An Overview on in vitro Culture of Genus Allium

1S. Gantait, 1N. Mandal and 2P.K. Das
1Department of Biotechnology, Instrumentation and Environmental Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, W.B. 741252, India
2Department of Genetics, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, W.B. 741252, India

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.

Keywords: 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 perspective 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, 2008). 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
drugs has improved exponentially in recent 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 Aliioideae). 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.

IN VITRO PROPAGATION 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 traits 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. (Bhoywani, 1980; Pandey et al., 1992; Seabrook, 1994; Silverland 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 (Bhoywani, 1980; Nagakubo et al., 1993; Zel et al., 1997).

Bhoywani (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>
<thead>
<tr>
<th>Allium sp.</th>
<th>Explant</th>
<th>Medium (PGR in mg L⁻¹)</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. sativum</td>
<td>Bulb</td>
<td>BS + 0.3 2,4-D + 0.1 NAA</td>
<td>Mult Sh</td>
<td>Bhajiwati (1980)</td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Shoot tip</td>
<td>MS + 1.1 NAA + 6 BA</td>
<td>Mult Sh</td>
<td>Zee et al. (1983)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Shoot tip</td>
<td>MS + 0.57 mM IAA + 0.46 mM Kn</td>
<td>Mult Sh</td>
<td>Conci et al. (1986)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MS + 8.9 mM 2IP + 1.7 mM NAA</td>
<td>Mult Sh</td>
<td>Kahane et al. (1992)</td>
</tr>
<tr>
<td>A. cepa</td>
<td>Basal plate</td>
<td>MS + 5 mM BA</td>
<td>Mult Sh</td>
<td>Pandey et al. (1992)</td>
</tr>
<tr>
<td>A. tuberosum</td>
<td>Shoot tip</td>
<td>MS + 0.5 BA</td>
<td>Mult Sh</td>
<td>Nagasubha et al. (1993)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Shoot tip</td>
<td>LS + 5 mM NAA + 10 mM BA +</td>
<td>Bulllet</td>
<td>Nagasubha et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56.5 mM KN03 + 3.5 mM NiCl₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Basal plate</td>
<td>MS + 0.1 NAA + 2 BA</td>
<td>Mult Sh</td>
<td>Seabrook (1994)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Shoot tip</td>
<td>MS + 8 mM BA + 0.1 mM NAA</td>
<td>Mult Sh</td>
<td>Mohamed-Yaseen et al. (1994)</td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Shoot tip</td>
<td>MS + 0.15 mM TDZ + 1.2 mM BAA</td>
<td>Mult Sh</td>
<td>Mohamed-Yaseen et al. (1994)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Shoot tip</td>
<td>MS + 0.5 NAA + 0.5 Kn</td>
<td>Mult Sh</td>
<td>Mohamed-Yaseen et al. (1994)</td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Seedling</td>
<td>MS + 4.4 mM BA</td>
<td>Mult Sh</td>
<td>Mohamed-Yaseen et al. (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MS + 0.5% AC</td>
<td>RI</td>
<td>(1995)</td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Flower stalk</td>
<td>MS + 1 NAA + 1 BA</td>
<td>Sh Reg</td>
<td>Silvertand et al. (1995)</td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Basal plate,</td>
<td>MS + 4.4 mM BA</td>
<td>Mult Sh</td>
<td>Barrett et al. (1996)</td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Root</td>
<td>MS + 10 mM BA + 10 mM 2,4-D</td>
<td>Mult Sh</td>
<td>Zee and Kurni (1997)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Root</td>
<td>MS + 1 μM NAA + 10 μM BA</td>
<td>Bulllet</td>
<td>Haque et al. (1997)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Basal plate</td>
<td>BS + 5 mM JA + 5 mM 2IP</td>
<td>Mult Sh</td>
<td>Zee et al. (1997)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Root tip</td>
<td>MS + 0.5 mM BA</td>
<td>Mult Sh</td>
<td>Haque et al. (1998a)</td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Flower pedicel</td>
<td>MS + 12% sucrose</td>
<td>Bulllet</td>
<td>Haque et al. (1998a)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Root</td>
<td>MS + 10 μM IAA</td>
<td>RI</td>
<td></td>
</tr>
<tr>
<td>A. sativum</td>
<td>Clove</td>
<td>MS + 0.2 2IP + 0.25 NAA</td>
<td>Bulllet</td>
<td>Rokkam et al. (2002)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Root</td>
<td>Liquid MS + 0.2 2IP + 2% sucrose</td>
<td>Bulllet</td>
<td>Kim et al. (2003)</td>
</tr>
<tr>
<td>A. sativum</td>
<td>Stem dome, basal plate</td>
<td>MS + 10.6 mM Kn</td>
<td>Mult Sh</td>
<td>Kamstaityte and Sturys (2004)</td>
</tr>
<tr>
<td>A. ampeloprasum</td>
<td>Shoot tip</td>
<td>MS + 2.5 Kn + 50 ADs</td>
<td>Mult Sh</td>
<td>Guntait et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MS + 0.5 IAA</td>
<td>RI</td>
<td></td>
</tr>
</tbody>
</table>

AC: Activated charcoal; ADs: Adenine sulphate; BS: Gamborg’s medium (Gamborg et al., 1968); JA: Jasmionic acid; L5: Linsmaier and Skoog medium (Linsmaier and Skoog, 1965); Mult Sh: Multiple shoot; RI: Root

and relatively higher cytokinin level promoted multiple shoot proliferation in Allium. This observation was further supported by the report of Zee 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-Yaseen et al., 1994; Silvertand et al., 1995; Haque et al., 1997; Wawrosch et al., 2001; Roksana et al., 2002; Mohamed-Yaseen and Nars, 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-Yaseen 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 1.6 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\(_4\)Cl) (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 BS medium supplemented with 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*

<table>
<thead>
<tr>
<th>Allium sp.</th>
<th>Explant</th>
<th>Medium (PGR in mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. sativum</em></td>
<td>In vitro shoot</td>
<td>MS + 0.3 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Nagasawa and Finer (1988)</td>
</tr>
<tr>
<td><em>A. ampeloprasum</em></td>
<td>Seed</td>
<td>MS + 1 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Buiterfeld et al. (1993)</td>
</tr>
<tr>
<td><em>A. ampeloprasum</em></td>
<td>Embryo</td>
<td>MS + 2.2 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Schaveemaker and Jacobsen (1995)</td>
</tr>
<tr>
<td><em>A. cepa</em></td>
<td>Flower bud</td>
<td>MS + 10 mg L&lt;sup&gt;-1&lt;/sup&gt; ADS + 10% sucrose + 2 mg L&lt;sup&gt;-1&lt;/sup&gt; TDZ</td>
<td>Em</td>
<td>Bohanse et al. (1995)</td>
</tr>
<tr>
<td><em>A. porrum</em></td>
<td>Shoot base</td>
<td>BDS + 9 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca, Em</td>
<td>Hong and Debergh (1995)</td>
</tr>
<tr>
<td><em>A. ampeloprasum</em></td>
<td>Zygotic embryo</td>
<td>MS + 0.25 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Silvertand et al. (1996)</td>
</tr>
<tr>
<td><em>A. cepa</em></td>
<td>Zygotic embryo</td>
<td>MS + 1 mg L&lt;sup&gt;-1&lt;/sup&gt; K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Ca</td>
<td>Zheng et al. (1998)</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>Root</td>
<td>RS + (4.7 mg L&lt;sup&gt;-1&lt;/sup&gt; picloram + 0.49 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D) or 4.5 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Myers and Simon (1998)</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>Basal plate</td>
<td>MS + 1.4 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Al-Zahim et al. (1999)</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>Root</td>
<td>MS + 0.3 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Barnebier et al. (1999)</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>Root-tip</td>
<td>MS + 0.3 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Robledo-Paz et al. (2000)</td>
</tr>
<tr>
<td><em>A. ampeloprasum</em></td>
<td>Inflorescence</td>
<td>MS + 3.2 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D + 1.5 mg L&lt;sup&gt;-1&lt;/sup&gt; K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Ca</td>
<td>Mohamed-Yasseen (2001)</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>Clove</td>
<td>MS + 4.44 mg L&lt;sup&gt;-1&lt;/sup&gt; NAA + 0.54 mg L&lt;sup&gt;-1&lt;/sup&gt; BA</td>
<td>Ca</td>
<td>Parisi et al. (2002)</td>
</tr>
<tr>
<td><em>A. ampeloprasum</em></td>
<td>Floret</td>
<td>MS + 0.5 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D + 3 mg L&lt;sup&gt;-1&lt;/sup&gt; picloram</td>
<td>Ca</td>
<td>Mohamed-Yasseen and Naur (2003)</td>
</tr>
<tr>
<td><em>A. ampeloprasum</em></td>
<td>Zygotic embryo</td>
<td>BDS + 1 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D + 0.5 mg L&lt;sup&gt;-1&lt;/sup&gt; BA</td>
<td>Ca</td>
<td>Toima et al. (2003)</td>
</tr>
<tr>
<td><em>A. ampeloprasum</em></td>
<td>Shoot tip</td>
<td>BDS + 0.5 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D + 1 mg L&lt;sup&gt;-1&lt;/sup&gt; BA</td>
<td>Ca</td>
<td>Zemoued et al. (2004)</td>
</tr>
<tr>
<td><em>A. cepa</em></td>
<td>Meristem</td>
<td>BDS + 0.45 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D + 4.43 mg L&lt;sup&gt;-1&lt;/sup&gt; BA</td>
<td>Ca</td>
<td>Luciani et al. (2006)</td>
</tr>
<tr>
<td><em>A. cepa</em></td>
<td>Seed</td>
<td>Liquid MS + 4.2 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D + 0.5 mg L&lt;sup&gt;-1&lt;/sup&gt; BA</td>
<td>Em</td>
<td>Tiwari et al. (2007)</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>Young bulb</td>
<td>MS + 0.5 mg L&lt;sup&gt;-1&lt;/sup&gt; NAA + 0.5 mg L&lt;sup&gt;-1&lt;/sup&gt; BAP + 0.5 mg L&lt;sup&gt;-1&lt;/sup&gt; K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Ca, Em</td>
<td>Rebilas and Rebilas (2008)</td>
</tr>
<tr>
<td><em>A. sativum</em></td>
<td>Basal plate</td>
<td>MS + 1 mg L&lt;sup&gt;-1&lt;/sup&gt; K&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt; + 1.2 mg L&lt;sup&gt;-1&lt;/sup&gt; 2,4-D</td>
<td>Ca</td>
<td>Neust et al. (2010)</td>
</tr>
</tbody>
</table>

ADS: Adenine sulphate; B5: Gamborg’s medium (Gamborg et al., 1968); BDS: BDS medium (Dunstan and Short, 1977); Ca: Callus; Em: Somatic embryogenesis; IA: IAA; MS: Nitsch’s basal medium (Nitsch, 1969); RS: Root; SEL: Adventitious shoot regeneration; RT: Root;

inflorescence or young bulb as explant sources (Nagasawa and Finer, 1988; Buiteveld et al., 1993; Schaveemaker and Jacobsen, 1995; Al-Zahir 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; Toima 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 callus 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<sup>-1</sup> 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 successes in *A. ampeloprasum* (Buiteveld et al., 1993; Schaveemaker 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 comparatively 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. ampoloprasum. 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. ampoloprasum and A. cepa, respectively.

Schimmer and Jacobsen (1995) put their best foot forward in regeneration of adventitious shoots in A. ampoloprasum. 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 study, 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 previously 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. ampoloprasum. 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-yi) urea (TDZ) with an increased (10%) level of 331
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.

**REFERENCES**


