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Review Article

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In vitro propagation techniques for some geophyte ornamental plants with high economic value

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Abstract: Propagation of some ornamental plants has increased tremendously due to the demand for them as cut flowers, in addition to their usage for interior and exterior landscaping purposes. Geophytes (bulbous-tuberous) are the most preferred group among the ornamental plants due to their aesthetic features, suitability to be cut flowers and their fragrance. These plants are highly propagated and consumed. The geophyte species which are highly profitable globally traded and constituting 90% of the flower bulb market are *Tulipa* (tulip), *Lilium* (lily), *Narcissus* (daffodil), *Gladiolus* (gladioli), *Iris* (iris) and *Hyacinthus* (hyacinth). *In vitro* propagation techniques, which provide disease-free mass production options, have started to be used increasingly to fulfil the demand for these species in the market. In this study, the results of *in vitro* propagation studies for some economically valuable tulip, lily, daffodil, gladiolus, iris and hyacinth species are provided.

Keywords: (Propagation, Geophyte, *In vitro*, Ornamental Plant

1. Introduction

Geophytes have economic value due to their attractive flowers and usage in the drug and perfume industries (Ekim et al., 2000). However, bulb formation rates are low and the formation periods are long for the plants, especially the ones belong to *Liliaceae*, *Iridaceae* and *Amaryllidaceae* families, in the natural environment (Nasırcılar and Karagüzel, 2006). It is reported that bulbous plants, especially *Tulip* and *Lilium*, lead cut flower sector worldwide (Buschman, 2005); while *Gladiolus, Fressia, Hyacinthus, Narcissus, Iris, Alstroemeria, Hippeastrum, Zantedeschia, Anemon* and *Ranunculus* follow these two plants (De Hertogh et al., 2012). *Tulip*, *Lilium*, *Narcissus*, *Gladiolus*, *Iris* and *Hyacinthus* plants build 90% of the market (Karagüzel et al., 2007).

Geophytes are of great economic value as they are used in food production, medicine and landscape sectors and are tried to be quickly propagated with tissue culture techniques (Zaidi et al, 2000). Plant tissue culture is a field including various botanical disciplines, techniques and methods used for research; and producing plantlets from plant cells, tissues or organs isolated from the main plant following these techniques (George, 2008).

This technique is used in the areas of horticulture, field crops, forestry and landscape architecture for commercial purposes (Mansuroğlu and Gurel, 2001). Eastern and Western Europe, including Poland and Hungary in particular, have become very important producers of

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in vitro propagation. Additionally, Far East Asia started to be playing a dominant role in the world market on micro propagation (Debergh, 1994).

Approximately more than 500 million plants in 50 thousand plant varieties are propagated annually by more than 600 firms using plant tissue culture techniques worldwide (Yu, 1998). However, commercial usage of micro propagation is still limited due to the high laboratory costs, low growth rates, non-uniform plant development and high production costs due to low survival rates (Özkaynak and Samancı, 2004). When performed *in vitro* studies are examined, factors such as used explant type, medium content, plant development regulators and physical and chemical properties of the mediums (light, temperature, moisture, pH, sugar, oxygen and carbon dioxide concentration) are studied in general. In this study, researchers carried using plant tissues of the five highly traded and propagated geophytes species are displayed (Table 1). The analysis aim at creating awareness on the propagation activities of the past and the possibilities for the future with an emphasis on the explants type used, medium, plant growth regular (PGR) and the results.

2. Results

Studies on *Lilium* species carried between 1976 and 2014 are examined. Bulb scales, roots, leaves and seeds were used as explant in these studies. Explants were planted in different nutrient mediums such as Linsmaier and Skoog, MS and Aartrijk. The impacts of NAA, BAP, 2,4-D, 2IP ,TDZ and other PGRs added in the mediums were determined according to the dosages used. Concentrations of PGs were mainly 0.1, 0.2, 0.5, 1, 1.5, 2 mg l^{-1} and 0.7, 1, 1.07, 4,4, 4,44, 5 µM, while different dosages were also tested. In addition to nutrient mediums and PGR concentrations, sucrose, light, explant size and phosphorus and potassium amounts in the mediums were examined. The results of the studies included information on the bulbs weight, number, quantity and quality of bulblet, callus, root and plantlet; chlorophyll, starch and sugar contents of scales, and their formation times.

Studies on *Gladious* species carried between 1977 and 2014 are examined. Petals, flower spike, flower and bulbous buds, terminal and lateral buds, axillary buds, peduncle, callus, roots, scapes, cormels, corms, bulbs, ovary tissue and stem sections were used as explant in these studies. MS, N_6, ½ MS, modified MS and Linsmaier and Skoog nutrient mediums were used in the studies and various chemicals (2,4-D, BA, NAA, BAP, Kinetin, Zeatin, IBA, ABA, CCC, GA_3, active carbon, TDZ, IAA, PP333, $AgNO_3$, sucrose) were added in different concentrations (0.0026, 0.0046, 0.01, 0.25, 0.1, 0.3, 0.4, 0.5, 0.6, 0.7, 1.1, 1.5, 2, 3 and 4 mg l^{-1} and 0.5, 1-2.5, 5 and 10 µM). The findings included information on the shoot growth, root development, rooted plantlet, number of bulblet and root, lengths of roots, size and fresh weight of the corms, callus, petal and corm formation, primary embryo development and number of somatic embryo.

Studies on *Iris* species carried between 1985 and 2014 are examined and it is determined that leaves, rhizoms, bulbs, bulb scales, green or mature embryos, adventitious and rhizome buds were used as *in vitro* explant. MS, ½ MS or different culture mediums were used as nutrient medium. Various PGRs (like BAP, 2,4-D, IBA, NAA, IAA, Kinetin, GA_3, TDZ and 6-furfurylaminopurine) at different concentrations (1 and 2 mg dm^{-1}, 0.2, 0.4, 0.5, 1, 2 and 3 mg l^{-1}) were added in the nutrient mediums. The studies included findings on callus, bud, bulblet and root formation, rooted plantlets, adventitious shoots, bulblet weights, number of bulblets and shoots, germination and shoot induction.

Studies on *Tulipa* species carried between 1983 and 2012 are examined. It is determined that explants such as floral stem, immature ovula and embryo, microbulbs, bulbs, scale, seed, flower, leaf and leaf stem were used. Different nutrient mediums such as MS, ½ MS, modified MS, N_6, B5, SH were used in the studies. GA_3, BAP, SA, BA, NAA, Kinetin, 2,4-D, Picloram, Active carbon were used as PGR, and cold incubation was also implemented. The PGRs are used at 25 ve 0.5 µM, 0.1 ve 0.5 mmol l^{-1}, 0.1, 0.2, 0.3, 0.4, 1, 1.5, 2 ve 3 mg l^{-1} concentrations

and data on callus, shoot, adventitious roots, bulblet and somatic embryo formation, fresh and dry weight of microbulbs and bulblet were obtained as a result of the studies.

Studies on *Narcissus* species carried between 1982 and 2013 are examined. Bulb, bulb scales, segments, twin scales, flower peduncle, leaves, flower stalk and scapes were used as explant in the studies. Materials such as BAP, 2,4-D, 6-BA, NAA, IAA, IBA and sucrose were added in different doses (0.01, 0.12, 0.1, 0.2, 0.5, 1, 1.5, 2, 3, 3.2, 4 and 8 mg l^{-1}, 176 mM, 0.5, 0.54, 4.9, 5, 5.4 and 27 µM) in N_6, MS, ½ MS, ¼ MS and different nutrient mediums. Callus, adventitious shoots, leaves, roots, bulbils, bulbs, bulblets, somatic embryogenesis, plantlets were obtained in the studies and data on lengths and thickness of roots, speed of bulblets growth, size of bulbs and their formation time is presented.

Studies on *Hyacinthus* species carried between 1974 and 2010 are examined. It is determined that bulb scale, basal plate, leaf, ovary, stem, peduncle, flower bud, pedicel and immature inflorescence were used as explant in the studies. These explants were planted in different nutrient mediums such as MS and modified Heller along with PGRs (NAA, BA, IAA, IBA, ABA, Kinetin, Fluridone and sugar forms) at various concentrations (0.54-5.4 and 4.4-44 µM, 360 mM, 0.2, 0.3, 0.5, 1, 1.5, 3, 5 and 10 mg l^{-1}). The results obtained were on callus formation, root differentiation, number and size of bulblets, bulblet primordia, length of root, tepals formation and tubers formation.

The most widely used explant source of *in vitro* propagation is bulb scale segments (Mirici et al., 2005). For bulb development, various explant sources such as ovary, flower stalk, leaf stalk and mature seeds are also used in addition to the bulb scale segments (Tıpırdamaz, 2003). In this review, it is found that the most widely used explant types in different plant species are bulb scale and leaf. Usage of MS as the medium is common, except for a couple of studies. GA_3, ABA, picloram and kinetin are rarely used as PGR in the studies, while NAA, BA and IBA are used in the same or similar range of concentrations almost in all the studies (Table 1). PGRs are known to have certain effects on the plants. On the other hand, they might have different effects on different parts on the plants. This is the reason of experiments on the impacts of PGR on callus, shoot and bulb formation in different species. In some studies, the impacts of the mediums with PGR or without PGR on the explants are examined, while in the others the impacts of culture conditions are examined. In this review, development of the explants in the mediums is taken into account. *In vitro* studies, especially the commercial ones, require disease free and mass production. In this review, it is aimed to build a basis for further studies by discussing *in vitro* protocols of some commercially important geophyte species and the results obtained.

Table 1. *In vitro* propagation techniques for some geophytes

Plant	Explant Types	Culture Medium	Results	References
			***Lilium* sp.**	
L. oriental hybrids	Bulb scales	MS+PGR	The highest yield was obtained by the cultivar '*Empress of India'* and during a six-month period 10 bulblets grew per bulb scale. It is estimated that a bulb could provide 500 bulblets.	Simmonds, J.A. and Cumming, B.G., 1976a.
Lilium hybrids	Bulb scales	Nutrient medium+different doses of 6-BA and NAA	Callus was induced by a combination of 5 μM BA and 5 μM 2,4-D on 12 lily cultivars. Once initiated, this callus grew vigorously on media without PGR. Continuous light and 0.5 μM NAA caused maximum production of plantlets.	Simmonds, J.A. and Cumming, B.G., 1976b.
L. rubellum	Leaf segments	MS+1 mg l^{-1} NAA+0.1 mg l^{-1} BA	NAA was important for bulblet formation. BA had a slight stimulatory effect only when NAA was also present. bulblet regeneration was affected by sucrose concentration and light.	Niimi, Y. and Onozawa, T., 1979.
Lilium cultivars	Bulb scales	Nutrient medium+different doses PGR	NAA strongly influenced the adventitious regeneration of plantlets. Adventitious regeneration was not affected BA and 2iP.	Van Aartrijk, J. and Barnhoorn, G.J.B., 1981.
L. oriental hybrid '*crimson beauty'.*	Bulb scales	Linsmaier and Skoog (1965) basal culture medium+different doses of NAA and BAP	The combination of 5 μM BAP and 1 μM NAA induced maximum bulblet formation per explant. On the same medium bud differentiation on the ovary- and leaf-derived explants was succeeded.	Novák, F.J. and Petrů, Ev A., 1981.
L. davidii var. *"unicolor cotton"*	Apical, middle or basal segment of bulb scales	MS + 0.2 mg l^{-1} NAA and 2 mg l^{-1} 6-BA	Adaxial surface belonged the bulb-scales emerged bulblets about at 9-10 days. It was also observed that the potential of the apical, middle or basal segment of a bulb-scale was similar. About after a month in culture, the result of stimulation of the roots on the same media plantlets were obtained.	Pifang, Z. et al., 1985a.
Lilium c.v. "*enchantement*"	Bulb scales	Aartrijk (1984) medium and contained MS salts, 0.1 mg l^{-1} NAA. Agars was selected among 7 commercially types	Maximum bulblets numbers were obtained on Merck 1614 and Difco Bacto (5.3 units). On BD purified agar, max. bulblets weights were obtained 471 mg. Minimum numbers and weights were obtained from BD (Becton Dickinson) grade A agar (3.2 unit and 120 mg).	Scholten H.J. and Pierik, R.L.M., 1998.

Orient lily hybrids of *L. tenuifolium*	Basal roots, roots of tube cultural seedlings and the base of scale leaves	Different nutrient mediums	The number of bulblet (2.25) and buldlet (3.13) were obtained by basal roots and the base of scale leaves. The number of buldlet of pretreatment of low temperature of 4℃ was 6.35, than significant higher the control. A4 culture medium induced bulblet 4.95 averagely.	Huang, H., 2000.
Lilium subsp.	Different size of bulb scale segments	Nutrient medium+sucrose	Bulb growth was influenced by explant size during the complete culture period and was stimulated by a high sucrose concentration. Together uptaking of medium components the percentage of bulb growth was 45-50% for large and 65–75% for small explants.	Langens-Gerrits, M. et al., 2003.
Lilium subsps.	Bulb scale	MS+different doses of plant growth regulations (PGR)	Scale color and shape changed distinctly along with milky white-pale yellow-pale purple-green purple-scale withering and bullet growing. Chlorophyll content of scale increased, but starch and sugar contents of scale decreased. The suitable increases of phosphorus and potassium concentrations in medium increased the quantity and quality of bullet.	Aiqin et al., 2004.
L. lancifolium	External, middle and internal bulb scales	MS+different doses of 6-BA and NAA	The regeneration ability of external scales was the best. MS + 1.5 mg l^{-1} 6-BA+0.2 mg l^{-1} NAA could induce more and stronger shoots. The best proliferation medium was MS+1.0 mg l^{-1} 6-BA +0.2 mg l^{-1} NAA.	Haibin, G. and Jiajun, L., 2006.
L. longiflorum	Leaf explants	MS+ different doses of BAP and NAA	All PGR combination succeded direct bulblet regeneration. Without callus formation, 0.5 or 2.0 mg l^{-1} NAA and 0.1 mg l^{-1} BAP added mediums developed bulblets and roots. Roots were formed by the shoots or bulblets on 0.2 mg l^{-1} NAA. Increasing the sucrose doses resulted in higher efficiency of bulblet enlargement.	Tang, D. et al., 2009.
L. tsingtauense	Bulb scales	MS+different doses of PGR	0.1 mg l^{-1} NAA+2.0 mg l^{-1} BA combination produced 52 plantlets regenerated from adventitious shoots; 0.1 mg l^{-1} NAA+2.0 mg l^{-1} TDZ had 2 plantlets and 2.0 mg l^{-1} BA+2.0 mg l^{-1} 2,4-D had none.	Yang, W. et al., 2010.
L. longliflorum	Bulbs scales and seeds	MS+different doses of PGR	The best combination was 2 mg l^{-1} BA and 0.2 mg l^{-1} NAA plus MS among 10 combinations of difference hormone. 14 indefinite bud seedings were obtained by 1 bud in 30-40 days. No hormone produced the rooting frequency of 99.0% and 6.9 average roots in one indefinite bud seeding.	Sun, L. and Jin, L., 2011.

Lilium cultivars	Roots from seedling	MS+different doses PGR	Picloram and cytokinins together affected callus formation positively. In three *Lilium* cultivars, the highest percentage of explants forming callus was obtained about 80-98% from 1.0 mg l^{-1} PIC and 0.5 mg l^{-1} BAP combination.	Zhou Y. et al., 2013.
Lily "*Siberia*" (c.v. *oriental*)	Bulb scales	MS+1.07µM NAA+4.44 µM BA and different doses of sucrose	The highest inducement rate, the most buds and induced fast were obtained from addition with MS+1.07µM NAA+4.44 µM BA+30 g l^{-1} sugar respectively 88.91%, 13.78/explants and 23.33 days. PGR showed slight difference with different light conditions on bulblets.	Zhang, M. and Jia, G., 2014.
Plant	**Explant Types**	**Culture Medium**	**Results**	**References**
			***Gladiolus* sp.**	
Cultivars of hybrid *Gladiolus*	Axillary buds excised from cormels	MS+different concentrations of BAP	BAP prevented dormancy, promoted shoot growth and inhibited root development.	Hussey, G., 1977.
Gladiolus subsp.	Bulbs	N6 or MS medium + 0.1-0.5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BA	Eye-bud of bulb in the N6 or Ms medium which contains 0.1-0.5 mg l^{-1} 2,4-D and 0.5 mg l^{-1} BA emergenced.	Bo, J. et al., 1984.
G. hybridus	Flower and bulbous buds	MS+different doses of BA and NAA. The young shoots were transplanted to ½ MS medium supplemented with 0.1-0.5 mg l^{-1} NAA	The perfectly rooted plantlets can be obainted.	Weiyan, Z. and Zhiran, S., 1986.
Gladiolus × *Homoglossum* hybrid	Corm	Linsmaier-Skoog medium+PGR	Up to 1000 plants could be produced. Large numbers of buds developed on transversely-cut halves of the corms when they were placed on Linsmaier-Skoog+BA. Transfer to medium containing NAA resulted in elongation of buds into shoots and formation of corms at the bases of the shoots.	Sutter, E.G., 1986.
Gladiolus hybridus	Ovary tissue	MS+different doses of PGR	Rootless seedlings, seedling with roots and bulbet can be obtained. Rootless seedling were transferred to ½ MS mudium supplemented 0.l mg l^{-1} NAA and plantlet with roots, bulblet can be obtained.	Gang, X. et al., 1992.

Gladiolus cultivars *"friendship", "gold Finch's"* and *"her majesty"*	Axillary buds	Modified MS+0.5 mg l^{-1} BAP and Kinetin (for shoot multiplication); MS+different doses of NAA, IBA and IAA (for rooting); ABA, CCC, Kinetin, GA_3 and AC (for corm formation)	Number of roots (per shoot), length of roots and the number of laterals formed with 0.25 and 0.5 mg 1^{-l} IAA appeared to be better than the other auxin treatments. BAP and Kinetin reduced the size and fresh weight of the corms. Except for 0,0026 mg l^{-1} ABA, all the other treatments proved inhibitory. 3% of AC inhibited corm formation (by 24%) and corm size (by 34%).	Dantu, P.K. and Bhojwani, S., 1995.
Gladiolus x grandiflorus cv. *"peter pears"*	Cormels	Formerly MS+2 mg l^{-1} 24-D. After 20 days, callusing medium was composed with different doses of zeatin and BA	Zeatin or with 0.25 µM BA induced primary embryo development. More plantlets were harvested per colony at the higher concentrations of BA and zeatin. Maximum plantlets were obtained as 12 from 0.5 mg l^{-1} BA and Zeatin.	Rernotti, P.C., 1995.
Gladiolus cultivars *"green bay", "wine & roses", "top brass"* and *"mornlo"*	Terminal and lateral buds of cultivars were excised from dormant corms	MS+1 mg l^{-1} BAP	The degree of response did not vary to a great extent in different cultivars, although "*green bay*" and "*wine & roses*" showed the lowest and the highest responses, respectively. The highest number of corms was obtained by apical bud as 144 while by nodal bud as 1.	Sen, J. and Sen, S., 1995.
Gladiolus c.v. "*topaz"*	Callus	Culture medium+different doses of 2,4-D	The percentage of adventitious root formation varied in culture media. It was suggested that the drop of the organogenic ability in callus could be related to the accumulation of 2,4-D in callus during subculture. Both adventitious roots and shoots were formed with 2,4-D 0-0.046 mg l^{-1}. No organogenesis was observed at higher concentrations.	JeongDoo, C. et al., 2000.
Gladiolus subsp.	Roots	For callus: MS+2 mg l^{-1} 2,4-D, 0.7 ppm kinetin, for plantlets: MS alone, containing either 0.2 mg l^{-1} BA or 1 mg l^{-1} kinetin. For root and corm formation: MS+0.1 mg l^{-1} IBA	Embryonic callus produced plantlets in to MS alone, containing either 0.2 mg l^{-1} BA or 1 mg l^{-1} kinetin. The produced plantlets were transferred to either root-induction medium composed of MS +0.1 mg l^{-1} IBA or to corm-induction medium composed of MS+90 g l^{-1} sucrose.	Mohamed-Yasseen, Y., 2000.
G. grandiflorus	Peduncle	MS+5 µM NAA+10 µM KN	3 buds were obtained per corm explant and 10 buds were obtained per inflorescence explant.	Ziv, M. and Lilien-Kipnis, H., 2000.

Gladiolus subsp.	Stem section	MS+different doses of BA and NAA	The callus and proliferation of the buds were beter while only in the presence of 1.0 mg l^{-1} 6-BA. The best treatment is of 0.5 mg l^{-1} 6-BA when deriving the roots.	Wang, Y. et al., 2003.
G. grandiflorus c.v.“*pink”*	Shoot-tip, corm section, basal plate and daughter corm	MS+different doses of BA. Supplemented IBA doses on root and corm formation	Shoots formed on all explant sources with 0.9, 2.2 or 4.5 µM BA. Basal plates gave the highest shoot formation as compared to other explants. Any explant were highest value by shoots at 0.9 µM BA. Formed roots by shoots on medium from 1.0 to 2.5 µM IBA. The highest plantlets with corm formation was 85% on 1.0 µM BA in 60 days.	Nhut, D.T. et al., 2004.
Gladiolus hybridus	Cormlets with buds	For callus induction: MS+3.0 mg l^{-1} 2,4-D. For shoot differentiation and proliferation: MS+different doses of 6-BA and NAA	The optimum media for shoot differentiation, proliferation and rooting were MS + 0.5 mg l^{-1} 6-BA + 0.1 mg l^{-1} NAA, MS + 0.5 mg l^{-1} 6-BA + 0.1 mg l^{-1} NAA and 1/2 MS+0.1 mg l^{-1} NAA.	Al, L. and Li, M., 2005.
Gladiolus c.v.s. *“rose supreme”* and *“advanced red”*	Scapes	MS+different doses of BA and IBA	The best medium for callus induction and adventitious shoot formation was MS+2 mg l^{-1} BA+0.4 mg l^{-1} IBA as 80.50% and 87.78% respectively “*rose supreme”* and “*advanced*”. Shooting percentage and average adventitious shoots per explants were 14.2 and 13.0 respectively. When the rooting medium was MS+0.5 mg l^{-1} IBA, the rate of rooting reached 86.67% and 92.44%, the number of roots per plantlet were 16.1 and 22.1. Roots grew strongly and the rate of corm formation was above 90%.	Hao, H. and Yi, M., 2007.
G. hybridus	Cormlet	Added paclobutrazol (PP333) on culture medium at different concentrations with PGR. For proliferation MS+0.1 mg l^{-1} NAA+0.4 mg l^{-1} 6-BA. For rooting ½ MS+1.5 mg l^{-1} IBA+ 0.01 mg l^{-1} NAA.	1 mg l^{-1} PP333 improved the proliferation of shoots. 0.1 mg l^{-1} PP333 in the medium for rooting improved rooting and growth.	Zheng, Y. et al., 2007.

Gladiolus c.v.s *"white friendship"* and *"fidelio"*	Axillary buds	MS+different doses of BAP	MS+BAP 0.5 mg l^{-1}, 1 mg l^{-1}, 2 mg l^{-1} and 3 mg l^{-1} gave the 100% establishment of explants. Concentrations of BAP at and above 2.0 mg l^{-1} resulted in the swelling of buds, more at the base and took longer time to produce leaves, which resulted in moderate growth of explants.	Akhare, A. A. et al., 2008.
Gladiolus c.v. *"advanced red"*	Cormlet and virus-free root	Nutrient mediım+different PGR and $AgNO_3$	0.6 mg l^{-1} 2,4-D+1.1 mg l^{-1} 6-BA+1.5 mg l^{-1} $AgNO_3$ medium was the optimum medium for direct regeneration of adventitious buds from the cormlet. Regeneration percentage of adventitious buds was 85.95%, and average adventitious buds was 14.10. The optimum medium for rooting was MS+0.5 mg l^{-1} IBA, the rate of rooting reached 91.81% and the number of roots per plantlet were 18.97.	Zhang, Z. et al., 2008.
G. anatolicus	Cormel segments	MS+ different doses of 2,4-D. After 8 weeks different doses of BA supplemented	At the lowest concentration of BA (0.5 µM), the highest number of somatic embryos (23.6 ± 3.6) was produced. The number of somatic embryos was significantly enhanced up to 31.6 ± 3.8 by adding 12% sucrose.	Erdag, B.B. et al., 2009.
Gladiolus c.v."*rose supreme"*	Petals	For callus induction: MS+ different concentrations of PGR. For somatic embryo induction: 2,4-D, 6-BA and TDZ doses	The best medium was MS+4.0 mg l^{-1}2,4-D+0.5 mg l^{-1} 6-BA for callus and the best medium was MS+1.0 mg l^{-1} 2,4-D+0.3 mg l^{-1} TDZ for somatic embryogenesis. Petal callus could form new petals directly. The highest Somatic embryo number was obtained 1.0 mg l^{-1} 2,4-D+ 0.5 mg l^{-1} 6-BA+ 0.3 mg l^{-1} TDZ as 26 and induction rate as 65%.	Cai-hua et al., 2012.
Gladiolus subsp.	Young adventitious shoots from cormels	MS+different doses of BA, Kinetin and sucrose	MS+3% sucrose and 0.1-0.5 mg l^{-1} BA gave the highest average number of plantlets per explants about 6.6-7.0 plantlets. The average number of cormels on MS+0.1-0.5 mg l^{-1} NAA and 3% sucrose was the highest about 5.8-5.6 cormels and their fresh weight about 144–144.2 mg per explants, respectively. On MS+1-2 mg l^{-1} NAA and 4% sucrose gave the highest average fresh weight of cormels per explant about 148.6–149.0 mg and the average cormel was the lowest about 4.2- 3.8cormels, respectively.	Jala, A., 2013.

Gladiolus c.v.s "*traderhon"*, "*white Friendship"* and "*peter pears"*	Nodal cultures of flower spike, flower buds, cormels, cormel sprouts**,** cormel sections/slices	MS+different doses PGR	Cormel sprouts were the best explant in terms of mean shoot induction (77.50%) and number of shoots (11.60) in "*white friendship".* The highest results for shoot induction (98.33%) and number of shoots (22.07) were observed from same cormel sprout on MS+BAP 4 mg l^{-1}. The heading stage of nodal cultures (7.67), medium size of cormels and cormel sprouts (11.60) each and top slice of cormels (3.65) were the best stages/sizes from each explant for efficient number of shoots.	Memon, N. et al., 2013.
G. grandiflorus	Corms	Culture medium+different doses of PGR	When 0.2 mg l^{-1} NAA was used, the highest rooted shoots percentage, number of roots, shoot length and 100% fungi-free plantlets were obtained.	González-Pérez, E. et al., 2014
Plant	**Explant Types**	**Culture Medium**	**Results**	**References**
			Iris sp.	
I. germenica var. *firecracker*	Leaves and rhizoms	MS+different doses of PGR	The highest percentage of bud formation, root formation and rooted plantlents was obtained from 2 mg dm^{-3} IAA 21%, 37.7% and 21% respectively. The highest callus formation was evaluated 52.6% on 2 mg dm^{-3} IAA+1 mg dm^{-3} 6-furfurylaminopurpurine.	Kromer, K.D., 1985.
Iris c.v. *"prof. blaauw"*	Bulbs	½ strenght MS	It was yielded 80 bulblets per bulb.	Van Der Linde, P.C.G., et al., 1986.
I. hollandica c.v. "Prof. Blaauw"	Bulb scales	MS and ½ MS	Explants produced adventitious shoots at the junction of the basal plate and the scales. The total bulblet weight produced per single outer-scale explant was higher successfully but not double inner-scale explants. The largest bulblets occurred on 1/2 MS. The number of bulblets was obtained by per explant varied from 2 to 6.	Linde, P.C.G. et al., 1988.
I. oxypetala	Green embryos	MS+different doses of PGR	For the initial growth of compact callus and formation, 2,4-D and kinetin was the best of the combination. It was concluded that without cytokinins the culture was depended on auxin.	Boltenkov, E.V. et al., 2000.
I. pumila and *I. reichenbachii*	Mature embryos and leaf bases	MS+different doses of PGR	On MS supplemented with 2,4-D alone or 2,4-D and kinetin in combination (1.0 mg l^{-1} each), embryogenic calli were developed. Somatic embryos provided germination (70%) on MS without hormones. Organogenic calli were cultured on MS+BAP and GA_3 (1.0, 0.1 mg l^{-1}, respectively).	Jevremović, S. et al., 2006.

Iris c.v.s *"white swan"* and *"black flag"*	Adventitious buds	Culture medium+different doses of NAA	Consisting of 1/2MS+0.5 mg l^{-1} NAA culture medium was the best one for rooting.	Zhao, C., 2012.
I. aphylla	Leaves and rhizome buds	MS+different doses of PGR	On MS+2.0 mg l^{-1} TDZ+3.0 mg l^{-1} NAA, Maximum percentage of callus was obtained from the leaf explants of *Iris aphylla* L. cultured.	Marinescu, M.V. et al., 2013.
I. sari and *I. schachtii*	Immature pods containing immature zygotic embryos	For shooting: MS+PGR For rooting: MS+1 mg l^{-1} IBA+0.2 or 0.4 mg l^{-1} NAA	The highest number of shoots per explant was obtained on MS+0.5 mg l^{-1} TDZ+0.5 mg l^{-1} NAA and MS+1 mg l^{-1}+TDZ+0.5 mg l^{-1} NAA, whereby 96.88% and 100% shoot induction with 9.55 and 11.34 shoots per explant of *I. sari* and *I. schachtii*. Regenerated shoots were successfully rooted on MS with either 1 mg l^{-1} IBA or 1 mg l^{-1} IBA+0.2 mg l^{-1} NAA.	Uzun, S. et al., 2014
Plant	**Explant Types**	**Culture Medium**	**Results**	**References**
			***Tulipa* sp.**	
T. c.v. *"merry widow"*	Floral stem	Nutrient medium+different doses of PGR	Shoots that had meristematic centres gave a variable bulbing response with applied gibberellins and cold incubation. Bulb production was gotten beter with a "soak" of 1.0 mg l^{-1} GA_3.	Rice, R.D. et al., 1983.
T. gesneriana	Immature ovula	Modified MS	Bulblet formation was obtained up to 90% under the improved conditions and with an average dry weight of approximately 50 mg.	Custers, J.B.M. et al., 1992.
T. gesneriana c.v. "*apeldoorn"*	Stalk and bulb scale	MS+1 mg l^{-1} 2,4-D, and 1.5 mg l^{-1} BAP	While some explants reacted well to tissue culture ('regenerating explants', showing callus and shoot formation and no extensive visible browning; mean explant score \2.5), the others didn't and a score <1.5. Stalk explants always had good regeneration and a faster increase in fresh weight.	Rossum, M. et al., 1997.
T. gesneriana	Micro bulbs	Nutrient medium+SA (salysilic acid)	Number of micro-bulb could be increased by 0.1 mmol l^{-1} SA and weight of micro-bulb could be increased by 0.5 mmol l^{-1} SA. Micro-bulb weighted 1.0-2.0 g.	Zhao, Y., 2005.
T. cultivars	Scales and stems	MS+different doses of PGR	The best medium was MS+0.4-1.0 mg l^{-1} BA+0.4 mg l^{-1} NAA, MS+2 mg l^{-1} BA+0.1 mg l^{-1} NAA and MS+1.0 mg l^{-1} BA+0.2 mg l^{-1} NAA for shoots; proliferation medium was MS+0.4 mg l^{-1} BA+0.2 mg l^{-1} NAA and MS+0.4 mg l^{-1} BA+0.2 mg l^{-1} IAA. It was better 1/2 MS+0.4 mg l^{-1} Kinetin+0.1-1.0 mg l^{-1} NAA for rooting.	Tian, Y., 2006.

T. karamanica, *T. sintenisii*, *T. humulis* and *T. armena*	Bulb scale, seed, immature embryo, flower, leave and leave stem	MS, N6 (Chu et. al.,1975), SH (Schenk and Hildebrandt, 1972) and B5 (Gamborg et. al., 1968)+ different doses of PGR for each explant types	*T. sintenisii* and *T. armena* species were found to be the best explant for immature embryos. *T. sintenisii and T. armena* had high number of bulblets regeneration as 22.67 and 16.42 bulblets per explant respectively.	Kalyoncu Doğan, D., 2007.
T. gesneriana c.v. *"apeldoorn"*	Floral stems	MS+different doses of PGR	The highest number of somatic embryos was produced in MS+25 µM Picloram+0.5 µM BA. 2,4-D induced adventitious roots.	Ptak, A. and Bach, A., 2007.
T. c.v.s *"little angel"* and *"christmas"*	Bulb scales	MS+different doses of PGR	Both varieties got best proliferation of the induced bulblet on MS+ 6-BA (3.0 mg l^{-1}) and NAA (0.2 mg l^{-1}).	Gong, M. et al., 2010.
T. c.v.s *"apeldoorn"* and *"leen van der Mark"*	Bulbs	MS+different doses of PGR	For "*apeldoorn*" MS+2 mg l^{-1} 6-BA + 2 mg l^{-1} NAA + 0.3 mg l^{-1} IAA was determined as the most suitable medium for bublets induction. For "*leen van der Mark*" MS+2 mg l^{-1} 6-BA + 2 mg l^{-1} NAA + 0.1 mg l^{-1} IAA was determined as the most suitable medium for bulblets induction. Activated carbon was been favourable effect on bulblets induction.	Mao, H. et al., 2012.
Plant	**Explant Types**	**Culture Medium**	**Results**	**References**
			***Narcissus* sp.**	
N. tazetta var. *chinensis*	Bulb sections	N6+2.0 mg l^{-1} BAP+0.1 mg l^{-1} 2,4-D	The section of ovary and flower stalk emergeced bulblets. The globules from callus were successfully induced, yielding regenerated plantlets.	Zhen-guang, C., 1982.
N. tazetta var. *chinensis*	Bulb scales	Nutrient medium+active charcoal	Formation and growth of bulblets was induced more efficient on active carbon in basic medium	Pifang, Z. et al., 1985b.
N. tazetta var. *chinensis*	Bulb segments	MS+different doses of PGR	The white compact callus was initiated from scale segments with the basal plate on MS+1 mg l^{-1} BA and 0.1 mg l^{-1} 2,4-D. Adventitious shoots were produced from callus cultures by transferring them on MS or with 1 mg l^{-1} BA or containing 0.1-0.5 mg l^{-1} NAA. Most of bulblets developed leaves and roots ½ MS+0.01-0.1 mg l^{-1} NAA or without any growth substance, particularly with 0.03 mg l^{-1} NAA.	Hengsen, G. and Cuihua, G., 1987.
N. tazetta var. *chinensis*	Scale segments	MS+different doses of PGR	MS+1 mg l^{-1} BA and 0.1 mg l^{-1} 2,4-D induced callus. Most of calli increased adventitious shoots on MS+0.1 mg l^{-1} NAA.	Hengsen, G. et al., 1987.

N. tazetta var. *chinensis*	Twin scales	MS+different doses of PGR	MS+0-5 mg l^{-1} BA, 0-1 mg l^{-1} NAA and a little activated-carbon induced bulbils. The percentage of induction reached 70%.	Yimin, H. and Guoning, Q., 1991.
N. tazetta var. *chinensis*	Flower peduncle	MS+NAA and 6-BA	The differentiation rate of puff callus induced by low concentration of NAA (0. 5 mg l^{-1})+6 BA (1-8 mg l^{-1}) is low.	Weilian, H. et al., 1993.
Narcissus c.v.s "*St. Keverne*" and "*hawera*"	Leaves	MS+different doses of PGR	Bulbil initiation and development were more strongly inhibited by BA in single leaf cultures than in shoot clump cultures. NAA stimulated bulbil formation on MS+176 mM sucrose for both cultivars. "*St Keverne*" showed good bulbil development with 0.54 µM NAA, 5.4 µM IAA and 5.4 µM lBA and "*Hawera*" responded only to 27 µM IAA.	Staikidou, I. et al., 1994.
N. bulbocodium	Twin scales	MS+different doses of PGR	MS+BAP (4 mg l^{-1})+NAA (0.12 mg l^{-1}) or BAP (2 mg l^{-1})+IBA (1 mg l^{-1}) resulted in shoot initiation and leaf development. Tiny bulbs were obtained with MS+BAP+IBA for a long period (70 days). The final size of the bulbs was not increased by the presence of NAA butlincrease but a better root system was developed by it.	Santos, J. et al., 1998.
N. pseudonarcissus c.v.s "golden harvest" and "St. Keverne"	Leaves, bulbs and flower stalk	MS+different doses of PGR	A range of 2,4-D and BAP concentrations started embryogenesis. 5 µM 2,4-D and 0.5 µM or 5 µM BAP was more efficieny on somatic embryogenesis (SEs) than other combinations. SEs were produced on scape explants earlier. SEs converted to plantlets with 4.9 µM IBA.	Sage, D.O. et al., 2000.
N. tazetta var. *chinensis*	Bulb scales	MS+different doses of PGR	It was found that the favorable medium was MS+1 mg l^{-1} BA+0.1 mg l^{-1} 2,4-D. The calli could differentiate on the media added BA and NAA. But the root differentiation rate could be increased, and the differentiation rate of buds decreased with increasing of NAA concentration in the media. The buds could grow up to small plants on the MS+BA and NAA.	Yu, W., 2001.
Narcissus c.v. "*pink charm"*	Young leaves	MS+different doses of PGR	MS+2.0 mg l^{-1} 6-BA+1.0 mg l^{-1} NAA was found to be the optimal medium for differentiation of rosette bud. For proliferation: MS+1.5 mg l^{-1} 6-BA +1.0 mg l^{-1} NAA and for induction of roots: ½ MS+0.2 mg l^{-1} 6-BA+0.5 mg l^{-1} NAA.	Zhu, H. et al., 2007.

Narcissus c.v. "*fortissimo"*	Bulblets	½ MS+different doses of PGR	The root of bulblets grew strongly on ½ MS+0.1 mg l^{-1} NAA or 0.1 mg l^{-1} IBA. The root was shorter and slimmer on ½ MS+0.05 mg l^{-1} IBA. The bulblets grew faster on ½ MS+1.0 mg l^{-1} NAA or 0.5 mg l^{-1} IBA. ½ MS+0.1 mg l^{-1} NAA or 0.1 mg l^{-1} IBA had the significant promotion on the rooting induction of tissue culture seedlings in narcissus.	Cui, W., 2008.
N. suzhou	Double scales	MS+different doses of PGR	The best medium was MS+3.2 mg l^{-1} 6-BA+.02 mg l^{-1} NAA for inducement. The average of inducing small bulbs was 4-5 and the increment rate was up to 322.22%.	Jiang, L. et al., 2010.
Narcissus c.v. *"delibes"*	Twin scales	MS+different doses of PGR	The optimum medium for shoot induction: MS+1.0 mg l^{-1} 6-BA and 15.0 g·l^{-1}sucrose; for bulbil formation: MS+4.0 mg l^{-1} 6-BA, 0.2 mg l^{-1} NAA, 2.0 mg l^{-1}activated charcoal and sucrose 60.0 g·l^{-1} or MS+ 2.0 mg l^{-1}6-BA, 1.0 mg l^{-1}2,4-D, 2.0 mg l^{-1} activated charcoal and 90.0 g·l^{-1}sucrose; for roots: MS +1.0 mg l^{-1} 6-BA, 0.5 mg l^{-1} 2,4-D, 0.5 mg l^{-1} NAA, 2.0 mg l^{-1} activated charcoal and 30.0 g·l^{-1} sucrose.	LV., X. et. al., 2010.
Narcissus c.v. "*arkle"*	Leaves, scapes and different parts of bulbs	MS, ½ MS and 1/4 MS + different doses of PGR	Twin-scale with basal plate was more suitable explant was described. The more appropriate medium for primary culture was MS+3.0 mg l^{-1} 6-BA+0.5 mg l^{-1} NAA+0.2 mg l^{-1} IBA. The proliferation medium was MS+1.5 mg l^{-1} 6-BA+0.3 mg l^{-1} NAA, its induction rate was 668%.The rooting rate of the bulblets was 80% on ½ MS+0.1 mg l^{-1} NAA+1 g·l^{-1} activated carbon. The combination of 6-BA and NAA had favourable effects on induction and multiplication of bulblets. ½ MS+ NAA and AC was beneficial to rooting.	Sun, X. et. al., 2010a.
N. tazetta var. *chinensis*	Bulb scales	MS+different doses of PGR	Higher concentration of 2,4-D (3.0-4.0 mg l^{-1})+6-BA demonstrated the capacity to induce colorless embryogenic calli. Production of shoot buds was stimulated with the moderate concentration of 2,4-D (0.5-1 mg l^{-1}). It was found that different calli induction and organogenesis were dependent on the auxin type and their concentrations in the medium.	Fang, Q. et al., 2013.
Plant	**Explant Types**	**Culture Medium**	**Results**	**References**
			***Hyacinthus* sp.**	
H. orientalis	Bulb scales segments	Nutrient medium	240-300 bulblets which are 3-4 cm long and 0.5 cm wide were obtained by basal scale segments.	Pierik, R.L.M. and Post, A.J.M., 1974.

H. orientalis c.v. *"lady derby"*	Bulb scales	MS+different doses of NAA+BA	MS+1 mg l^{-1} NAA and 10 mg l^{-1} BA was the best combination for bulblet initiation It was found that NAA was the only necessary hormone for callus formation and root differentiation.	Saniewski, M. et al., 1974.
Hyacinthus subsp.	Bulb scales, basal plates, leaf, ovary and stem	Nutrient medium+different doses of PGR	Bulb scale and basal plate didn't require PGR but leaf and ovary responded to low doses of IAA and NAA.	Hussey, G., 1975.
Hyacinthus subsp.	Peduncle, rachis, pedicel and flower bud	Nutrient medium+different doses of PGR	Only small bulblet primordia (about 1 mm in length) regenerated at the basal end of the rachis segments with without hormones. When NAA+BA added NAA dose is less than that of BA, the bulblets are differentiated from explants.	Jinyu, D. and Hong, H., 1983.
Hyacinthus subsp.	Bulb scales and leaflet explants	Nutrient medium+different doses of IBA and BA	Politarity of the regeneration sites on the explants was affected by IBA as well as BA. On the same condition, the number of plantlets formed, and the fresh weight of their bulblets were affected.	Van Aartrijk, J. et al., 1986.
H. orientalis	Bulb scales	MS+different doses of NAA or BA	Bulblet regeneration was stimulated on solid MS+4.4–44 μM BA and 0.54–5.4 μM NAA, but their growth was slow. Based on the results, a mass propagation scheme for *H. orientalis* using shake culture had been established.	Takayama, S. et al., 1991.
H. orientalis	Leaves	Nutrient medium+different doses of PGR+different types of sugars	Media containing glucose and sucrose gave better results for formation of shoots and bulbs than containing fructose medium. Sugar varieties and concentrations affected the regeneration of shoots and tubers.	Bach, A. et al., 1992.
Hyacinthus orientalis	Ovary explant	MS+different doses of NAA and BA	MS+5 mg l^{-1} BA and 1 mg l^{-1} NAA was more adaptable to induce callus and shoot bud. MS+2 mg l^{-1} BA+2 mg l^{-1} NAA was found to be suitable for callus and shoot bud. But without hormones medium was suitable root induction.	Yanbo, L. et al., 1998.
H. orientalis c.v. "white pearl"	Flower buds	Nutrient medium	Continuous differentiation of tepals was successively induced. In 250 days, each flower bud differentiated an average of more than 70 tepals. It was found that the first whorled organ of the flower bud was perianth which consisted of perianth tube and tepals grown at the top of the perianth tube, which is the same as the flower bud of the wild type in *H. orentalis.*	Wenliang, L. et al.,1999.
H. orientalis c.v. "delft blue"	Leaves	MS+ different types sand doses of sugars	The highest number of bulbings was obtained under low and medium doses sugars. The highest proliferation rate of adventitious buds were obtained by 360 mM glucose.	Bach, A. and Swiderski, A., 2000.

Hyacinthus orientalis	Peduncle and pedicel peduncle	MS+NAA 5 µM +BA 10 µM	5 buds were obtained per bulb explant and 12 buds were obtained per inflorescence explant.	Ziv, M. and Lilien-Kipnis, H., 2000.
H. orientalis c.v. "delft blue"	Shoot explants from *in vitro* immature leaves	MS+different doses of PGR	Bulb formation was promoted by adding of ABA to the medium although adding of fluridone inhibited it.	Li, H. et. al., 2002.
H. orientalis c.v. "carnegie"	Bulb scale	Modified Heller (1953) medium+ different doses of IAA+IBA	The max. number of root was (1.3) was obtained medium+3 mg l^{-1} IBA when the max. root lenght was 0.4 cm on medium+1.5 mg l^{-1} IBA. The highest bulblet height was 0.9 cm on medium+1.5 mg l^{-1} IAA while bulblet diameter was 0.8 cm in medium+1.5 mg l^{-1} IAA or medium+1.5 mg l^{-1} IBA.	Y.B. et al., 2002.
H. orientalis c.v. "*blue jacket"*	Bulb scales	MS+2 ppm BAP+0.25 ppm NAA	In first subsculture it was observed formation of callus and in third subsculture bulblet. At the end of study, 111 calli and 22 bulblets were obtained.	Çığ, A. et al., 2006.
H. orientalis	Bulb scale, leaf primordia and immature inflorescence	MS+different doses of PGR	The scale explant was found to be appropriate for callus. Calli were obtained MS+0.5 mg l^{-1} IBA or MS+1 mg l^{-1} IBA. In indirect organogenesis and MS+3 mg l^{-1} BAP+0.3 mg l^{-1} IBA, the best results of producing bulblet (3.06 bulblets) were achieved.	Salehzadeh, S. et al., 2008.
H. orientalis c.v. "gipsy queen"	Scale segments and tender leaves	Nutrient medium+different doses of PGR	Twin-scale was better than single-scale for initial culture and the appropriate medium for them was MS+5.0 mg l^{-1} 6-BA+0.1 mg l^{-1} NAA. The appropriate medium for tender leafs was: MS+3.0 mg l^{-1} 6-BA+0.2 mg l^{-1} NAA, and its effect was better than that of scale segments. The best medium on subculture multiplication was MS+2.0 mg l^{-1} 6-BA+0.5 mg l^{-1} NAA+0.2 mg l^{-1} Kinetin. Root induction for the bulblets was beter on ½ MS+0.2 mg l^{-1} NAA.	Sun, X. et al., 2010b.

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