Standard Review

Plant regeneration of garlic (Allium sativum L.) via somatic embryogenesis

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Allium sativum L. belongs to a member of the onion family (Alliaceae) and has been used for both culinary and medical purpose. Extensive research works have been carried out on the health promoting and medicinal properties of garlic. A. sativum has shown the wide range of biological and pharmacological activities including antioxidant, cancer prevention, liver protection, immunomodulation and reduction of cardiovascular disease risk factors. The propagation of garlic is by division of the individual cloves of its bulbs. Because garlic almost never produces fertile seeds, it must be propagated vegetatively. Therefore, a variety of studies have reported in vitro plant regeneration of A. sativum from the culture of several explants for multiple propagations. In this review paper, we summarize previous and current information regarding somatic embryogenesis, in vitro plant regeneration, long-term culture and somaclonal variation of A. sativum and provide new insights for future study in this discipline.

Key words: Allium sativum L., garlic, long-term culture, plant regeneration, somaclonal variation, somatic embryogenesis.

INTRODUCTION

Allium sativum L., commonly known as garlic, belongs to a member of the onion family (Alliaceae). Garlic has been used throughout the ages for both culinary and medical purpose (Tapsell et al., 2006). Garlic was in use at the beginning of recorded history and interest in the potential benefits of garlic is one of the earliest documented examples of plants employed for treatment of disease and maintenance of health (Rivlin, 2001, 2006).

Extensive research work has been carried out on the health promoting and medicinal properties of garlic. A. sativum has shown a variety of biological activities including antioxidant, cancer prevention, liver protection, immunomodulation and reduction of cardiovascular disease risk factors (Butt et al., 2009; Iciek et al., 2009; Pittler and Ernst, 2007). Garlic is characterized by medicinal properties due to the content of over 2000 biologically active compounds (Swiderski et al., 2007). Garlic has an unusually high concentration of sulfur-containing compounds. Sulfur compounds, including allicin (thio-2-propene-1-sulfinic acid S-allyl ester) were confirmed to be the main active components in the root bulb of the garlic plant (Tattelman, 2005). Allicin has the wide range of biological and pharmacological activities, such as anticoagulation, antihypertensive, antimicrobial, antibiotic, antiparasitic, antymycotic, antiviral, antitumoral, anti-oxidant, anti-aging, antiplatelet, detoxifies heavy metals, fibrinolysis, hypolipidaemic (lipid-lowering) and immune enhancer and modulator (Amagase, 2006; Iciek et al., 2009; Jacob, 2006; Munchberg et al., 2007).

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fore, a variety of studies have reported in vitro somatic embryogenesis, plant regeneration and micropropagation of *Allium sativum* from the culture of several explants for multiple propagations (Bockish et al., 1997; Novak, 1990).

Recently, we establish the plant regeneration of garlic through somatic embryogenesis using various explants (Figure 1). In this paper, we reviewed various studies about somatic embryogenesis, in vitro plant regeneration, long-term culture and somaclonal variation of *A. sativum* for crop improvement.

**PLANT REGENERATION OF GARLIC VIA SOMATIC EMBRYOGENESIS**

**Plant regeneration through somatic embryogenesis**

Early this century, Haberlandt (1902) demonstrated that individual nucleated plant cells have the genetic capacity to be converted to complete plants either directly or through an intervening callus stage. This phenomenon has been termed 'totipotency'. More recently, the term 'regeneration' has been generally used in the context of plant tissue culture to indicate the recovery of a whole plant from cells, tissues, organs, meristems or zygotic embryos cultivated in vitro. There are a number of pathways for the regeneration of whole plants from excised plant tissues or cells. In general, there are two main pathways which can be considered, that is, plant regeneration through organogenesis and somatic embryogenesis (Phillips et al., 1995). Steward et al. (1958) originally observed plant regeneration by somatic embryogenesis from cultured carrot cells. In somatic embryogenesis, somatic cells develop by division to form complete embryos analogous to the development of zygotic embryos. The bipolar structure of the somatic embryo contains both shoot and root meristems. As the embryos develop, they progress through the distinct structural steps of the globular, heart, torpedo, cotyledonary and mature stages. Somatic embryogenesis can occur directly from cells of the explant tissue without an intervening callus phase. However, the indirect embryogenesis pathway, where somatic embryos are induced and develop from a proliferated callus, is generally more common (Pierik, 1987; Rashid, 1988).

During the initiation of embryogenic cultures, the supplied high concentration of auxin (usually 2, 4-Dichlorophenoxyacetic acid (2, 4-D)) will induce both cellular proliferation (callus induction) and the embryogenic pathway of development. It is generally thought that the embryogenic pathway is induced and becomes determined very early in embryogenic cultures and this clearly seems to be the case in carrot, which is considered the model species. The high concentration of auxin used for induction, however, is usually inhibitory to the development of somatic embryos into advanced stages. A hormone-free medium often is used for the development of globular-staged somatic embryos into plantlets. Sometimes low concentrations of hormones in the development medium can be beneficial or even required, depending on the species, to promote normal development of the embryos. Cytokinin usually is not required for induction of somatic embryogenesis, but cer-

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**Figure 1.** Somatic embryogenesis of *Allium sativum* L. Embryogenic callus and early stage somatic embryos (A) and numerous somatic embryos (B) are shown developing on the surface of shoot tip cultured on solid MS medium supplemented with 2.0 mg/l 2, 4-D and 0.1 mg/l BAP. Magnification: A and B, x15.
tain monocotyledonous species do have a specific requirement for cytokinin. Thus, induction of somatic embryogenesis requires a single hormonal signal to induce a bipolar structure capable of forming a complete plant. In contrast, organogenesis requires two different hormonal signals to induce first a shoot organ, then a root organ, using two different culture media. Somatic embryogenesis does not require a different medium and uses lower concentrations of hormones, if any, to promote the development of embryogenic cells into plantlets (von Arnold et al., 2002; Zimmerman, 1993).

Somatic embryogenesis has the potential for rapid and efficient in vitro plant regeneration. The yield and quality of somatic embryos produced in cell culture depends on the optimization of media constituents and plant growth regulators.

**Somatic embryogenesis of *Allium sativum* from the various explants culture**

Plant cell and tissue culture plays an important role in the plant regeneration, micropropagation and manipulation of plants for improved crop varieties. Cells and tissues of many plant species are difficult to culture and establish optimal growing conditions in vitro. Therefore, there continues to be an urgent need for extensive work in the field of basic tissue culture protocols for many crop plants before any practical utilization (Birch, 1997; Hicks, 1980). Lots of reports have documented plant regeneration through embryogenesis in garlic tissues cultured in vitro using various explants (Bhagyalakshmi et al., 2005).

Early studies reported in vitro plant regeneration of garlic through somatic embryogenesis using shoot tips as explants (Abo El-nil, 1977; Kehr et al., 1976; Koul et al., 1994; Novak, 1981). Nagakubo et al. (1993) developed a micropropagation system of garlic by the combination of initial shoot-tip culture, shoot multiplication and in vitro bulblet formation. Garlic shoot tips were cultured on LS medium (Linsmaier et al., 1965) containing 1 μM indole-3-acetic acid (IAA) and 1 μM 6-benzyladenine (BA) to regenerate proliferative shoots.

Haque et al. (1997) established an efficient and novel method of direct shoot regeneration from root tips of garlic. The best condition of plant growth regulator treatment was 1-naphthaleneacetic acid (NAA) and BA at 1 and 10 μM, respectively, inducing shoot initiation from 75% of the explants. Haque et al. (1998) also developed an efficient protocol of plantlet regeneration through somatic embryogenesis in *A. sativum* using root tips as explants. Root tips cultured on agar-solidified MS medium containing various concentrations of 2,4-D for callus and embryo formation. The optimum concentration of 2, 4 -D was 0.5 μM. Embryos germinated and converted rooted plantlets on MS (Murashige et al., 1962) solid medium containing 5.0 μM kinetin. Anatomical changes during in vitro direct formation of shoot bud from root tips of garlic were observed under a light microscope by Haque et al. (1999). Barandiari et al. (1999a) tested the effect of different callus induction media on the regeneration process in garlic. Root tips of cv. Rojo de Cuenca were cultured on B5 (Gamborg et al., 1968) medium containing various combinations of benzyladenine, 2, 4 -D, NAA and 2iP (isopentenyladenine) for callus induction. For regeneration, media containing various combination of benzyladenine, kinetin, IAA and TIBA were tested. The best results were obtained in induction media containing 13.3 μM benzyladenine + 0.14 μM 2,4-D + 10.7 μM NAA. Barandiari et al. (1999b) analyzed twenty garlic genotypes for genetic variation in their ability to form callus and regenerate shoots. Genotypes showed significant differences in the analysis of variance of all the traits tested. Myers et al. (1998) used root segments from shoot tip-derived plantlets of the garlic as an explant for continuous, friable callus production. For plant regeneration, embryogenic and friable call was transferred to liquid medium for 1 month and then transferred to solid regeneration medium for 14 weeks. The best shoot and root regeneration (85.3 and 35.8%, respectively) achieved on 4-month-old calli from the clone ‘DDR7099’.

Robledo-Paz et al. (2000) also used root apices from in vitro cultured garlic cloves of cvs. ABEN and GT96-1 for organogenic callus production and plant regeneration experiments. Root explants cultured in media based on those of N6 (Chu, 1978; Chu et al., 1975) or MS could induce organogenic callus after 8 wk culture in darkness. Fereol et al. (2002) developed a protocol for somatic embryogenesis and plant regeneration of the garlic. They used young leaf explants or root explants from in vitro plants as explants source. Callus production was optimal on explants derived from root sections. However, callus from young leaves expressed higher embryogenic potential. Up to 75% of such embryogenic callus differentiated globular somatic embryos after 2 months on B5 medium supplemented with 2, 4 -D (0.1 mg l⁻¹) and kinetin (0.5 mg l⁻¹) and then up to 30% of the somatic embryos were converted into plants with shoots and roots after 8 weeks on a BDS medium with Benzyaminopurine (BAP) (0.3 mg l⁻¹). Zheng et al. (2003) developed an efficient cultivar-independent plant regeneration system from callus derived from both apical and non-apical root segments of garlic. They demonstrated that callus induction on apical root segments was significantly higher compared to callus induction on non-apical root segments. In contrast, shoot regeneration from callus induced on non-apical segments was higher, although not significant, compared to callus induction from apical root segments. Martin-Urdiroz et al. (2004) investigated the effect of light on the organogenic ability of garlic roots using a one-step in vitro system. The application of light from the beginning of the culture process did not affect the callus induction rate but did significantly improve the explant regeneration ability. In a 2-month period it was possible to obtain up to 250 shoots.
per gram of callus. The basal plates were used as explants for tissue culture of garlic in vitro. Xue et al. (1991) established somatic embryogenesis and plant regeneration in basal plate and receptacle derived-callus cultures of garlic. Koch et al. (1995) reported improved regeneration of shoots from garlic callus induced from basal plate culture. Barandiaran et al. (1998) developed the biolistic transfer and expression of a uid-A reporter gene in different tissues of Allium sativum, including basal plates.

Several reports studied callus induction, somatic embryogenesis, plant regeneration of garlic using first primordial leaves (Dolezel and Novák, 1985; Novák, 1980; Sata et al., 2000). Suh and Park, (1988) investigated necessary cultural conditions and factors for induction of somatic embryogenesis in garlic. Somatic embryogenesis only occurred from very immature flower organ such as pedicels and flower buds but not from foliage leaf, stem and root when these different explants were cultured on MS medium containing with NAA and BAP. However, adventitious shoots were developed from all explants cultured. Nagasawa And Finer, (1988) developed rapidly growing and regenerable suspension cultures from meristem-derived callus cultures of garlic for plant regeneration. Masuda et al. (1994) established a micropropagation system of virus-free plants has been achieved for garlic. The virus-free plants were regenerated from the cultures of shoot apices in cloves and maintained in axenic cultures. Explants were then excised from bulblets of these in vitro grown plantlets and subjected to micropropagation experiments. Ayabe et al. (1998) established a novel tissue culture method, stem-disc culture and its practical application to micropropagation of garlic. A restricted part of the undeveloped stem of the garlic clove, called the "stem disc", which is just under the basement of the immature foliage leaves, proved to be a very potent explant for the plant regeneration and micropropagation of garlic. Kim and Kim, (2002) developed high frequency plant regeneration of garlic calli immobilized in calcium alginate gel. The effects of different explants and growth regulators on callus induction and plant regeneration in garlic were evaluated by Luciani et al. (2006). They demonstrated that the optimized plant regeneration system allow to develop a protocol suitable for further transformation experiments in garlic.

A long-term regeneration system of Allium sativum

Robledo-Paz et al. (2006) demonstrated callus and cell suspension of plants can be used for long-term cell cultures maintenance. They reported procedures for the induction of somatic embryos of garlic, keeping a regeneration capacity for more than 5 years. Myers et al. (1999) developed a long-term regeneration system for garlic clones of diverse origin. Regeneration rate decrea-
the other hand, callus maintained in solid medium for long-term culture showed abnormality in chromosome. In contrast, Sudarmonowati et al. (2001) demonstrated in vitro cultured embryogenic callus showed genetic stability after six years maintenance.

**CONCLUSION**

The ability to regenerate whole plants from individual cells and various tissues provides the application of plant cell and tissue culture to the improvement of plants. Extensive research has improved plant regeneration system in garlic and has applied to breeding program for crop improvement. However, much of the progress has been empirically based and plant regeneration is still devoid of a satisfactory theoretical foundation. The desired applications of transformation techniques have placed extra demands on optimized regeneration systems. The purpose of this review to collect all the possible information regarding systems of plant regeneration in *A. sativum* thus will help students and scientists to take action for future study in this discipline.

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