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An Overview on *in vitro* Culture of Genus *Allium*

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Abstract: The genus *Allium*, consists of hundreds of medicinal plant species, is one of the most imperative sources of life supporting drugs. The *in vitro* biotechnological interventions are vital to choose, multiply, store up and improve the major *Allium* sp. *In vitro* culture of *Allium* has performed an incredibly crucial role in accelerated growth of several species with desirable traits and production of healthy and disinfectant propagules as well as paved the way towards cultivar improvement. During the last quite a few years, several moves have been made for *in vitro* propagation of *Allium*. *In vitro* regeneration via direct and indirect organogenesis using different explants and plant growth regulator formulations has been comprehensively covered in the literature. Recent challenges for establishment of protocols for genetic transformation have gained preference in the recent past reports. This review article comprehensively describes the exploitation of biotechnology for *in vitro* regeneration and genetic transformation for enhancement of the genus *Allium*.

Key words: Callus, *in vitro*, micropropagation, organogenesis, regeneration, somatic embryogenesis, transformation

INTRODUCTION

Prior to the commencement of the 19th century, a lot of herbs were regarded as traditional medicines and were comprised in medicinal prospective and formularies simultaneously. Hundreds of genera are used in herbal remedies and in traditional medicines all over the globe. As per one estimate 35-70,000 species have been used in some cultures for medicinal purposes (Sebastian *et al*., 2007). According to another estimate of the World Health Organization, up to 80% of people are still reliant primarily on conventional therapy, for instance herbs for their medicines (Gantait, 2009). Plants have long been a key resource of curative agents for mitigation or healing of human diseases. It has been assessed that plant extracts or active component obtained from plant materials comprise around one fourth of approved medicines (Tripathi and Tripathi, 2003). Recent studies point out that the herbal drug industries continue to raise by the rate of 15% per annum. India and China are two mega producers of medicinal plants and they have the competence to be paid by export of herbs as well as herbal products. The worldwide trade in medicinal plant materials and plant-based

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drugs has improved exponentially in current years. The convenient Research and Development compulsions of medicinal plant industry clearly insist a steady supply of planting materials. Medicinal plants having well-known market demands are quick attracting the attention of the tissue culture industry to meet these demands (Gantait, 2009). *In vitro* propagation harbours great potential for the production of premium herbal medicines (Murch *et al*., 2000).

The genus *Allium* secures its place amid the top ranked multipurpose medicinal plants with huge commercial value. *Allium* is an imperative member of onion family (Alliaceae; subfamily Allioideae). It serves human being both in the therapeutic as well as culinary purpose (Tapsell *et al*., 2006). At the eve of recorded history and awareness the prospective benefits of garlic (*A. sativum*), the key species of the genus *Allium* was one of the initial recognized instances of plants exploited for medicinal activities (Rivlin, 2001). A comprehensive exploration has been carried out with garlic on its health promoting and healing properties. *Allium* has been used since ancient times for innumerable complaints and amongst the properties attributed to it are diaphoretic, diuretic, expectorant and intestinally antispasmodic. It is considered to be Nature's very own antibiotic. Unlike most antibiotics, it will not deplete the body of flora and is considered to be the cure-all herb because of its effectiveness on the entire body (Gantait *et al*., 2009a). An array of biological activities including antioxidant, cancer prevention, liver protection and reduction of cardiovascular risk factors have been shown by *A. sativum* (Butt *et al*., 2009; Iciek *et al*., 2009; Pittler and Ernst, 2007). Elephant garlic (*A. ampeloprasum* L.) has also been used for lowering cholesterol, reducing high blood pressure and treating respiratory problems such as bronchitis and asthma (Gantait *et al*., 2009a). *Allium* has an extraordinarily high concentration of sulphur-containing compounds. Sulphur compounds, counting allicin were established to be the chief active components in the bulb of the plant (Tattelman, 2005). Allicin particularly, exhibits an ample array of pharmacological performance, for instance antibiotic, antihypertensive, antitumor, anticoagulation, anti-aging, immune enhancer and modulator and detoxifies heavy metals (Amagase, 2006; Iciek *et al*., 2009; Jacob, 2006; Munchberg *et al*., 2007).

It is significant that the majority of marketable *Allium* cultivars are contaminated by virus. The transmission of viral infections such as Garlic Mosaic Virus constantly occurs through clonal propagation and results in an inferior product and diminishes the total production (Ma *et al*., 1994). For *Allium* cultivars the rate of propagation in the field is around five to ten per year and hence to generate an adequate figure of seed cloves for convenient cultivation it takes several years (Nagakubo *et al*., 1993). Since, *Allium* almost never generates fertile seeds, it has to be propagated asexually through splitting up the individual cloves of the bulbs (Lee *et al*., 2009). This attribute restricts the breeding of *Allium* to clonal assortment. *In vitro* propagation and genetic transformation proved to be competent equipment for the breeding of several crops and could be promising in the potential enhancement of *Allium* (Martin-Urdiroz *et al*., 2004). Consequently, a wide range of experiments were conducted over *in vitro* micropropagation, somatic embryogenesis and transformation and regeneration of *Allium* using a number of explants (Bockish *et al*., 1997; Novak, 1990).

In our recent study, we developed the plant regeneration of *A. ampeloprasum* through direct organogenesis using shoot tip explants. In this study, we reviewed a variety of studies concerning micropropagation via direct organogenesis, callus culture, somatic embryogenesis, *in vitro* plant regeneration and genetic transformation of *Allium*.

Amid the key biotechnological tools *in vitro* cell and tissue culture proved its uniqueness in utilization of 'totipotent' nature of plant cells. Near the beginning of 19th century, the demonstration by Haberlandt (1902) revealed the genetic competence of uninucleated plant cells to be transformed into entire plants either through direct organ development or through an intermediate callus growth stage. In recent times, the exclusive term regeneration has been employed to designate the revival of a complete plant from *in vitro* cultured cells, tissues, organs, meristems or zygotic embryos (Lee *et al*., 2009). Among the number of trails for the regeneration of whole plants from plant cell or excised tissues there are two central pathways, (1) plant regeneration through organogenesis and (2) regeneration from somatic embryogenesis can be followed in general (Phillips and Hubstenberger, 1995).

In vitro propagation of *Allium* is increasing worldwide to satisfy the demand of its medicinal values both domestic as well as global markets. To attain this target and to assure the requirement of herbal medicine prepared from numerous class propagules, arrays of research has been conducted in investigating the regeneration competence and in progression to achieve innovative improvement in the advanced biotechnological facet. The *in vitro* regeneration of *Allium* have been attained primarily through direct organogenesis from excised explants or indirect organogenesis via callus growth stages, but the other modes of regeneration such as somatic and zygotic embryogenesis have also been reported.

Direct Organogenesis

Plant tissue culture displays a significant assignment in micropropagation, regeneration and manipulation of plants introducing desirable characteristics to improve their acceptance and popularity. Sometimes it is tricky for many plant species to culture and ascertain an optimum growing condition *in vitro* (Lee *et al*., 2009). Consequently, there is always an imperative requirement for widespread experimental effort in fundamental protocols of tissue culture for loads of crop plants prior to any practical exploitation (Hicks, 1980; Birch, 1997). Plenty of reports have documented plant regeneration through direct organogenesis from several explants such as bulb, shoot tip, basal plate, flower stalk, peduncle-pedicel, root *etc*. (Bhojwanii, 1980; Pandey *et al*., 1992; Seabrook, 1994; Silvertand *et al*., 1995; Ziv and Kipnis, 1997; Kim *et al*., 2003) have been listed comprehensively in Table 1. Apart from the explant sources, Plant Growth Regulators (PGRs) along with additive compounds play their decisive parts which helped *Allium* to regenerate and multiply *in vitro*. All these factors drive the tempo of *in vitro* organogenesis and regeneration of complete plants by integrating the individual organs (Gantait and Mandal, 2010). Recently, Kothari *et al.* (2010) emphasized on the synergistic obligation of both cytokinins and auxins for initiation of cell division and growth in plant tissue cultures; where an array of experiments have fundamentally recognized the continuation of antagonistic as well as additive interactions involving these two types of PGRs. Though MS liquid or semi-solid media in half or full concentration (Murashige and Skoog, 1962) have been extensively used as the basal medium, introduction of B5 or Gamborg's medium (Gamborg *et al.,* 1968) and LS (Linsmaier and Skoog, 1965) medium also served as basal media for successful *in vitro* regeneration of *Allium* (Bhojwanii, 1980; Nagakubo *et al*., 1993; Zel *et al*., 1997).

Bhojwanii (1980) was the first to test different hormonal regimes for multiple shoot regeneration through direct organogenesis in *A. sativum* using 0.1 mg L^{-1} naphthalene acetic acid (NAA) and 0.5 mg L^{-1} 6-(g-dimethylallylamino) purine (2iP). The lower auxin

Table 1: *In vitro* direct organogenesis of *Allium*

Allium sp.	Explant	Medium (PGR in mg L^{-1})	Result	Reference
A. sativum	Bulb	B5 + 0.5 2-iP + 0.1 NAA	Mult Sht	Bhojwanii (1980)
A. ampeloprasum	Shoot tip	MS + 1 NAA + 6 BA	Mult Sht	Ziv *et al.* (1983)
A. sativum	Shoot tip	MS + 0.57 μM IAA + 0.46 μM Kn		
		MS + 8.9 μM 2iP + 1.7 μM NAA	Mult Sht	Conci *et al.* (1986)
A. cepa	Basal plate	MS + 5 μM BA	Mult Sht	Kahane *et al.* (1992)
A. tuberosum	Shoot tip	MS + 0.5 BA	Mult Sht	Pandey *et al.* (1992)
A. sativum	Shoot tip	LS + 5 μM NAA + 10 μM BA + 56.5 mM KNO_3 + 3.5 mM NH_4Cl	Bulblet	Nagakubo *et al.* (1993)
		LS		
A. ampeloprasum	Basal plate	MS + 0.1 NAA + 2 BA	Mult Sht	Seabrook (1994)
A. sativum	Shoot tip	MS + 8 μM BA + 0.1 μM NAA	Mult Sht, Bulblet	Mohamed-Yasseen *et al.* (1994)
A. ascalonicum	Shoot tip	MS + 0.15 μM TDZ + 1.2 μM IBA	Mult Sht, Bulblet	Mohamed-Yasseen *et al.* (1994)
A. sativum	Shoot/scape tip	MS + 0.5 NAA + 0.5 Kn	Mult Sht	Ma *et al.* (1994)
		MS	Rt	
A. ampeloprasum	Seedling	MS + 4.4 μM BA	Mult Sht	Mohamed-Yasseen *et al.* (1995)
		MS + 0.5% AC	Rt	
A. ampeloprasum	Flower stalk	MS + 1 NAA + 1 BA	Sht Reg	Silvertand *et al.* (1995)
A. ampeloprasum	Basal plate, bulb	MS + 4.4 μM BA	Mult Sht	Barringer *et al.* (1996)
A. ampeloprasum	Peduncle-pedicel	MS + 10 μM BA + 10 μM 2,4-D	Mult Sht	Ziv and Kipnis (1997)
A. sativum	Root	MS + 1 μM NAA + 10 μM BA	Mult Sht	Haque *et al.* (1997)
		MS	Rt	
A. sativum	Basal plate	B5 + 5 μM JA + 5 μM 2iP	Mult Sht	Zel *et al.* (1997)
		B5 + 8% sucrose	Bulblet	
A. sativum	Root tip	MS + 0.5 μM BA	Mult Sht	Haque *et al.* (1998a)
		MS + 12% sucrose	Bulblet	
A. ampeloprasum	Flower pedicel	MS + NAA + Kn + ADS	Mult Sht	Ziv (2000)
A. wallichii	Seed	MS + 20 μM BA + 5 μM IAA	Mult Sht	Wawrosch *et al.* (2001)
		½ MS + 10 μM IAA	Rt	
A. sativum	Clove	MS + 0.5 2iP + 0.25 NAA	Bulblet	Roksana *et al.* (2002)
A. ampeloprasum	Floret	MS + 1 BA + 0.5 NAA	Mult Sht	Mohammad-Yasseen
		MS + 0.2 BA	Rt	and Nasr (2003)
A. sativum	Root	Liquid MS + 0.5 2iP + 2% sucrose	Mult Sht	Kim *et al.* (2003)
		Liquid MS + 0.1 NAA + 11% sucrose + 10 μM JA	Bulblet	
A. cepa	Stem dome, basal plate	MS + 10.6 μM Kn	Mult Sht	Kamstaityte and Stanys (2004)
A. ampeloprasum	Shoot tip	MS + 2.5 Kn + 60 ADS	Mult Sht	Gantait *et al.*
		MS + 0.5 IAA	Rt	(2009a)

AC: Activated charcoal; ADS: Adenine sulphate; B5: Gamborg's medium (Gamborg *et al.*, 1968); JA: Jasmonic acid; LS: Linsmaier and Skoog medium (Linsmaier and Skoog, 1965); Mult Sht: Multiple shoot; Rt: Root

and relatively higher cytokinin level promoted multiple shoot proliferation in *Allium*. This observation was further supported by the report of Ziv *et al.* (1983) where, 1 mg L^{-1} NAA plus 6 mg L^{-1} 6-benzylaminopurine (BA) induced multiple axillary shoots in *A. ampeloprasum*. Later, several authors reported these cytokinin: auxin combinations in a higher ratio resulted direct shoot initiation and proliferation in *A. sativum*, *A. ampeloprasum*, *A. ascalonicum* and *A. wallichii* (Conci *et al.*, 1986; Seabrook, 1994; Mohamed-Yasseen *et al.*, 1994; Silvertand *et al.*, 1995; Haque *et al.*, 1997; Wawrosch *et al.*, 2001; Roksana *et al.*, 2002; Mohammad-Yasseen and Nasr, 2003). Single cytokinin source without any supplementation of auxin successfully promoted multiple shoot culture in *Allium*. Initially Kahane *et al.* (1992) introduced 5 μM BA as the only PGR for initiation of multiple shoots in *A. cepa*, simultaneously Pandey *et al.* (1992) also, reported the sufficiency of 0.5 mg L^{-1} BA for the same task in *A. tuberosum*. Later on, the studies of Mohamed-Yasseen *et al.* (1995) and Barringer *et al.* (1996) in *A. ampeloprasum* and Haque *et al.* (1998a) in *A. sativum* recognized the competency of BA alone in direct organogenesis. Kamstaityte

and Stanys (2004) employed 10.6 μM kinetin or 6-furfurylaminopurine (Kn) as an alternative cytokinin source to BA for direct shoot regeneration from stem dome and basal plate of *A. cepa*. Later, corresponding to this report Gantait *et al.* (2009a) observed the efficiency of 2.5 mg L^{-1} Kn in initiation and enhancement of multiple shoots in *A. ampeloprasum*. In this study they supplemented adenine sulphate (ADS) as an additive growth factor which supports the earlier observation of Ziv (2000) in the same species. There are several other instances holding up the immense efficacy of ADS over boosting up the shoot growth and multiplication in *A. ampeloprasum* (Gantait *et al*., 2010a) and other genus like *Anthurium* (Gantait *et al.,* 2008). It appears that ADS used as an elicitor or enhancer of plant growth, works collectively or synergistically with endogenous or exogenously supplemented PGRs. Accumulation of ADS to the medium improved the multiple shoot elongation considerably (Gantait and Mandal, 2010). Apart from ADS as an additive to the PGRs several other compounds like potassium nitrate (KNO_3) in combination with ammonium chloride (NH_4Cl) (Nagakubo *et al*., 1993) or jasmonic acid (JA) (Zel *et al*., 1997) have been reported to induce direct shoot regeneration in *A. sativum*.

Nagakubo *et al.* (1993) was first to report the *in vitro* bulblet formation directly from excised shoot tips of *A. sativum* inoculated in LS basal medium. Later, Zel *et al.* (1997) documented successful bulblet production in B5 medium supplemented w ith 8% sucrose in *A. sativum*. A higher sucrose supplementation (12%) in MS medium regenerated *Allium* bulblets directly from root tip explants (Haque *et al*., 1998a). Roksana *et al.* (2002) inoculated surface sterilized individual clove of *A. sativum* in MS medium plus 0.5 mg L^{-1} 2iP along with 0.25 mg L^{-1} NAA to regenerate multiple bulblets. Supplementation of 10 μM JA in liquid MS medium with 0.1 mg L^{-1} NAA and 11% sucrose helped in direct bulblet regeneration from root tip of *A. sativum* in a better frequency (Kim *et al*., 2003).

For successful rhizogenesis from *A. sativum*, Ma *et al.* (1994) transferred *in vitro* regenerated multiple shoots in MS basal medium devoid of any PGR, which corresponds with the report of Haque *et al.* (1997). Wawrosch *et al.* (2001) for the first time experienced impact of indole-3-acetic acid (IAA) for direct root induction and elongation in *A. wallichii* when ½ MS was fortified with 10 μM IAA. With the progression of the preceding idea Gantait *et al.* (2009a) reported well developed rhizosphere in *A. ampeloprasum* using MS plus 0.5 mg L^{-1} IAA. Mohamed-Yasseen *et al.* (1995) carried out the pioneer work in *A. ampeloprasum* introducing 0.5% Activated Charcoal (AC) in MS medium to improve direct root development. In accordance to this report on *Allium* successive studies of Gantait *et al.* (2009b, c) in *Dendrobium* and *Vanilla* along with another medicinal plant *Aloe* (Gantait *et al*., 2010b), supplementation with AC proved indispensable for *in vitro* rooting. Nevertheless, the fortification with AC appears to offer a bonus advantage. It is evident that AC avoids illumination and endow with a rational atmosphere for the rhizosphere expansion (Gantait and Mandal, 2010).

Indirect Organogenesis

Plant regeneration *in vitro* is usually the most noteworthy movement for triumphant execution of a variety of biotechnological skills exploited for crop improvement curricula. Initiation of adventitious shoots and regeneration from callus cultures are significance for somaclonal variation and consequently, for breeding (Pati *et al*., 2006). In *Allium*, there are scores of reports which specify rapid regeneration and multiplication via indirect organogenesis or somatic embryogenesis are discussed concisely in Table 2.

For organogenic development via callus proliferation and regeneration MS medium was widely employed as basal medium using *in vitro* shoot, seed, embryo, basal plate,

Table 2: *In vitro* indirect organogenesis of *Allium*

Allium sp.	Explant	Medium (PGR in mg L^{-1})	Result	Reference
A. sativum	*In vitro* shoot	MS + 0.3 2,4-D	Ca	Nagasawa and Finer (1988)
A. ampeloprasum	Seed	MS + 1 2,4-D	Ca	
		MS + 1 Kn	Em	Buiteveld *et al.* (1993)
A. ampeloprasum	Embryo	MS + 2 2,4-D	Ca	Schavemaker and Jacobsen
		MS + 2 BA	Sht Reg	(1995)
A. cepa	Flower bud	MS + 10 ADS + 10% sucrose + 2 TDZ	Em	Bohanec *et al.* (1995)
		MS + 4% sucrose	Rt	
A. porrum	Shoot base	BDS + 9 mM 2,4-D	Ca, Em	Hong and Debergh (1995)
		BDS + 1.9 mM ABA + 17.6 mM 2iP	Sht Reg	
		BDS + 9 mM IBA	Rt	
A. ampeloprasum	Zygotic embryo	MS + 0.25-0.5 2,4-D	Ca	Silvertand *et al.* (1996)
		MS + 1 Kn	Sht Reg	
A. cepa	Zygotic embryo	MS + 1 2,4-D	Ca	Zheng *et al.* (1998)
		MS	Sht Reg	
A. sativum	Root	B5 + (4.7 μM picloram + 0.49 μM 2iP)	Ca	Myers and Simon (1998)
		or 4.5 μM 2,4-D		
		B5 + 1.4 μM picloram + 13.3 μM BA	Sht Reg	
A. sativum	Basal plate	MS + 1 2,4-D	Ca	Al-Zahim *et al.* (1999)
		MS + 1 IAA + 10 2iP	Sht Reg	
A. sativum	Root	MS + 0.3 ppm 2,4-D	Ca	Barandiaran *et al.*
		MS + 3 ppm BA	Sht Reg	(1999a)
A. sativum	Root-tip	N6 + 2.2 μM 2,4-D	Ca	Robledo-Paz *et al.* (2000)
		MS + 4.5 μM 2,4-D + 4.6 μM Kn	Sht Reg	
A. ampeloprasum	Infloresence	MS + 3 2,4-D + 1.5 Kn	Ca	Mohamed-Yasseen (2001)
		MS + 0.1 BA	Sht Reg	
		MS + 1 IAA	Rt	
A. sativum	Clove	MS + 4.44 μM NAA + 0.54 μM BA	Ca	Parisi *et al.* (2002)
A. ampeloprasum	Floret	MS + 0.5-10 2,4-D + 3 Picloram	Ca	Mohammad-Yasseen and
				Nasr (2003)
A. ampeloprasum	Zygotic embryo	BDS + 1 2,4-D + 0.5 BA	Ca	Toaima *et al.* (2003)
A. ampeloprasum		MS+NAA+BA	Ca	Evenor *et al.* (1997)
		MS + IAA +Kn	Sht Reg	
A. cepa	Shoot tip	BDS + 0.5 2,4-D + 1 BAP	Ca	Zhang *et al.* (2004)
		MS	Sht Reg	
A. sativum	Meristem	BDS + 0.45 μM 2,4-D + 4.43 μM BAP	Ca	Luciani *et al.* (2006)
			Sht Reg	
A. cepa	Seed	Liquid MS + 4 2,4-D + 0.5 BAP	Em	Tiwari *et al.* (2007)
		MS + 0.5 NAA + 0.5 BAP + 0.5 Kn	Sht Reg	
A. sativum				
A. cepa	Young bulb	MS + 1 Kn + 1 2,4-D	Ca	Rêbilas and Rêbilas (2008)
A. sativum	Basal plate	MS + 1 BAP + 0.25 2,4-D	Em	Nasim *et al.* (2010)

ADS: Adenine sulphate; B5: Gamborg's medium (Gamborg *et al*., 1968); BDS: BDS medium (Dunstan and Short, 1977); Ca: Callus; Em: Somatic embryo; JA: Jasmonic acid; N6: Nitsch's basal medium (Nitsch, 1969); Sht Reg: Adventitious shoot regeneration; Rt: Root

inflorescence or young bulb as explant sources (Nagasawa and Finer, 1988; Buiteveld *et al*., 1993; Schavemaker and Jacobsen, 1995; Al-Zahim *et al*., 1999; Mohamed-Yasseen, 2001; Evenor *et al*., 1997; Rebilas and Rebilas, 2008) for *Allium*; although there are plenty of reports showing the potentiality of BDS (BDS medium; Dunstan and Short, 1977) (Hong and Debergh, 1995; Toaima *et al*., 2003; Zhang *et al*., 2004; Luciani *et al*., 2006), B5 medium (Myers and Simon, 1998) and N6 medium (Robledo-Paz *et al*., 2000) to serve as the basal medium for indirect organogenesis in *A. porrum*, *A. ampeloprasum*, *A. cepa* and *A. sativum*. For induction or organogenic calli in *Allium* 2,4-dichlorophenoxyacetic acid (2,4-D) served as the only auxin source in the media. Nagasawa and Finer (1988) for the first time successfully demonstrated the callus initiation and proliferation using MS media plus 0.3 mg L^{-1} 2,4-D in *A. sativum*. This report on efficacy of 2,4-D as the only PGR for callus culture was further favoured by several successors in *A. ampeloprasum* (Buiteveld *et al*., 1993; Schavemaker and Jacobsen, 1995; Silvertand *et al*., 1996), *A. porrum* (Hong and Debergh,

1995), *A. cepa* (Zheng *et al.*, 1998) and in *A. sativum* (Al-Zahim *et al.*, 1999; Robledo *et al.*, 2000). Addition of a lower level of cytokinin with a comperatively higher level of 2,4-D resulted in enhancement of callus culture than 2,4-D alone. Mohamed-Yasseen (2001) was the earliest author to report the combined impact of auxin-cytokinin (3 mg L^{-1} 2,4-D plus 1.5 mg L^{-1} Kn) in callus initiation of *A. ampeloprasum*. Afterwards, Mohammad-Yasseen and Nasr (2003) continued to obtain similar result replacing Kn by picloram as the cytokinin source. Introduction of BA (Toaima *et al.*, 2003) or BAP (Zhang *et al.*, 2004) as the alternative cytokinin source with 2,4-D proved best for *A. ampeloprasum* and *A. cepa*, respectively.

Schavemaker and Jacobsen (1995) put their best foot forward in regeneration of adventitious shoots in *A. ampeloprasum*. They fortified 2 mg L^{-1} BA with MS basal media for this purpose. This report finds its absolute correspondence with the subsequent experiments of Barandiaran *et al.* (1999a) and Mohamed-Yasseen (2001) who observed the maximum regeneration potential of *Allium* in MS medium plus 3 ppm or 0.1 mg L^{-1} BA respectively. In contradiction to this theory of single cytokinin source in shoot regeneration Hong and Debergh (1995) initiated auxin-cytokinin (1.9 mM ABA with 17.6 mM 2iP) concoction, which was later established by Robledo *et al.* (2000) and Evenor *et al.* (1997) who supplemented 2,4-D and IAA as the auxin source with Kn.

Somatic Embryogenesis

Near the mid-19th century Steward *et al.* (1958) at first established the plant regeneration from *in vitro* cultured carrot cells through somatic embryogenesis. Somatic embryogenesis is a developmental procedure of somatic cells from single cell or a group of cells kicking off the pathway that directs to reproducible regeneration of complete embryos analogous to the development of zygotic embryos competent to form entire plants. During *in vitro* culture somatic embryogenesis crops up most recurrently as a substitute to organogenesis for whole plants regeneration (Kanwar and Kumar, 2008). The somatic embryo develops through the discrete structural stages of the globular, heart, torpedo, cotyledonary and maturity. Escaping an intermediate callus phase somatic embryogenesis can occur straight from cells of the explant. However, the development of somatic embryo from proliferated callus in an indirect embryogenesis pathway is more frequent (Pierik, 1987; Rashid, 1988) and potential as a competent regeneration scheme with reasonably high genetic truthfulness. It has also been measured lucrative over other *in vitro* propagation systems as it shortens the extent multiplication time (Kothari *et al.*, 2010). This report raises a noteworthy concern for *in vitro* regeneration through somatic embryogenesis, which is still under progress in *Allium*. The somatic embryogenesis in *A. sativum* has been reviewed by Lee *et al.* (2009). What follows is a concise overview on somatic embryogenesis of genus *Allium* including most recent literature listed in Table 2.

Earlier studies of eminent researchers described the *in vitro* plant regeneration of *A. sativum* via somatic embryogenesis from shoot tip explants (Kehr and Schaeffer, 1976; El-Nil, 1977; Novak, 1981; Koul *et al.*, 1994). *In vitro* plants were readily attained from callus but large scale regeneration from somatic embryos was not well established previous to Xue *et al.* (1991) developed somatic embryogenesis and plant regeneration in basal plate and receptacle derived-callus cultures of *A. sativum*. During *in vitro* regeneration of *Allium* via callus growth, followed by adventitious bud formation, has been proposed as an attractive option for large scale production by Buiteveld *et al.* (1993) in *A. ampeloprasum*. Bohanec *et al.* (1995) reported somatic embryogenesis and plant regeneration in *A. cepa* from flower bud using an induction medium containing MS supplemented with 10 mg L^{-1} ADS plus 2 mg L^{-1} 1-phenyl-3-(1,2,3-thiadiazol-5-yl) urea (TDZ) with an increased (10%) level of

sucrose. Haque *et al.* (1998b) established a competent practice of plant regeneration from root tip of *A. sativum* via somatic embryogenesis in MS medium containing 0.5 μM 2,4-D. Embryos germinated and converted to complete plantlets on MS medium with 5.0 μM kinetin. Fereol *et al.* (2002) established a unique scheme for somatic embryogenesis and plant regeneration in *A. sativum* using young leaf or root explants from *in vitro* plants as the sources of explants. They reported that the embryogenic potential was higher in callus proliferated from young leaves in B5 medium supplemented with 0.1 mg L^{-1} 2,4-D and 0.5 mg L^{-1} Kn. The regeneration of somatic embryos to plants with shoots and roots was observed on BDS medium with 0.3 mg L^{-1} BAP. Direct or indirect somatic embryogenesis and plant regeneration from *A. cepa* seed using as explants have been achieved by Tiwari *et al.* (2007) in liquid MS medium fortified with 4 mg L^{-1} 2,4-D plus 0.5 mg L^{-1} BAP. Contrary to this earlier report, a lower level of 2,4-D (0.25 mg L^{-1}) with comparatively higher level of BAP (1 mg L^{-1}) supplementation in MS medium significantly affected or even they boost up the rate of regeneration from somatic embryo in *A. sativum* (Nasim *et al.*, 2010).

GENETIC TRANSFORMATION IN *ALLIUM*

Genetic engineering and *in vitro* regeneration protocols are two collaborative equipments to complement conventional *Allium* breeding and to develop high yielding, biotic/abiotic stress resistant/tolerant cultivars, adapted to local ecological conditions (Barandiaran *et al.*, 1999a-c; Martin-Urdiroz *et al.*, 2004).

It is utmost significant to establish an efficient genetic transformation system for the further advancement of traditional crops.

Eady *et al.* (2000) were the pioneer in *Agrobacterium tumefaciens* mediated gene transformation and regeneration of *A. cepa*. Simultaneously, Kondo *et al.* (2000) also standardised the unique scheme on transformation and regeneration of *A. sativum* by means of *Agrobacterium*-mediated gene transfer. Afterwards, Park *et al.* (2002) generated the chlorosulfuron-resistant transgenic on *A. sativum* employing particle bombardment methodology which proved as better alternative to the complicated intervention of *Agrobacterium*. Eady *et al.* (2003, 2005) momentously achieved herbicide resistant onion *A. cepa* and developed transgenics in *A. porrum* and *A. sativum* using *Agrobacterium tumefaciens*-mediated transformation. Zheng *et al.* (2004) followed similar methodology for development of a reproducible transformation system in *A. sativum* and the production of transgenics resistant to beet armyworm (*Spodoptera exigua* Hubner). In the most recent experiment Kenel *et al.* (2010) developed transgenic *A. sativum* plants straight from juvenile leaf tissue by selective culture subsequently *Agrobacterium*-mediated gene transformation. Their technique involved the exploitation of a binary vector holding the *mgfp-ER* reporter gene and *hpt* selectable marker.

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