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Research article

PROPAGATION AND BULB FORMATION OF *FRITILLARIA* (*FRITILLARIA IMPERIALIS* L.) VIA *IN VITRO* CULTURE

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ABSTRACT: The *Fritillariaimperialis* is grown natively in Iran. Between ornamental bulblet plants, this plant has special place, therefore this plant needs more attention, otherwise this plant will be destroyed in future. For this reason *in vitro* culture of *Fritillariaimperialis* was studied. Tissue culture techniques have high potential for mass propagation. The multiplication efficacy of the method by bulb is low; the plantlets are easy to get diseases and depended on the crop. Plant tissue culture is a technique that has ability of production of a large number of high quality plants. Factors influencing on the regeneration ability and *in vitro* plantlet growth were examined. The number of regenerated shoots was the highest on MS medium supplemented with 0.5 mg/l TDZ and 30 g/l sucrose; the number of roots was the highest on MS medium supplemented with 0.2 mg/l NAA and 30 g/l sucrose; the *in vitro* bulb diameter is largest. *In vitro* plantlets survived in greenhouse at a high rate. A whole process was established for rapid multiplication of *Fritillariaimperialis* L.
Keywords: Bulb scale, bulblet, *Fritillariaimperialis*, *in vitro*, micropropagation

INTRODUCTION

Fritillariaimperialis L. which is called ‘‘Tears of Mary’’, because of great drops of nectar at the petal base, is aperennial plant with high medicinal and ornamental importance [18]. The fascinating Fritillaria genus, belonging to Lilliaceae family, includes 100 species, of which 14 important species are native to Iran [1, 16, 17]. In Iran, wild populations of important species, like F. imperial is and F. *persica*, are at the risk of rapid eradication, because of irregular grazing of *Fritillaria* stands, lack of protecting rules, changing the pastures to dry farmlands, and pest overflow [3].Wild populations of *F. imperialis* are mostly found in high altitudes (>2,000 m) of western parts of Iran, particularly in two provinces, ChaharMahal-va-Bakhtiari and Kohkyluyeh-va-Bouyrahmad. *In vitro* cloning of endangered plants can offer considerable benefits for rapid propagation of species that are at the risk, that have limited reproductive capacity and exist in threatened habitats [5].*In vitro* culture using bulb scale segments, the most commonly used explants in tissue culture of bulbous plants, has been reported for a defined cultivar of F. imperial is [19].The use of bulbs as a source of explants is often associated with the heavy bacterial and fungal contamination. In the case of *F imperialis*, because of a limited number (3–5) of scales per bulb, the problem is more serious. In addition, since the bulb of *F. Imperialis* has a fewer number of active meristematic cells in comparison with other Lilliaceae family like lilies; the tissue culture system based on bulb segments is not efficient [1].Moreover, the use of bulb scale pieces destroys the perennial endangered parent plant. The used petal at green-closed flower bud stage for callus production and indirect regeneration of wild population of F. imperialis by using regular sub culturing in PGRs-containing media [7]. The major limitation of indirect regeneration pathways is genotype-dependency. Because of self-incompatibility nature of *F. imperialis*, a wild population of F. imperialis is highly heterozygote and non-uniform, so, for conservation of different F. imperialis populations, the regeneration pathway must be highly genotype-independent and applicable for a wide number of genotypes. Direct regeneration pathways (regeneration without passing callus phase) and indirect regeneration pathways (regeneration with passing callus phase) have been used for overcoming genotype-dependency and retaining long-time to impotency in different plants like cereals [4] and cumin [2]. However, up to know, there is no report either on indirect organogenesis/bulblet regeneration in *F. imperialis*.

The establish indirect bulblet regeneration pathway by application of different proportions of auxins (NAA and IAA) to cytokinins (BAP and TDZ) in the culturing media [13]. Fritillariami crop propagation by bulb plants as an alternative to the conventional methods for vegetative propagation is becoming important owing to certain advantages such as increased multiplication rates and the attention of plant material free of viruses and other pathogens.

The *in vitro* micropropagation of Lilium species using bulb scales as an explants source for the production of bulblets has been reported[15].In this paper, a protocol from shoot multiplication, plantlet regeneration to acclimatization plants in greenhouse was was standardized by studying effect of different concentrations of cytokinin, auxin and activated charcoal on bud multiplication, bulb and root formation.

MATERIAL AND METHODS

Plant materials

Bulb scales derived from in vitro bulb of *Fritillariaimperialis* L. cultured in Murashige and skoog [8] supplemented with 0.5 mg/l 6-benzyladenine (BA), 30 g/l sucrose and 8 g/l agar.

Hoot multiplication

MS modified ($^1/_2$ MS) medium supplemented with different concentrations of BA or kinetin (Kin) or thidiazuron (TDZ), 30 g/l sucrose and 8 g/l agar was used for bud multiplication from Fritillaria bulb scale *in vitro.*

Root formation

MS and 1/2 MS medium supplemented with different concentrations of a-naphthalen acetic (NAA), 30 g/l sucrose, 8 g/l agar with or without 1.0 g/l activated charcoal (AC) were used for root formation shoots derived from culturing *Fritillariaimperialis* bulb scales *in vitro.*

Media were dispensed in 250-ml culture vessels at a volume of 40 ml per vessel; the pH of media was adjusted to 5.8 before autoclaving at 121 ^{0}C, 1.0 atm for 30 minutes.

Fritillaria bulb scales and plantlets were placed at 25 ± 1.0 ^{0}Cand 70 – 80 % relative humidity, with a light intensity of 45 µmolm^{-2}s^{-1}provided by cool white fluorescent lamps for a light period of 10 hours per day.

Acclimatization

Uniform *Fritillariaimperialis* plantlets derived from bulb scale were collected and transferred in greenhouse in order to observe their growth and development.

Result and discussion

Effect of BA, TDZ, Kinetin on shoot regeneration and bulb formation of *Fritillariaimperialis* bulb scales

After six week culture, shoot regeneration and bulb formation were carefully observed, data will be showed in table 1.

Table 1. Effects of BA, TDZ, Kinetin concentrations on shoot regeneration and bulb formation of *Fritillariaimperialis* bulb scales

BA (mg/l)	TDZ (mg/l)	Kinetin (mg/l)	Shoot height (mm)	N. of shoots	N. of leaves	Leaf diameter (mm)	N. of roots	Root length (mm)	Bulb diameter (mm)	Fresh weight (mg)
0.1			39.3	3.1	2.0	4.6	5.2	8.3	7.1	281.2
0.2			42.1	4.1	2.3	5.3	7.0	24.3	10.1	789.0
0.5			24.1	4.2	1.3	4.3	4.1	7.2	9.2	657.4
1.0			9.5	4.1	-	-	-	-	6.1	420.2
	0.1		27.2	3.1	1.0	0.5	4.4	60.3	6.2	398.0
	0.2		22.2	6.2	0.02	1.3	4.0	57.1	4.5	460.5
	0.5		-	10.1	-	1.2	3.1	22.2	-	708.1
	1.0		14.1	5.5	0.9	-	2.5	10.5	4.8	456.3
		0.1	67.3	2.0	1.1	9.3	6.0	48.1	8.2	605.0
		0.2	68.1	1.1	1.0	8.2	6.4	39.6	8.0	636.8
		0.5	65.5	2.1	2.2	11.2	7.7	33.3	8.6	762.4
		1.0	70.3	2.2	2.4	11.3	8.3	21.4	8.7	776.6

A significant development was observed in growth regulator media of 0.2 mg/l BA with the best fresh weight (0.804 mg/shoot), shoot height (40.8 mm), number of shoots (4), number of leaves (1.5), leaf diameter (5.7 mm), number of roots (5.33), root length (30.20 mm), bulb diameter (7.4 mm) (Table 1).

In term of shoot regeneration, it has no general pattern that the amount of shoot steadily increased with increasing BA even to 1.0 mg/l. It was also observed that all of the explants produced roots when BA was present, and medium which is supplemented with 0.2 mg/l BA appeared to be best inductive with the highest number of roots and root length (Table 1).

In assessing the root-forming abilities the results clearly indicated that the quality of bulb steadily increased with the increasing of root-forming (Table 1).In consistent with our result, Indirect Organogenesis of has been previously reported [13, 14].Among various concentrations of BA the cultured explants showed best bulb formation on the medium containing 0.2 mg/l.

This concentration showed highest for bulb diameter and fresh weight production.Paek and Murthy [9] reported the of bulblet regeneration from *F. Thunbergii* using bulb scale segments and combination of different cytokinins with NAA. The result also conveyed that the relationship between bulb formation and cytokinin concentration is consistent with previous studies that using cytokinin in inducing bulb formation was successful than using auxin and cytokinin has an important role in bulb formation stage which activated starch synthesizing process and inhibited the enzyme which analyzed starch. These observations support the findings of Pierik and Post [12]; Pierik and Woet [11]; Pierik and Ruibing [10]; Young Byung et al. [20], who showed noticeable bulblets regeneration of hyacinth from scale explant.

With bulb scales culture in MS medium supplemented with different concentrations of TDZ, shoot initiation was observed every one week after culture. The optimum response in terms of percentage of explants producing shoots and the highest number of shoot buds per explant was recorded on MS medium supplemented was 0.5 mg/l TDZ. On this medium 100% culture responded with an average 9 shoots per culture. The regenerated shoots attained fresh weight of about 723.7 mg/shoot in about 42 days of culture. Hussey [6] has approved the importance of time in regeneration of bulblet from hyacinth explant and suggested that the appropriate period for regeneration of bulblet in 6 to 7 weeks.

The results showed that there was no significant difference in shoot regeneration or bulb formation of explants in MS medium plus kinetin with different concentration. The number of shoot was just 1. A few bulbs were produced. However, the media containing kinetin was found to be more effective in producing the complete plants compared with other media tested.

Effect of NAA and activated charcoal on *Fritillariaimperialis* root and bulb growth

After six week culture, root and bulb formation were carefully observed. Root length, bulb diameter and fresh weight of *Fritillariaimperialis* were obtained and data in detail will be showed in Table 2.

Table 2. Effect of NAA and activated charcoal on *Fritillariaimperialis* root and bulb growth of *Fritillariaimperialis* plantlets derived form bulb scales

Medium	NAA (mg/l)	Activated charcoal (g/l)	Number of roots	Root length (mm)	Bulb diameter (mm)	Fresh weight (mg)
MS	-	-	3.2	39.2	7.3	587.5
½ MS	-	1	7.1	35.4	6.2	613.3
½ MS	-	-	10.5	40.4	9.1	450.2
½ MS	0.1	-	20.3	39.1	9.5	930.7
½ MS	0.2	-	30.3	57.3	10.2	1329.7

Of the treatments, ½ MS supplemented with 0.2 mg/l NAA gave the best results. Explants cultured in this medium had higher number of roots, root length, bulb diameter and fresh weight than than those cultured in the other medium (Table 2).

Activated charcoal (AC) has an important role in inducing cell development and differentiation in some plants such as orchids, onion, carrot…In this study, 1.0 g/l AC was added to ½ MS media to investigate that whether or not it enhances *Fritillariaimperialis* root and bulb growth of *Fritillariaimperialis* plantlets derived from bulb scales. The results indicated that there were not significant differences between treatment with AC and other with no AC (Table 2). The same result was also obtained with the number of roots, root length, bulb diameter, fresh weight compared with MS and ½ MS without AC.

Acclimatization

In the present experiment, about 1000 regenerated plants *Fritillariaimperialis* were taken out from *in vitro* condition and planted into plastic pots containing garden soil, sand in a ratio of 2:1. Survival frequency of the plantlets under *ex vitro* condition on soil was 97%.

CONCLUSION

This paper examined factors influencing on the regeneration ability, bulb formation and growth *in vitro* of *Fritillariaimperialis* plantlets derived from bulb scales. The number of regenerated buds was the highest on MS medium supplemented with 0.5 mg/l TDZ; the number of roots was the highest on½MS medium supplemented with 0.2 mg/l NAA; the *in vitro* bulb diameter is largest on MS medium supplemented with 0.2 mg/l BA. *In vitro* plantlets survived in the arboretum at a high rate. A whole process was established for rapid multiplication of *Fritillariaimperialis* L.

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