BULBOUS AND CORMOUS MONOCOTYLEDONOUS ORNAMENTAL PLANTS *IN VITRO*

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ABSTRACT

Literature on bulbous/cormous plants propagated through tissue-cutlure technique is reviewed. Murashige and Skoog (1962) medium (MS) was frequently used to study the different modes of micropropagation. For somatic embryogenesis (SE) explants from aerial parts and somatic organogenesis (SO)/direct somatic organogenesis (DSO) from subterranean parts of the plant were found to be suitable.

Species-wise exhibition of SE, SO and DSO analysed literature is tabulated, showing culture media, growth regulators and organic addenda in primary and secondary medias, relevant explants and the author index.

Effective growth-regulators, their quantity-wise ranges and combinations are also tabulated in terms of percentage, drawing a comparative picture, helpful for their most probable application, to achieve obvious results in quick succession of experiments in the case of newly explored species through in vitro cutlure.

BAP, Kin., 2,4-D and NAA, in different combinations, induced different in vitro features of SE, SO and DSO In the primary culture-medium (1^o) effective range of 2,4-D was 0.1-6.0 mg/L; that of NAA was 0.075-10.0 mg/L; that of Kin was 0.1-4.0 mg/L; and that of BAP was 0.1-3.0 mg/L. In secondary culture mediums (2^o), effective ranges of 2,4-D, NAA, Kin and BAP were 0.5-2.0 mg/L, 0.01-2.5 mg/L, 0.2-2.0 mg/L and 0.5-10 mg/L, respectively.

ABBREVIATIONS

IAA	= **Indole Acetic acid**
2,4-D	= **2,4-Dichlorophenoxyacetic acid**
NAA	= **Naphthelene acetic acid**
PCPA	= **Para chlorophenoxy acetic acid**
IBA	= **Indole butyric acid**
TIBA	= **Tertiary Indole butyric acid**
Pic	= **Picloram**
Kin	= **Kinetin**
BAP	= **Benzylaminopurine**
2-iP	= **Isopentenyl adenine**
GBA & GA_3	= **Gibbrellic acid**
Ade	= **Adenin sulphate**
CH	= **Casein hydrolysate**
CM & CW	= **Coconut water**
Suc	= **Sucrose**
aa	= **Aminoacid**
AC	= **Activated charcoal**

INTRODUCTION

Comparatively, monocotyledons are regarded as difficult *in vitro* material. Initially, Gautheret [1], mentioned only ten monocotyledons out of the hundred odd species to be described for tissue-cutlure. Partenan [2], Castor et al., [3] and Krikorian and Berquam [4] commented on the alleged refractory nature of monocotyledons, as regards *in vitro* culture. Within the last few decades, an increasing number of monocotyledons have been successfully cultured. This included members of the family Graminae and a number of temperate grasses, as well as some bulbous and related species. Bulbs and corms belonging to the Liliaceae, Iridaceae and Amaryllidaceae include species with slow natural propagation e.g. *Hyacinthus* and *Narcissus*, as well as species which can proliferate more freely e.g. *Ornithogallum* and *Ipheion*.

Tissue-culture technology greatly influenced the demand of rapid multiplication and clonal propagation of slow-growing monocots. G. Hussey's work [5] on twelve species of Amaryllidaceae, Iridaceae and Liliaceae establiszhed totipotency in monocots and made a break-through for propagation of ornamentals by tissue-culture. In 1975 Holdgate [6], with co-workers at Twyford Labortories, worked on a number of plants and published numerous papers in the same year.

Thence onwards, several economically important monocot species, whether bulbous, cormous,

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rhizomatous or tuberous, constituting nutritional, medicinal or ornamental groups of plants were investigated *in vitro* for clonal propagation, hybridization [7], disease control [8,9] and production of secondary metabolites [10]. Most of the bulbous and cormous species belonged to the families Amaryllidaceae, Iridaceae and Liliaceae. The explants obtained from storage tissue and growing tissue responded actively. The developing leaves of growing inflorescence stem also responded very well to the growth-regulators and formed plantlets and callus.

The stems of monocotyledons lack a cambium and thus new vascular differentiation after growth due to the apical and intercalary meristem terminates and this limits considerably the possibilities of regeneration. Later on, attempts proved otherwise. The floral stem-tissue transformed into meristematic cells, giving rise to bulblets of adventitious origin. Pierik and Steegmans [11] have reported the regeneration of adventitious sprouts and bulblets on explants of young inflorescence and floral stem explants of *Nerine bowdenii*, N. *sarniensis* and *Eucharis amazonica*. It was thus concluded that floral stem explant could be an excellent source of *in vitro* propagation of Amaryllids.

Although dedifferentiation is relatively rare in monoctyledons, yet there are reports on totiopotency of leaves and buds restricted on floral stalks. There are reports on regenerations of buds and roots on intact or detached leaves and excised leaf fragments of *Heloniopsis orientalis*. Adventitious bud-formation, in etiolated stem segments and in callus tissues arisen from leaf segments of the same, was studied by Kato [12].

Utilization of underground portion of ornamental monocots was introduced by Stone and Holling [13]. Bulblet formation was induced by planting the vertically divided segemnts of the bulbs on suitable culture media. Meanwhile, pieces of single scale with some basal portion were also tried, but the percentage success was just 2 to 3 [14,15,16].

Known chipping technique was introduced by Stone [17] and Stone et al., [18]. The whole bulbs were radially divided into 8 to 16 pieces, each piece consisting of 7-8 scales joined with a piece of basal plate. Formerly, chips were sown in soil in polyethylene bags. Afterwards, soil was replaced with defined cutlure-media for *in vitro* propagated chips [19 & 20].

Twin scaling, the most advanced technique was developed from earlier work on the propagation of *Hippeastrum, Narcissus* and other amaryllids [21]. From one large bulb, 30 - 60 twin scales could be obtained. Each twin scale consisted of two scale pieces, with a small piece of basal plate. The twin scaling technique has been described in detail by Alkema and Leeuwen [22]; Anon [23]; Stone [17]; Stone et al., [18]; and Tompset [24]. It has been successful for a wide range of amaryllidaceous species, as well as for irises and certain Liliaceae [25,26,27,24,28,29 & 30].

Somatic embryogenesis or organogenesis from differentiated tissues of bulbous and cormous monocots is also frequently reported in the literature.

Somatic embryogenesis (SE) through root callus cutlure of rhizomatous irises [31], plantlet regeneration of Gladiolus from inflorescence stalk callus [32] and calli of cormal slices, young leaf bases and whole intact plants was reported by Stefariak [33]. Globular embryos of *Crocus sativus* from callus of meristematic region [34] and plantlets from calli of bud [35] were also reported.

Several species of *Allium* regenerated through callus mediated somatic organogenesis *viz*. A. *sativum* from bud, stem tip and bulbs leaf disc tissue [36,37]; A. *senescences* from flower bud callus [38], somatic organogenesis in Gladiolus cultivars through inflorescence stalk and bud callus cultures [39,40]; similarly *Litium japonicum* and *L. Longiflorum* regenerated through somatic organogenesis (50) from calli of bulblets [41] and mother bulb scale [42] respectively; *Nerine bowdenii* from floral stem callus [11] and *Fritilleria thunberg* had also undergone somatic organogenesis from bulb scale callus [43].

Direct somatic organogenesis (DSO) i.e. organ-to-organ regeneration was also exhibited by several species, such as *Lilium auratum, L.*

speciosum and L. longiflorum formed bulblets on their bulb scales [44]. Bulblets formed on segments of immature floral stem of *Nerine bowdenii* [11], leaves of *Ornithogallum* regenerated shoots [45]. Cormlets formed on young inflorescence stalks of Gladiolus [39]. Multiple shoot formation on substranean shoot apices of *Iris rossii* [46] and shoots regenerated from young inflorescences stalk leaves and immature zygotic embryos of *Freezia refracta* [47].

Bulblet regeneration from chip and twin scale of *Polianthes tuberosa* was reported by Khan et al., [48] and pseudo embryogeney by Naraayanaswamy and Prabhudasai [49] and Zaidi et al., [50].

A detailed survey on micropropagation of crop, non-crop and graminecious species [51] revealed that any one of the species may be regenerated through somatic embryogenesis (SE), somatic organogenesis (SO) (callus mediated) or direct somatic organogenesis (DSO). It was also evident that some species regenerated by two means as well, depending on the nature of the plant and primary and secondary cutlure media. A peer look onto the concise review on ornamental bulbous and cormous monocotyledonous species also indicated similar facts governing their micropropagation. Brief analyses are presented hereforth.

In pursuance to interpret the most probable, applicable culture-medium, source of explant material and effective range of growth-regulator quantities, to establish and micropropagate the bulbous and cormous monocotyledonous ornamental plants the review-cum-analysis of the concerned literature led to the conclusions, suggesting some very likely and helpful parameters to investigate the newer species through tissues-cutlural techniques.

ANALYSIS OF LITERATURE ON ORNAMENTAL MONOCOTYLEDONS*

Culture Media

Among the nutritional media, Murashige and Skoog medium [52] was most frequently used for the monocot species *viz*. *Amaryllis* and *Hippeastrum* [53], *Lilium rubelium* [54] *L. formilonga* [55] and *Leucojum aestivum* [56].

The presented review of *in vitro* studies on monocots is regarding cutlure-media, mode of cutlure, morphogenetic potential of explants and *in vitro* expression of the cultures based on data collected from ornamental bulbous and cormous monocotyledonous species. The information was derived through a literature-survey and included twenty nine *in vitro* cultured species (Table 15). Data analysis, such as, explant source, media or growth-regulator concentration and combinations to ascertain the necessary information for plant regeneration experiments, is based on the lines of Evans, et al. [51].

In literature the following media were mentioned for regeneration of bulbous and cormous monocotyledonous species.

Medium	**Reference**	**Ref.No.**
MS	Murashige and Skoog (1962)	[52]
B5	Gamborg et al. (1968)	[57]
AZ	Abo-El-Nil and Zettler (1976)	[58]
BDS	Modified B5, Dunstan and Short (1977)	[59]
LS	Linsmeir and Skoog (1965)	[60]
Nitsch [61]	Nitsch and Nitsch (1969)	
Nel	Nel (1981)	[45]

Frequency of MS medium used for regeneration through somatic embryogenesis (SE) or somatic organogenesis i.e. direct somatic organogenesis (DSO) and callus mediated somatic organogenesis (SO) of bulbous and cormous monocotyledonous species was found to be maximum. It was 75% for SE, 68.4% for DSO and 69.2% for SO (Table 1). B5 medium [57] was far less frequently employed i.e. only 10.5% for DSO. For SE and SO, B5 was not reported. Frequency of modified B5 medium (BDS) [59] was 23.0% for SO and 8.3% was successful for regenerating the species through SE. Frequency of the rest of the media LS [60], Nitsch [61] or Nel [45] was 5.2% each for DSO. 3.8% Nel medium was employed for SO, but none

* Alliums were also included, as they comprised a number of species investigated *in vitro*

for SE. Regeneration of species through SO and SE on LS or Nitsch mediums was not reported.

Modes of Culture

For regeneration of ornamental monocot species, micropropagation through stationary (agar solidified or mounted on a paper bridge), suspension or both culture techniques have been reported. However, *Tissue Cultures* were more generally established on agar solidifed mediums, as reported for establishment of *Crocus sativus* [34] and *Lilium Longiflorum* [62].

Organ Culture of monocotyledonous plants included quiescent buds from underground storage organs, as in case of *Narcissus* [63], axillary buds of the floral stalks e.g., *Nerine bowdenii* [64] and shoot apices of subterranean parts e.g. *Iris rosii* [46]. Evidence of shoot multiplication in both solid and liquid agitated medium was reported for *Lilium auratum, L. speciosum*, and *L. longiflorum* [44]. Bulb scales of *L. rubelium were* cultured on modified MS liquid and agar mediums, behaved differently, as the growth of bulbs was enhanced in liquid medium [54]. *Gladiolus* bud explants when cultured in liqiuid agitated medium formed plantlets [40].

The technique of cell culture was generally applied for clonal propagation and production of secondary metabolites. Cell cultures of *Iris siberica* and *Polianthes tuberosa* were established for the production of essential oils and polysaccharides, respectively [10,65]. Cell-suspension cutlures of *Gladiolus* cultivars established with friable calli, regenerated plantlets from two month old suspensions [32].

Protoplast culture, usually applied for somatic hybridization of known species to create new varieties, was also reported for certain monocotyledonous bulbs. Creamy white calli with embryogenic potential were induced from the seeds of *Lilium formilonga* compact cell clumps formed in liquid medium of the same composition. Protoplasts isolated from meristematic nodular clumps were successfully colonized and then planted in gellengun solidified medium, which then formed shoots and roots [55].

Morphogenetic Potential of Explants

Morphogenetic potential of the plants cultured *in vitro* depended on the nature of the explant, age of the organ and status of the donor plant. Explants are generally obtained from the parts which retain meristematic activities.

Bulbous, cormous, tuberous, rhizomatous and pseudobulbous ornamental monocots are perennial herbaceous plants, which propagate by specialized vegetative structures. Aerial and underground parts of the plants regenerate annually. Almost all parts of the plant from 'root to inflorescence tip' have been reported in literature as being utilzied as a source of explant.

Parts of the bulbs, stem, leaf, basal plate and carpels of endangered plants *Lilium rhodopoeum* and *Leucojum aestivum* [56] were cultured *in vitro*. The basal part of the bulbs of former and leaves of the latter possessed highest regenerative activity.

Tissue cultures of *Fritillaria thunbergii* were obtained from the various parts of the plant during germination stage. The bulb cells showed a higher growth potential than cells from root, stem or leaf [43].

Segments of young inflorescence, young leaves and immature zygotic embryos of *Freezia refracta* were cultured by Wang et. al. [47]. Morphogenetic response of the explants, either directly or mediated through callus, depended on the exogenous hormonal conditions in the cutlure media. Ishioka and Tanimoto [30] successfully induced differentiation in bulb scale segments, cultured cells and leaf segments of *Lilium longiflorum*.

Root and leaf pieces from *in vitro* grown plants of several genotypes of rhizomatous *Iris spp*. were cultured *in vitro*. Callus induction occured only on root cultures. Somatic embryogenesis, which occurred in regeneration medium, was reported by Genvieve et al. [31].

Azuma and Yokoyama [66] used leaf explants of *Iris palleda* for regenerating whole plants. *Ornithogallum maculatum* was rapidly propagated

in vitro from leaf tissues, provided the donor plants, of which the first floret had opened [67].

Induction of Adventitious buds on undetached leaves, excised leaves, leaf fragments and, in etiolated stem segemnts of young *Heloniopsis orientalis* plants were reported by Kato [12,68]. Culture of young flower stalks of *Narcissus tazetta* was reported as an efficient explant source by Hosoki and Asahira [35].

Relation between differentiation and phytohormones in scales of *Lilium daviaii* was determined by Yang et. al., [69]. The upper part of the scale had no differntiating ability, lower and middle parts however differentiated at a frequency of 80 and 10 percent respectively. It was assessed that differentiating ability in different parts of the Lily bulb scale was closely related to the contents of the endogenous growth regulators.

Inflorescence stalk of *Gladiolus* plants cultured on MS basal salts media formed compact type of callus that regenerated plantlets [32].

Excised stamens of *Lilium rega* were grown *in vitro* to obtain haploids from its pollen grains. Another sac, connective tissue, and filaments showed variable proliferative capacities and organogenic potentialiites [70]. Calli were formed on the cut ends of the filament, while the buds embedded in the callus formed plantlets in continuous light.

Table-2 shows a variety of explants used in regeneration experiments on bulbous and cormous monocots. The frequency of regeneration as SE, SO and DSO indicated the morphogenetic potential of the explants.

In literature, almost every part of the whole plant was employed for *in vitro culture*. From top to root, six groups of explants were identified belonging to aerial and sub-terrestrial zones of the plant (Table-2). Shoot apex and axillary buds on floral stem belonged to group 1 of Aerial Zone. Group 2 of the same zone comprised floral stem, peduncle, inflorescence segments, flower bud, carpel, stamen/filament and mature/immature embryo served as a source of explant. Group 3 to 6 belonged to Subterrestrial Zone of the plant. Explant from mother bulb scale, bulb scale and leaf base/leaf blade were gathered in group 3. Fourth group explants were obtained from the meristematic region, twin scales, basal plate, corm slice and shoot bases. Bulblets, cormlets and young pseudobulblets constituted the group 5 explant source. Roots were the only organ providing group 6 explants.

Regenerative potential of explants of group 1 of Aerial Zone was 22% SE, 17% SO and 10% DSO, that of group 2 explants of the same zone was 31% SE, 26% SO and 14% DSO (Table-2).

Group 3 explants of underground zone expressed the potential of regeneration in terms of percentage i.e. 15,31 and 29 for SE, SO and DSO respectively. Frequency of Regenerative potential of explants of groups 4 and 5 was 15 and 8 each and 12, 33 and 14 percent for SE, SO and DSO respectively. Root expalnts exhibited only SE which was 8% (Table 3).

In Vitro Expression of Culture

Phenotypic expression of culture *in vitro* depends on modified behaviour of the explants in the pretext of genetic format induced by micro environment, comprising medium constituents including growth regulators and physical factors. Differentiated culture may redifferentiate via callogenesis or directly through organogenesis. Adaptation of a pathway for a variety of morphological, biochemical, physiological and genetic expression, normally commences with a prelude to sterile culture establishments.

Plant Regeneration

Growth regulator concentrations in the culture medium are critical to the control of growth and morphogenesis. Generally, a high concentration of auxin and a low concentration of cytokinin in the medium promotes abundant cell proliferation with the formation of callus. Often 2,4-D is used to initiate callus. On the other hand, low auxin and high cytokinin concentration in the medium results in the induction of shoot morphogenesis. Auxin alone or with a very low concentration of cytokinin is important in the induction of root primordia.

Embryogenesis

General and specific information pertaining to the culture regimens for initiation of SE for the bulb and corm species have been summarized in Tables-3,4,5 and 6. Table-3 shows species which have undergone somatic embryogenesis. Information regarding culture media, growth regulator for primary and secondary media (1° and 2° medium) and the explants, being mentioned with particular references, respectively.

It was notable that *Ca* 30% of species had somatic embryogenesis, originating from explants excised from distil end of the floral stem, comprising shoot apices, rachis and floral parts. 75% of the explants cultured on MS medium or on mofidication of MS medium (Table-2). These medium regimens included 2,4-D in 83.3% primary culture mediums and Kin, in 50% of the primary culture mediums. NAA was used in primary culture mediums to lesser extent at a frequency of 33.3% (Table-4). Effective concentration ranges for these growth regulators were 0.1-6.0 mg/L for 2,4-D, 0.1 - 1.0 mg/L for Kin., and 0.075-10.0 mg/L for NAA (Table -6). Although, 2,4-D was used more frequently (Table-5) but range of NAA concentration was wider than other ones *viz* pCPA, Pic., BAP, Kin., and 2iP (Table-6). In primary culture media, occasional use of casein hydrolysate and coconut water was also reported.

The culture media used for induction of embryo development during secondary culture (20) of bulbous species contained growth regulators, while the 2° mediums for the cormous species were devoid of growth regulators. In secondary culture media, growth regulators other than 2,4-D and NAA were used. IAA at a concentration range of 1.75-2.0 mg/L, Kin., 0.2-2.0 mg/L and BAP 1.0-10 mg/L with other additives, GA_3, adenine sulphate and ascorbic acid, were supplemented in the media (Table-6).

Examination of Table-5 revealed that 2,4-D alone or in combination of one or two cytokinins dominated the list of growth regulator combinations such as 2,4-D with pCPA, NAA and/or BAP. In 50% combinations 2,4-D was present. In secondary culture mediums 2,4-D was not required in any concentration. Kin was present with the combination of IAA, TIBA or alone in 14.2%, 7.1% and 9.7% respectively in 2° mediums. BAP alone was present in 14.2% secondary culture mediums and in combination with GA_3, TIBA and Pic, at a frequency of 7.1% each (Table-5).

Organogenesis

In vitro data of ornamental monocotyledonous species with specialized stems, show that 56% of them exhibited somatic organogenesis and 50% species manifested direct somatic organogenesis. MS medium was [52] equally important and popular for inducing direct somatic organogenesis (DSO) i.e. 68.4% and 69.2% respectively as compared to somatic embryogenesis (Table-1). Frequency of BDS [59] medium was 23.0% for callus mediated somatic organogenesis.

Explants from bulb/crom rachial portion of the floral stem (Group 2) and scale/leaf (Group 3), had undergone 26% and 31% callus mediated somatic organogenesis. Table-2 indicates direct somatic organogenesis by the explants excised from scale/ leaves (Group 3) and basal disc or meristematic region (Group 4) of the bulbs/corms. Frequency of direct somatic organogenesis from group 3 and 4 explant was 29% and 33% respectively.

Callus mediated somatic organogenesis being comparable to somatic embryogenesis in case of primary and secondary culture mediums, either of the two (1° and 2° mediums) were necessary for regeneration among monocotyledonous bulbous and cormous species (Table-7). It was the direct somatic organogenesis of the same species that was sometimes expressed after a subculture on secondary culture medium. This type fo feature is not common among the dicotyledonous and graminecious species.

Somatic Organogenesis

Growth regulator regimen in primary and secondary cutlure mediums for somatic organogenesis included IAA, IBA, 2,4-D NAA, pCPA and Pic, among the auxins. Whereas Kin, BAP and 2iP from the cytokinin group were prominent in the Table-8, showing frequency of individual growth regulator supplemented in

primary and secondary culture mediums. 2,4-D was individually present in 40.7% primary and 22.2% secondary culture mediums. The other important auxin, NAA was 33.7% in 1^{o} mediums and 29.6% in 2^{o} mediums.

Among the cytokinins, BAP and Kin were present frequently. Frequency of BAP was 3-4 times more than Kin. in primary and secondary culture mediums, i.e., 51.8% in 1^{o} medium and 48.1% in 2^{o} medium, while those of Kin were 18.5% and 11.1% respectively.

The Table-9 shows that combinations of 2,4-D or NAA supplements with other growth regulators in primary culture mediums for somatic organogenesis were almost equal. But BAP with either of the two was more frequently used i.e. 2,4-D + BAP 14.8% and NAA + BAP 18.5%. In secondary culture medium, combination of 2,4-D with BAP was equally important and its frequency was again 14.8% as that in primary culture medium, NAA remained important in 2^{o} medium. Cumulative frequency of NAA with BAP and certain additives or Kin. was 25.9%.

There are 14.8% secondary culture mediums which were devoid of growth regulators. 11.1% primary cutlure mediums are sufficient for somatic organogenesis of bulbous and cormous monocotyledonus species.

Table 10 shows range of 2,4-D concentration in 1^{o} mediums i.e. 0.5-5.0 mg/L and 0.5-2 mg/L in 2^{o} mediums. NAA range in primary and secondary culture mediums was 0.01-10.0 mg/L and 0.01-2.5 mg/L respectively. Among the cytokinins, Kin. and BAP were 0.1-4 mg/L and 0.1-16 mg/L respectively in primary culture mediums, but 0.5-2.0 mg/L and 0.5-4.0 mg/L respectively in secondary culture mediums.

Among the other additives range of sucrose was more than 3% i.e. 5% and 5-7% in 1^{o} and 2^{o} culture mediums respectively.

Direct Somaatic Organogenesis

Table - 11 shows species that have undergone direct somatic organogenesis and were influenced by a limited, yet very much known, members of the auxins and cytokinins such as IAA, IBA, 2,4-D and NAA; Kin., BAP and 2iP respectively. Among the auxins, NAA was present in 63.1% and BAP in 73.6% primary culture mediums (Table 12). Frequency of 2,4-D was 10.5% and Kin. 15.7% in 1^{o} media. Rest of the auxins were far less frequent. In 2^{o} culture media, only NAA, BAP and 2iP were used at a frequency of 10.5% each.

Supplementation of additives for direct somatic organogenesis was not required for the bulbous/ cormous species, as observed in literature.

For induction of DSO there were six combinations of NAA and BAP, five of Kin., two each of 2,4-D and 2iP and three each of GA, and sucrose (Table-13). Frequency of 1^{o} culture medium with a successful combination of growth regulators i.e., NAA and BAP was 31.5%. Next combination was that of BAP with sucrose present in 10.4% mediums.

Frequencies of 2^{o} media with NAA and BAP/2iP or BAP and 2iP were only 5.2% each. 20.8% secondary culture mediums were devoid of growth regulators. For 57.8% regeneration of several species via callus mediated somatic organogenesis secondary culture mediums were not required.

Overview of bulbous and cormous species exhibiting somatic regeneration is shown in Table-15 i.e., 56% species expressed somatic organogenesis SO *in vitro*. 50% had direct somatic organogenesis (DSO), while 30% species regenerated throguh somatic embryogenesis (SE).

CONCLUSION

Inclusion of 2,4-D, NAA, Kin. and BAP into MS medium was found to be remarkably suitable for both somatic organogenesis as well as somatic embryogenesis.

Explants from aerial zone of the plants were suitable for somatic embryogenesis (SE). The explants from floral stem and mother scales were good for somatic organogenesis (SO), while mother bulb scale and twin scale explants from

sub-terrestrial zones exhibited direct somatic organogenesis (DSO).

Among the growth regulators 2,4-D, Kin, NAA and BAP were found to be employed in cutlure mediums in different combinations to study various features of micropropagation i.e. SE, SO and DSO. 2,4-D alone or in combinaton with Kin and/or NAA supplemented in primary or secondary culture mediums was effective for callogenesis and SE. Combination of BAP with 2,4-D/NAA in primary and secondary culture mediums was effective for SO. BAP with NAA and occasionally sucrose in primary culture mediums promoted DSO.

Range of growth-regulator quantities i.e. 0.1-6.0 mg/L for 2,4-D; 0.015-10.00 mg/L NAA; 0.1-4.0 mg/L Kin. and 0.1-30.0 mg/L BAP were observed in primary culture mediums. However, 0.5-2 mg/L 2,4-D; 0.01-2.5 mg/L NAA; 0.2-2.0 mg/L Kin. and 0.1-10 mg/L BAP were effective growth-regulator concentrations in secondary culture mediums.

Table 1: Frequency of Culture Media used for Regeneration of Bulbous and Cormous Monocotyledonous Species *In Vitro*

Media	Somatic Organogenesis		Somatic (SE) Embryogenesis
	Direct	Callus Mediated	
MS	68.4%	69.2%	75.0%
B5	10.5%	--	--
BDS	--	23.0%	8.3%
AZ	--	3.0%	8.3%
LS	5.2%	--	--
Nitsch	5.2%	--	--
Nel	5.2%	8.8%	--

Table 2: Frequency of Somatic Embryogenesis and Organogenesis (Callus Mediated and Direct) Exhibited through *in vitro* Culture of Explants of Monocolyledonous Bulbous and Cormous Plants

Explants From	Regenerants		
	Somatic Embryogenesis (SE)	Somatic Organogenesis (SO)	Direct Somatic Organogenesis (DSO)
Aerial Zone			
1. Shoot apex	22%	17%	10%
Axillary bud			
2. Floral stem	31%	26%	14%
Peduncle			
Inflorescence segments			
Flower bud			
Carpel			
Anther			
Embryo			
Sub-terrestrial Zone			
3. Mother bulb scale	15%	31%	29%
Bulb scale			
Leaf base			
4. Meristematic region			
Twin scale	15%	12%	33%
Basal plate			
Corm slices			
Shoot base			
5. Bulblet	8%	12%	14%
Cormlet			
Pseudobulb			
6. Root	8%	--	--

Table 3: Bulbous and Cormous Monocotyledonous Species Capable of Somatic Embryogenesis (SE) after Primary and Secondary Culture

Bulbous Species	Growth Regulators and Organic addenda (mg/L)		Medium	Explant	Reference	Ref.No.
	Primary medium 1°	Secondary medium 2°				
Allium sativum	2.0 pCPA 2.2 2,4-D 0.10 Kin	0.2 Kin 1.75 IAA	AZ	Bud Stem tip basal leaf disc	Abo-El-Nil and Mustafa, (1977)	36
A.sativum	0.1 2iP 0.5 NAA 0.1 2,4-D	10.0 BA		Shoot tip	Choi, (1993)	37
A. fistulosum X A. cepa	0.75 Pic 2.00 BAP 90.00 CH	0.75 Pic 2.00 BAP 25.0 Glycine 25.0 Proline+Vit.	BDS	Basal plate	Lu et. al., (1989)	72
Iris setosa	A: 5.0 2,4-D, 1.0 Kin, 250 Proline, 250 CH, 5% Suc. B: 1.0 2,4-D, 1.0 Kin, 1.0 GA_3, 250 Proline, 250 CH, 5% Suc.	1.0 BAP 100 Ade 2% Sucrose	MS embryo	Mature	Radojevic and Subotic (1989)	73
Iris SPP (Rhizomatous)	1.0 2,4-D 0.075 NAA 0.1 Kin or 5.4 2,4-D or 0.075 NAA 0.1 Kin	2.0-5.0 BAP or 1.0 Kin 2 mMTIBA 2.0 BAP 2 mMTIBA	MS	Root (1991)	Genevieve etl.al	31
Polianthes tuberosa	2.0 2,4-D 1.0 Kin 6.0 2,4-D -- 15 %CW 2.0 2,4-D --	-- -- Ibid	MS	Bract/ petal Ibid Ibid	Narayanaswamy & Prabhudesai (1979)	49
Cormous						
Gladiolus cu Jennylee	10 NAA 0.5 Kin	2.0 Kin or None	MS	Basal Leaf	Kamo (1994)	74
Gladiolus	2,4-D	None	MS	Inflore scence stalk	kamo et. al. (1990)	32
Crocus sativas	2.0 2,4-D 0.5 Kin	2 IAA 2 Kin 100 Ascorbic	MS	Merist- ematic region	George et al.	34

Table 4: Frequency of Individual Growth Regualtors Supplements in Primary and Secondary Culture Mediums Supplemented to Initiate Somatic Embryogenesis (SE) in Monocotyledonous Bulbous and Cormous Species

Growth	Primary Culture Medium (1º)	Secondary Culture Medium (2º)
Auxin		
1 AA	--	16.6%
2,4-D	83.3%	--
NAA	33.3%	--
pCPA	8.3%	--
TIBA	--	16.6%
Pic.	8.3%	8.3%
Cytokinin		
Kin	50.0%	33.3%
BAP	8.3%	41.6%
2iP	8.3%	--
Others		
GA_3	--	0.3%
Ade	--	0.3%

Table 5: Frequency of Specific Growth Regulator's Supplements and or Combination in Primary and Secondary Culture Mediums used for the Somatic Emrbyogenesis (SE) of Bulbous and Cormous Monocotyledonous species

Growth Regulators	Culture Mediums for Somatic Embryogenesis	
	Primary Culture Medium (1º)	Secondary Culture Medium (2º)
IAA + kin	--	14.2%
2,4-D	16.6%	--
2,4-D+pCPA+Kin.	8.3%	--
2,4-D + Kin.	16.6%	--
2,4-D + Kin. + CH	8.3%	--
2,4-D + Kin. + NAA	16.6%	--
2,4-D + NAA	8.3%	--
2,4-D + CW	8.3%	--
Pic. + BAP	--	7.1%
Pic. + BAP + CH	8.3%	--
NAA + Kin.	8.3%	--
BAP	--	14.2%
BAP + GA_3 + Ade.	--	7.1%
BAP + TIBA	--	7.1%
Kin. + TIBA	--	7.1%
Kin.	--	7.1%

Table 6: Growth Regulator Concentration Range in Primary and Secondary Culture Mediums used for Induction of Somatic Embryogenesis (SE) in Monocotyledonous Bulbs and Corms

Growth Regulators	Effctive Concentration (mg/L)	
	Primary Culture (Medium (1º)	Secondary Culture Medium (2º)
1 AA	--	1.75 - 2
1 BAP	--	--
2,4-D	0.1 - 6	--
NAA	0.075 - 10	--
pCPA	2	--
Pic.	0.75	0.75
BAP	2	1 - 10
Kin.	0.1 - 1.0	0.2 - 2
2iP	0.1	--
TIBA	--	2 - 4 µM
GA_3	--	1.0
Ade.	--	100
Ascorbic acid	--	100
CH	250	--

Table 8: Frequency of Individual Growth Regulator Supplements in Primary and Secondary Culture Medium inducing Somatic Organogeneis (SO) through Calli of Bulbous and Cormous Monocots/ledonous Plants

Growth Regulators	Callus Mediated Somatic Organogenesis (SO)	
	Primary Culture Medium (1º)	Secondary Culture Medium (2º)
1 AA	--	7.4%
IBA	3.7%	--
2,4-D	40.7%	22.2%
NAA	33.3%	29.6%
pCPA	3.7%	--
Pic.	3.7%	3.7%
TIBA	--	--
Cytokinin		
Kin	18.5%	11.1%
BAP	51.8%	48.1%
2iP	3.7%	--
Others		
GA_3	--	3.7%
Ade	3.7%	--
Media with growth retardants	--	--
Media with no growth regulators	3.7%	22.2%

Table 9: Frequency of Specific Growth Regulator Supplements on Combination of Primary and Secondary Culture Media used for Somatic Organogenesis (SO) of Bulbous and Cormous Monocotyledonous Species

Growth Regulators	Direct Somatic Organogenesis	
	Primary Culture Medium (1°)	Secondary Culture Medium (2°)
IAA + kin	--	3.7%
IAA + Kin. + Ascorb	--	3.7%
IBA	3.7%	--
2,4-D	3.7%	--
2,4-D + NAA	3.7%	--
2,4-D + Kin.	3.7%	3.7%
2,4-D + Kin. + pCPA	3.7%	--
2,4-D + Kin. + Suc + CH	3.7%	--
2,4-D + BAP	14.8%	14.8%%
2,4-D + BAP + CM	3.7%	3.7%
2,4-D + 2iP + NAA	3.7%	--
NAA	--	3.7%
NAA + BAP	18.5%	11.1%
NAA + BAP + Ade.	3.7%	--
NAA + BAP + CH	--	3.7%
NAA + BAP + Suc	--	3.7%
NAA + Kin.	7.4%	3.7%
Pic. + BAP + aa	--	3.7%
Kin.	--	3.7%
BAP	3.7%	3.7%
BAP + GA_3	--	3.7%
Growth inhibitors	3.7%	--
No growth regulators	--	14.8%
No 2° medium	--	11.1%

Table 10: Growth Regulator Concentration Range in Primary and Secondary Culture Mediums used for Somatic Organogenesis (SO) for Regeneration of bulbous and Cormous Monocotysyledonous Species

Growth Regulators	Concentration Range (mg/L)	
	Primary Medium (1°)	SecondaryMedium (2°)
1 AA	--	1.75 - 2
1 BAP	10	--
2,4-D	0.5 - 5.0	0.5 - 2
NAA	0.01 - 10	0.01 - 2.5
pCPA	2	--
Pic.	0.75	0.75
Kin.	0.1 - 4	0.5 - 2.0
BAP	0.1 - 16	0.5 - 4.0
2iP	0.1	--
GA_3	--	0.1
CH	90 - 250	100.0
Ade.	62.5	--
CM	2%	2%
Ascorbic acid	--	100
Sucrose	5%	5 - 7%

Table 11: Monocot Yledonous Bulbous and Cormous Species Capable of Direct Somatic Organogenesis (DSO) through Primary and/or Secondary Culture

Species	Growth Regulators Organic addenda (mg/L)		Medium	Explant	Reference	Ref. No.
	Primary Medium (1°)	Secondary Medium (2°)				
Bulbous						
Allium sativum	0.1 NAA 9-12 % Suc	None	-	Vegetative bud	Park *et al.*, (1993)	85
	2.0 NAA 4.0 BAP 4.0 2,4-D	-	MS	Bulblet	Sub & Park, (1993)	77
A.tuberousum	0.5 BAP 0.5 2iP	0.5 BAP 0.5 2iP	B5	Halved shoot base	Panday *et al.*, (1992)	86
	1.0 NAA 0.5 2iP	1.0 NNA 0.5 2iP	B5			
Iris spp.	16.0 IBA 0.1 2,4-D	None	MS	Mature embryo	Stoltz (1977)	87
	2.0 Kin. 12.0 GA3	None	MS	Emryo	*Ibid.*	
I. rossii	0.2 NAA 5.0 BAP	-	MS or Nitsch	Shoot apex	Furuya (1992).	46
Leucojam aestivum	1.0 BAP 1.0 Kin.	-	MS	Leat	Stanilova *et al.*, (1994).	56
Lilium daviai	1 1AA 0.4 µMBA 0.3 µMGA$_3$	-	-	Bulb scale	Yang *et al.*, (1989).	70

Table - 11 (Cont'd)

Table 11 Cont'd

Species	Growth Regulators Organic addenda (mg/L)		Medium	Explant	Reference	Ref. No.
	Primary Medium (1°)	Secondary Medium (2°)				
Lilium rhodopeaum	0.5 NAA 0.1 Kin.	- -	LS	Basal plate	Stanilova *et al.*, (1994)	56
Lycoris aurea	3.0 NAA 30.0 BAP	3.0 NAA 10.0 BAP	MS	Twin Scale	Huang & Lila (1989)	29
Ornithogallum maculatum	2.0 NAA 2.0 BAP 3.0 % suc	-	Nel	Leaf	Rensburg *et al.*, (1989)	67
Narcissus	2-16 BAP 0.25-4 NAA	-	MS	Bulbscale leaf	Hussey, (1982)	63
N.tazetta cv Geranium and fortune	5 BAP 1 NAA	- Ring & Nitsch	MS + Plate	Overy, leaf, basal (1980)	Hosoki & Asahira,	69
N.tazetta cv Grand Soleil d Or	6 BAP 1.0 NAA 3 % Suc 0.5 % AC	-	MS	Twin scale	Stenitz and Yahel, (1982)	28
Dendrobium	0.1 NAA 0.1 BAP		MS	Pseudobulb	Yasugi & Shinto (1994)	88
Cormous						
Gadiolus dalenii	2 BAP 3 % Suc	None	MS	Corm slice	De Bruyn & Ferreire, (1992).	89
G. trista	0.5-1.0 BAP 6-9 % Suc	6-9 Suc only	MS	Corm slice	*Ibid.*	

Table 12: Frequency of Individual Growth Regulator Supplements in Primary and Secondary Culture Medium in Initiation of Somatic Organogenesis induced Directly in Bulb and Corm Forming Monocotydedonous Plants

Growth Regulators	Direct Somatic Organogenesis	
	Primary Culture Medium (1°)	Secondary Culture Medium (2°)
Auxins		
1 AA	5.2%	--
IBA	5.2%	--
2,4-D	10.5%	29.6%
NAA	63.1%	10.5%
pCPA	--	--
Pic.	--	--
TIBA	--	--
Cytokinin		
Kin.	15.7%	--
BAP	73.6%	10.5%
2iP	10.5%	10.5%
GA_3	70.6%	--
Ade.	--	--
Media with growth retardants	--	--
Media without growth regulators	--	--

Table 13: Frequency of Specific Growth Regulator's Supplements and Concentration in Primary and Secondary Culture Mediums used for Direct Somatic Organogenesis (DSO) of Monocotyledonous Bulbs and Corm forming plants

Growth Regulators	Culture Media for Direct Somatic Organogenesis	
	Primary Culture Medium (1°)	Secondary Culture Medium (2°)
IAA + kin + GA_3	5.2%	--
IAA + BAP + GA_3	5.2%	--
IBA + 2,4-D	5.2%	--
2,4-D + Kin +BAP	5.2%	--
NAA + Kin.	5.2%	--
NAA + BAP	31.5%	5.2%
NAA+BAP+Suc.	5.2%	--
NAA+BAP+Suc.+AC	5.2%	--
NAA + Suc.	5.2%	--
NAA + 2iP	5.2%	5.2%
Kin. + BAP	5.2%	--
Kin. + GA_3	5.2%	--
BAP + 2iP	5.2%	5.2%
BAP + Suc.	10.4%	--
Suc.	--	5.2%
None	--	20.8%
2° Medium		57.8%

Table 14: Growth Regulator Concentration Range in Primary and Secondary Culture mediums used for Direct Somatic Organogenesis (DSO) in Monocotyledonous Bulbous and Cormous Plants

Growth Regulators	Concentration ange (mg/L)	
	Primary Culture Medium (1°)	Secondary Culture Medium (2°)
1 AA	1.0	--
1 BAP	15.0	--
2,4-D	0.1 - 1.0	--
NAA	0.25 - 3.0	1 - 3.0
Kin.	0.1 - 2.0	--
BAP	0.1 - 30.0	0.5 - 10.0
2iP	0.5	--
Suc.	8 - 12%	6 - 9%

Table 15: *In Vitro* Regenerated Bulbous and Cormous Species

Species	Mode of Somatic Regeneration		
	SE	DSO	SO
Allium Sativum	✓	✓	✓
A. fislulosum and S.L. epa	✓		✓
A. tuberouse			
A.Senecens			✓
A.trifolium hirsulam			✓
Iris setosa	✓		✓
I. spp.	✓	✓	
I. rossii		✓	
Lilium japonicum			✓
L. longiflorum			✓
L. daviaii		✓	
L. rhodopeaum		✓	
Gladiolus sp.	✓		✓
Gladiolus dalenii		✓	
G. trista		✓	
G. cv jannylee	✓		
Narcissus cv.		✓	
Narcissus spp.		✓	
Narcissus tazetta		✓	✓
Crocus sativas	✓		✓
Lycoris aurea		✓	
Leucojam aestivum			
Ornithogallum maculatum		✓	
Fritilaria thunbergi			✓
Nerine bowdenii			✓
Muscari armenicum			✓
Hippeastrum hybrid			✓
Plianthes tuberosa	✓		
Dendrobium		✓	

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