punctata* (Li *et al.*, 2004) *Ludwigia repens* (Öztürk *et al.*, 2004) and *B. monnieri* (Praveen *et al.*, 2009). Results further revealed that increase of TDZ siginificantly decreased the palntlets per explants in line with Karataş *et al.*(2013b) for dwarf hygro and *B. monnieri* (Karataş and Aasim, 2014).

It was interested to noted that direct plantlets regeneration was observed during culture and these plantlets with roots were separated easily from mother explant Therefore, there was no need of rooting and these plantlets were directly transferred to aquariums provided with oxygen supply where plants acclimatized and produced plantlets within two months.

Conclusion: This study reports the successful developement of direct whole plant regeneration under *in vitro* conditions followed by successful adaptation of these plantlets in the aquariums. This protocol can be use for secondary metabolites production, genetic tranformation or for phytoremediation studies.

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