Journal of Medicinal Plants Research Vol. 5(17), pp. 4009-4020, 9 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Review

Use of somatic embryogenesis as a vehicle for cotton transformation

Olawole O. Obembe[1]*, Tanveer Khan[2] and Jacob O. Popoola[1]

[1]Department of Biological Sciences, Covenant University, P. M. B. 1023 Ota, Ogun State, Nigeria.
[2]Department of Plant Physiology, CBSH, GBPUAT, Pantnagar, Uttaranchal-263145, India.

Accepted 23 May, 2011

Cotton has been aptly described as the prosperity plant owing to its unrivalled economic importance as a source of feedstock, food and oil, as well as raw material for diverse industrial applications, ranging from textile and footwear to automobiles, energy, medical and pharmaceutical. As such, over 180 million people of the world depend on its production for livelihood. However, cotton production is grossly hampered, and has long been peaked in many regions where it is being grown. Without prejudice to the genetic improvement already made by conventional breeding with respect to yield and quality over the years, genetic transformation is arguably the last recourse for further development of cotton, especially with respect to the prevailing production constraints of insect pests, weeds, environmental stresses and diseases. This review therefore focuses on the use of somatic embryogenesis as a vehicle for cotton genetic transformation. It indeed attempts to overview the challenges of cotton transformation with respect to narrow genetic base coupled with the recalcitrant nature of the crop species, as well as the research success achieved so far. It then discusses the underlying mechanisms of somatic embryogenesis as well as the current constraints and various strategies being used to overcome them; all with the aim of motivating interest groups to initiate research activities in cotton biotechnology and to strive for its optimization for further genetic improvement.

Key words: Somatic embryos, genetic transformation, plant regeneration, genetic regulation, classical breeding.

INTRODUCTION

There are four domesticated species of the cotton, two tetraploid cultivars from Americas, *Gossypium hirsutum* and *G. barbadense*, and two diploid cultivars from Africa-Asia, namely *G. arboretum* and *G. herbaceum* (Wendel and Cronn, 2003). Of the four species, *G. hirsutum* (upland cotton) has dominated world cotton commerce, being responsible for 95% of the annual cotton, with approximate annual plantation of 35 million ha worldwide and grown in over 50 countries (Wilkins et al., 2000). Cotton is the world's most important natural textile fiber and an important source of feed, foodstuff, and oil, with approximate world consumption put at 27 million metric tons per year (Chen et al., 2007). The seed has approximately 25,000 cotton fibers, which are specialized single-celled trichomes that occur on the epidermal layer of the ovule (Wendel and Cronn, 2003; Chen et al., 2007). Cotton production provides income for approximately 180 million people (Benedict and Altman, 2001), with the fiber industry producing $30 billion worth of raw cotton and its economic impact estimated to be approximately $500 billion/year worldwide (Chen et al., 2007). China is the largest producer of cotton followed by the United States, which grows cotton worth $6 billion/year for fiber and approximately $1 billion/year for cottonseed oil and meal, on 12 million acres (Chen et al., 2007). Additionally, cotton is a major economic driver for some developing countries like India, Pakistan and Uzbekistan, among others (Wendel and Cronn, 2003). However, yield and quality of this all-important crop have declined over the last decade due largely to general erosion in genetic diversity of cotton varieties (Meredith,

*Corresponding author. E-mail: odun_wole@yahoo.co.uk. Tel: +234-8130928965.

2000). Hence, it was rational that the genetic improvement of this high-valued multi-purpose crop would not only enhance the nutrition and livelihoods of millions of people in food challenged economies but also that its natural fiber would be competitive with petroleum derived synthetic fibers (Chen et al., 2007).

This review discusses the narrow genetic base in cotton with respect to the progress made through conventional breeding as well as its limitation as a means of genetic improvement. It then discusses the several approaches being pursued to bring about the cotton genetic transformation as recourse for classical breeding techniques. Finally, it gives an overview of the underlying mechanisms of somatic embryogenesis as well as the current constraints and various strategies being used to overcome them; all of which have made this plant regeneration system one of the major potent vehicles for cotton transformation.

SUCCESS AND LIMITATION OF CONVENTIONAL BREEDING FOR COTTON GENETIC IMPROVEMENT

The agriculturally elite types of the most extensively cultivated cotton, *Gossypium hirsutum* have low level of genetic diversity (Ulloa, 2000; Gutiérrez et al., 2002), hence they have been target of several breeding efforts with respect to increasing the diversity (Saha, 2006). Conventional breeding programs through classical genetics have made great progress in cotton improvement, over the years, with respect to fiber yield and quality, even though there is still a negative correlation between the two (Shen et al., 2005). The cotton breeding strategies, which include varietal breeding and interspecific introgression have, however, reached their limits of applicability due partly to sexual incompatibility between some cultivars and wild cotton (Fu et al., 2009) and worse still, due to narrow genetic base because of non-availability of wild species containing desired traits (Kumria et al., 2003a). The narrow genetic base in cotton, in particular, has made the more promising and more robust marker assisted selection-aided interspecific introgression of multigenic traits into the elite cultivars of little relevance. Meanwhile, protoplast fusion method has been explored recently, with certain degree of success, to overcome the incompatibility challenge between wild and cultivated cotton species (Sun et al., 2004; Sun et al., 2005a; 2005b; Yang et al., 2007a; Fu et al., 2009). However, the major factors influencing cotton yield, which include pathogen and insect attacks as well as environmental stresses still remain elusive for conventional breeding and protoplast fusion, largely because of the lack of genetic resources that are adaptable to these approaches.

The foregoing hence form the basis for employing genetic transformation approaches for the genetic improvement of the four world cotton species, especially.

IN VITRO REGENERATION AND *IN PLANTA* STRATEGIES FOR COTTON GENETIC TRANSFORMATION

The genetic transformation of most crop species, including cotton has been majorly through *in vitro* plant regeneration approaches using tissue culture method. Cotton transformation in particular was originally based on the somatic embryogenesis (SE) pathway-mediated protocol of Umbeck et al. (1987) and Firoozabady et al. (1987), even though the approach is characterized by long culture period and is applicable to limited numbers of cultivars (Kumria et al., 2003a; Obembe et al., 2010; Wilkins et al., 2000). The prolonged culture duration, in particular, has potential problems of somaclonal variation, increased difficulty of plant regeneration, and plant infertility (Duncan, 2010). These limitations have inspired several research efforts including the use of shoot organogenesis (ORG). Various techniques have been employed to achieve *in vitro* organogenesis in cotton, which include direct organogenesis from pre-existing meristems (Hazra et al., 2001; Sharma et al., 2007; Ozyigit and Gozukirmizi, 2008; Farahani et al., 2010; Yang et al., 2010; Obembe et al., 2011) and indirect organogenesis via callus phase (Efe, 2005; Ganesan and Jayabalan, 2005). To this extent, there are various cotton transformation methodologies in the literature that have used the ORG approach successfully (Hazra et al., 2001; Satyavathi et al., 2002; Ouma et al., 2004; Tohidfar et al., 2005; Balasubramani et al., 2005; Yuceer and Koc, 2006). Even though plant regeneration is more convenient and faster with the ORG approach, however, in many cases when coupled to transformation procedures, it is time consuming, labor intensive and frequently generates chimeric plants that are not transformed in germ-line cells (Duncan, 2010). In addition, low transformation efficiency of 0.5-1% has been reported for this approach when coupled to either *Agrobacterium*-mediated- (Katageri et al., 2007) or particle bombardment transformation (Aragão et al., 2005; Rech et al., 2008). However this approach has an inherent potential of being applicable to a wide range of genotypes (Duncan, 2010).

An alternative approach that avoids the tissue culture platform for cotton transformation is the *in planta* platform via pollen or pollen tube pathway transformation (Li et al., 2004; Aragão et al., 2005; Zhang et al., 2009; Chen et al., 2010). It is envisaged that transforming a cotton gamete would be one means to avoid the chimeric issues associated with meristem transformations and may possibly be the most rapid means to produce transgenic plants from a wide variety of cotton (Duncan, 2010). However, gamete transformation of cotton is still labor intensive, time consuming and requires significant space for growing mature cotton plants and for isolating them from other plants during and after a transformation, as such for it to be considered as practical options for routine production of transgenic cotton plants, it will also

need to become an easier and a much more efficient procedure (Duncan, 2010).

The *in vitro* regeneration system that is based on the SE pathway gives rise to somatic embryos, which are of single cell origin; an advantage that is now being exploited for cotton transformation in different cultivars (Ganesan et al., 2006; Kouakou et al., 2007; Wang et al., 2008; Wu et al., 2009). Apart from the advantage of its single cell origin, the SE approach also has a more scale-up capacity for use in large scale vegetative propagation. Additionally, in most cases, the somatic embryos or the embryogenic cultures can be cryopreserved, which makes it possible to establish gene banks (von Arnold et al., 2002). As such, SE promises to be one of the very vital components of cotton transformation. The first report of cotton regeneration via the SE was by Davidonis and Hamilton (1983), who obtained somatic embryos from immature cotyledons of cultivar Coker310. This feat was followed by the results of Rangan (1984) in which they reported cotton SE and plant regeneration from non-Coker cultivars (Rangan et al., 1984). The afore-mentioned genotype-dependence in cotton SE was first reported by Trolinder and Xhixian (1989) and has continued to generate great research interest to date. Other landmark advances in cotton SE, regeneration and transformation are summarized in Table 1.

COTTON SE AND TRANSFORMATION- THE PROTOCOL

The first reports of cotton transformation (Umbeck et al., 1987; Firoozabady et al., 1987), which were based on SE pathway involved *Agrobacterium*-cocultivation of cotyledonary and hypocotyl explants of cotton and subsequent selection of transformation events on kanamycin-containing Murashige-Skoog (MS) media (Murashige and Skoog, 1962). The addition of cefotaxime or carbenicillin to the medium was necessary to control the growth of the bacterial cells. The tissue culture procedure involved the addition of 100 mg L^{-1} myo-inositol, 0.4 mg L^{-1} thiamine HCl and glucose to reduce tissue browning, and a combination of an auxin and a cytokinin, e.g. 2 mg L^{-1} naphthaleneacetic acid (NAA) and 0.5 mg L^{-1} benzyladenine (BA) (Smith et al., 1977), to induce callus formation. Even though the two traditionally used tissues (cotyledons and hypocotyls) are the most widely used explant sources for cotton SE, other cotton tissues including immature zygotic embryos (Hussain et al., 2004), roots (Sun et al., 2005c) as well as leaves and petioles (Trolinder and Goodin, 1988a; Zhang et al., 2000; Kumria et al., 2003b) and even protoplasts (Sun et al., 2005a, b) have been used successfully to generate somatic embryos. The well-developed callus could then be moved to hormone-free embryogenic-callus induction medium, which would develop into proembryo masses, which would in turn develop into embryos after 3-4 subcultures and eventually into plants when placed on Stewart and Hsu's (1977) low salt medium, specially formulated for cotton embryo germination and seedling development.

The work of Leelavathi et al. (2004), which involved Agrobacterium-mediated transformation of established embryogenic callus (EC) has been able to reduce the time between the introduction of DNA into cotton cells and the regeneration of transgenic plants by six months (Wu et al., 2005). Nonetheless, neither did it reduce the overall time that the tissues were in culture nor increase the number of cotton cultivars that can be transformed (Duncan, 2010). This modified protocol, which is now being routinely used, consists in making several subcultures of the initial EC to produce a rapidly growing, friable, loose white to yellow callus (Leelavathi et al., 2004; Jin et al., 2005; Wu et al., 2005, 2008). This fast growing EC was exposed to A. tumefaciens with (Jin et al., 2005; Wu et al., 2008) or without (Leelavathi et al., 2004; Wu et al., 2005) acetosyringone induction of the bacteria and then placed on kanamycin-containing medium with several subcultures to facilitate embryo induction and maturation.

In addition to *Agrobacterium*-mediated transformation, the EC (as suspension culture) is also compatible with other transformation techniques such as particle bombardment (Finer and McMullen, 1990), silicon carbide micro-fibers (WHISKERS™) (Beringer et al., 2004) and chemically induced direct transformation (Sawahel, 2001). The particle bombardment was carried out on an embryogenic suspension of Coker 310 and resulted in hygromycin resistant cotton plants while the WHISKERS™ strategy involved vigorous agitation of suspended pro-embryo masses with silicon carbide micro-fibers coated with DNA containing the PAT gene, which resulted in herbicide resistant plants. Similarly, the chemically induced direct transformation consists in exposing embryogenic cotton suspension to MS medium containing 0.25 M spermidine and the polycation hexadimethrine bromide and the DNA containing the hpt and the gus genes and selection with hygromycin, which resulted in the identification of transgenic cells (Sawahel, 2001). The use of liquid phase for establishing cotton embryogenic suspension cultures has been touted as having potential to reduce the length of time to produce somatic embryos (Sakhanokho et al., 2004; Ganesan and Jayabalan, 2005). Besides, the possibility of cryo-preserving the established cultures could further reduce the amount of work involved in the overall process, as such embryogenic cultures could be maintained for multiple transformation projects (Beringer et al., 2004).

THE PHENOMENON OF SE

SE occurs in nature as one of the evolutionary strategies for asexual embryogenesis, to overcome various environmental and genetic factors that prevent fertilization

Table 1. Major landmarks in cotton embryogenesis, plant regeneration and transformation.

Year	Major advance	Reference
1979	First demonstration of somatic embryogenesis in cotton	Price and Smith (1979)
1983	First successful regeneration of whole plants from somatic embryos using immature cotyledons of cultivar Coker310	Davidonis and Hamilton (1983)
1983	Report on the need to withdraw plant growth regulators at the onset of embryogenic callus (EC) proliferation	Gayle and Hamilton (1983)
1984	First report of SE from non-Coker cultivars and using explants other than the cotyledons	Rangan et al. (1984)
1987	First report on genetic transformation of cotton (Coker210)	Firoozabady et al. (1987)
1987	First report on genetic transformation of commercial varieties of cotton	Umbeck et al. (1987)
1988	Somatic embryos development in auxin-free glutamine-fortified liquid medium	Finer (1988)
1988	2,4-D and kinetin were recommended for initiation and maintenance of EC	Trolinder and Goodin (1988a, 1988b)
1989	First report on the genotype-dependence in cotton regeneration via SE pathway	Trolinder and Xhixian (1989)
1990	First report on cotton transformation via particle bombardment of embryogenic suspension culture	Finer and McMullen (1990)
1994	The first report on protoplast-to-plant via the formation of EC	Peeters et al. (1994)
1996	Succesuful regeneration of cotton plants from cryo-preserved embyogenic callus	Rajasekaran (1996)
1996	First report of plant regeneration of a wild species *G. Klotzschianum* A.	Zhang et al. (1996)
1999	First major attempt of developing SE-based plant regeneration protocols for broadening the range of regenerable cotton lines	Sakhanokho et al. (1998)
2000	High frequency stable transformation of cotton using embryogenic cell suspension cultures	Rajasekaran et al. (2000)
2001	First demonstration of expression of GFP in cotton that revealed the timing and localization of transient transformation events.	Sunilkumar and Rathore (2001)
2004	Rapid *Agrobacterium*-mediated transformation of cotton using EC	Leelavathi et al. (2004)
2008	Enhanced *Agrobacterium*-mediated transformation of using EC	Wu et al. (2008)
2010	High-frequency regeneration and efficient *Agrobacterium*-mediated transformation via somatic embryogenesis commercial cotton	Khan et al. (2010)

(von Arnold et al., 2002). Active cell divisions in the cultured explants result in a callus, in which shoot buds differentiate, this callus is called organogenic. An intermediate region of callus cells always separates the shoot bud and root meristem. The two together subsequently give rise to a new plant after establishing vacuolar connections between them (Merkle et al., 1995). In SE either single cells of the explants give rise to an embryo like structure directly without any intervening callus stage (direct SE) or such embryo-like structures are produced often from single cells of the callus derived from the explants (indirect SE), such callus is called embryogenic callus (EC) (Merkle et al., 1995).

SE is an ideal system to investigate the process of differentiation in plants and particularly zygotic embryogenesis (ZE) because zygotic embryos are encased by maternal tissues and are difficult to access using biochemical and molecular tools. For simplicity, SE may be defined as a process in which bipolar structures resembling zygotic embryos develop from somatic (non-zygotic) cells with independently originating vascular connections. SE is a multi-step regeneration process starting with the formation of proembryogenic cell mass, followed by somatic embryo induction, their maturation, dessication and finally plant regeneration (von Arnold et al., 2002). The formation of somatic embryos involves an induction- and an expression phase. The induction phase consists in the acquisition of embryogenic competence by the differentiated somatic cells and proliferation as embryogenic cells. In the expression phase, the embryogenic cells display their embryogenic competence and differentiate to form somatic embryos (Jiménez, 2001). It is important to mention that the development of somatic embryos resembles closely that of zygotic embryos both morphologically and temporally. These similarities include polarity and asymmetric cell division, pattern formation, meristem formation, maturation (globular, heart-shaped and torpedo) and germination. Nonetheless, the two phenomena are triggered by different mechanisms; whereas the process of ZE commences as a result of the formation of the zygote following sexual fertilization, the somatic cells acquire embryogenic competence as a result of different chemical and physical stimuli that trigger the expression of specific genes (von Arnold et al., 2002). The fact that structurally and developmentally normal embryos can develop from somatic cells demonstrates that the genetic program for embryogenesis is completely contained within the cell and can function without the influence of the gene products from the maternal environment (von Arnold et al., 2002). During this transition from the somatic to the embryogenic state, cells have to dedifferentiate, activate their cell division cycle and reorganize their physiology, metabolism and gene expression patterns (Fehér et al., 2003). The unraveling of the in depth molecular basis for this sequence of coordinated events has been an interesting research focus, which could ease the process of cotton SE and transformation.

MOLECULAR GENETIC BASIS OF SE

Efforts have been made in the past few years to unravel the molecular genetic basis of SE, which have produced evidence of involvement of few genes that may serve as embryogenic markers. For example, the over-expression of SERK (Somatic embryogenesis receptor kinase) gene gave a 3- to 4-fold increase in embryogenic competence, indicating that the gene does not only mark embryogenic competence but also promotes the transition of somatic cells to an embryogenic state (Hecht et al., 2001). Others include the LEC and LEC-related genes such as FUS3, that were identified as loss of function mutations resulting in defects in both embryo identity and seed maturation process in *Arabidopsis* and which play a central role in controlling many aspects of SE (Kwong et al., 2003; Gaj et al., 2005; Kwaaitaal et al., 2005), the BBM (babyboom) gene, which was isolated from microspore embryo culture of *Brassica napus* (Boutilier et al., 2002) and was found to bypass the requirement for plant growth regulators to induce embryogenesis (Souter and Lindsey, 2000). Also identified are the AGL15 gene (AGAMOUS like 15), which is expressed in response to auxin treatment (Zeng et al., 2006), the WUS gene (Wuschel), which promotes vegetative to embryonic transition from different plant organs of *Arabidopsis* in the presence of auxin (Zuo et al., 2002) and the PKL (PICKLE) gene, which qualifies as the master regulator of embryogenesis (Karami et al., 2009), as it ensures that traits expressed during embryogenesis and seed formation are not expressed after germination (Ogas et al., 1999). There is enough evidence as discussed in the next section, which validates the activation of these genes by certain external signaling stimuli.

GENETIC REGULATION OF THE EMBRYOGENIC PATHWAY

The induction of embryogenic pathway involves a reprogramming of gene expression from the original pattern in certain responsive cell to an embryogenic gene expression program through a signaling cascade, which turns on and off the expression of specific genes (for comprehensive reviews see (von Arnold et al., 2002; Fehér et al., 2003; Karami et al., 2009). As such, DNA methylation by auxins had been proposed as a plausible mechanism for downregulation of the existing gene expression, as it has been shown in many reports that the formation of an embryogenic cell is related to nuclear DNA methylation in the presence of 2,4 dichlorophenoxy acetic acid (2,4-D), an auxin (Xiao et al., 2006; Legrand et al., 2007). It has also been established that the establishment of auxin synthesis and polar auxin

transport is a key step in meristem formation underlying embryo development (Nawy et al., 2008). Additionally, auxins and stress have also been implicated in mediating the signal transduction cascade leading to this reprogramming of gene expression, which results in induction and proliferation of embryogenic cells in the auxin-sensitive cells (Dudits et al., 1991). The cascade involves the binding of auxin to an auxin-receptor protein TRANSPORT INHIBITOR RESPONSE 1 (TIR1) (Dharmasiri et al., 2005; Kepinski and Leyser, 2005) leading to the forming of a complex with and the eventual degradation of certain auxin/indole-3-acetic acid (Aux/IAA) repressor proteins through the action of the ubiquitin protein ligase SCFTIR1 (Woodward and Bartel, 2005). The repressor proteins are believed to block the auxin response factors, thereby allowing transcriptional activation (Woodward and Bartel, 2005). Available evidence suggests that 2,4-D, which is also a strong herbicide and associated with up-regulation of oxidative stress and defense genes, functions as a stress substance rather than a phytohormone, triggering the acquisition of embryogenic competence by plant cells (Thibaud-Nissen et al., 2003; Sharma et al., 2008).

One of the genes implicated in the transition of somatic to embryogenic state, WUS gene has been shown to be activated by cytokinins for the regulation of the stem cells in the apical meristem (Leibfried et al., 2005), however, a similar signal transduction cascade involving the cytokinins remains to be elucidated. Meanwhile, abscisic acid (ABA) and giberellic acid (GA) have been proposed to be involved in auxin-like signaling cascade (Gazzarrini and McCourt, 2003; Kobayashi et al., 2005; Ueguchi-Tanaka, 2005). Since ABA is known to increase after various stresses, it has been proposed that increased levels of ABA might induce SE (Satoh et al., 1986). However, the genetic and biochemical evidence for the involvement of ABA in the induction and development of plant embryogenesis remain to be elucidated (Karami and Saidi, 2010). Another important gene implicated in SE is the SOMATIC EMBRYO RELATED FACTOR1 (MtSERF1), which is induced by ethylene and expressed in embryogenic calli (Nakano et al). The gene has been found to be a member of the gene family ethylene response element (ERF), whose members play an important role in hormone signal transduction, and interconnect different hormone pathways (Vogler and Kuhlemeier, 2003). Wu et al. (2009) have found several genes related to ethylene signaling, e.g. ACC oxidase 2, ACC oxidase3, EIN2, ethylene-responsive factor, and ethylene-responsive transcriptional co-activator, which are also implicated in cotton SE. Hence, the ability of a single cell to undergo somatic-to-embryonic transition leading to the formation of somatic embryos is genetically determined. This genetically determined embryogenic potential may allow the expression of embryogenic competence under appropriate conditions, which will result in the initiation of embryo development in response to an appropriate developmental signal in those cells only where the physiological conditions are favorable, which explains why only certain genotyoes and certain cells can go throught the whole process of SE (Fehér, 2008). Needless to say therefore that, the choice of suitable genotype is the pre-requisite condition for all other factors influencing SE, as will be discussed hereafter.

OTHER FACTORS INFLUENCING SE

In addition to genotype, the stage of development of the plant and the nature of the explant also contribute significantly to the embryogenic potential of several species (Litz and Gray, 1995). For instance, explants with high levels of endogenous auxin may be more responsive (Jiménez and Thomas, 2006), and may not require exogenous auxin, as discusses below, for induction (Ikeda-Iwai et al., 2003)

Exogenous application of auxin into the culture medium usually results in the formation of EC, even though auxin has to be absent for its further development into somatic embryos (Filonova et al., 2000). This then necessitates the transfer of the cultures to auxin-free medium at the onset of EC proliferation, especially when using synthetic auxin, such as 2,4-D, which does not easily breakdown. This important step is especially necessary for the activation of gene expression required for the transition to the heart stage of the embryo development (Zimmerman, 1993). The appropriate amount of auxin needed to elicit this embryogenic transition is usually determined empirically for each species and explant. As such, for different cotton cultivars, different auxin types including 2,4-D, α-Naphthalene acetic acid (NAA), 4-amino-3,5,6-trichloropicolinic acid (picloram), Indoleacetic acid (IAA), etc. have been used at varied concentrations to induce SE from different explants such as hypocotyls, cotyledons and protoplasts (Trolinder and Goodin,1988b; Kumria et al., 2003b; Wilkins et al., 2004; Wu et al., 2004; Ganesan and Jayabalan, 2005; Jin et al., 2005; Sun et al., 2006). 2,4-D has been adjudged the best auxin, as well as the principal controlling factor for induction of SE (Cheong and Pooler, 2004; Sharma et al., 2008), which is why it is being preferred over other plant growth regulators (PGRs), to such an extent that over 65% of the available reports on SE as of 2004 used it either alone or in combination with other PGRs (Gaj, 2004). It had also been long established that the Coker variety of *G. hirsutum* L. is the most highly embryogenic, as such it is now being used as the reference for determining embryonic competence in other cultivars and species.

The application of cytokinins for the formation of SE has also been reported severally, even though its treatment is largely dependent on the auxin type used. BAP has been sparingly used with 2,4-D for SE induction in cotton (Kumar and Tuli, 2004), however, available reports for cotton somewhat suggest parings that favors

kinetin and 2,4-D or NAA (Trolinder and Goodin, 1998a; Kumria et al., 2003a; Khan et al., 2010) and isopentenyladenine (2iP) and 2,4-D or NAA (Kumria et al., 2003a; Khan et al., 2006). Nonetheless, Zhang et al. (2001) were able to induce high frequency SE on medium supplemented with zeatin only.

Other PGRS being used for inducing SE include ABA, GA, ethylene and Thidiazuron (TDZ; N-phenyl-N-[1,2,3 thidiazolyl]-urea), even though there has not been any report on their use in cotton SE. Other factors influencing SE include stress, as such the introduction of stress inducing agents, such as the addition of Fe into the medium (Yoshimura et al., 2000; Pasternak et al., 2002), using one-fifth strength MS medium,) (Kumria et al., 2003b), using dark grown and etiolated hypocotyl tissue (Kouadio et al., 2004), tightly sealing Petri dishes of tissue with Parafilm™ (Leelavathi et al., 2004), introducing a brief regime of myo-inositol starvation (Kumar and Tuli, 2004) and a gradual desiccation with ventilation (Wu et al., 2008) have been reported to enhance development of somatic embryos and plant regeneration in cotton. In addition, external environment, which includes media type and composition (pH, nitrogen and carbon sources) and physical culture conditions (light, temperature) are also important determining factors for SE (Gaj, 2004; Jin et al., 2005). Of particular interest is the replacement of the traditional MS vitamins with Nitsch vitamins (Nitsch and Nitsch, 1969) (Divya et al. (2008) or with Gamborg's B5 vitamins (Gamborg et al. 1968) (Ganesan and Jayabalan, 2005), for the culture of different cotton cultivars. It is generally understood that it is the interaction between all of these factors that lead to the induction and expression of a specific mode of cell differentiation and development (Gaj, 2004).

LIMITATION AND OPTIMIZATION OF COTTON SE AND TRANSFORMATION

Despite the advances made so far in cotton genetic engineering, especially for creating new insect- and herbicide resistance lines, genotype-dependence and efficiency of the regeneration and transformation procedures remain the two limiting factors in the development of cotton (Wilkins et al., 2000). As such, this challenge has restricted the impact of biotechnology on cotton production, which had long been peaked in many regions of the world. Worse still, there has been an over-reliance on the obsolete varieties (Coker or Coker-derived) for biotechnological improvement of cotton (Agrawal et al., 1997; Sunilkumar and Rathore, 2001; Sakhanokho et al., 2001; Leelavathi et al., 2004). This lack of variability in genetically modified cotton could potentially contribute towards a narrow genetic base. To introduce a gene into commercially important cultivars, the standard international practice is to transform a Coker cultivar, produce plants that are homozygous for the gene inserted and then enter these plants into a backcross breeding program (Wilkins et al., 2000). This process delays the commercial release of transgenic cotton by several years (Rech et al., 2008). Hence, it is imperative to develop standard protocols of regeneration for a wide array of commercial varieties (Rajasekaran et al., 2001).

The challenge of genotype-dependence in cotton SE especially, is due in part to the enormous intra-varietal differences with respect to the sensitivity to embryogenesis induction factors and the capability to undergo SE (Jin et al., 2005; Kouakou et al., 2007). Consequently, several research strategies are being targeted at increasing the range of genotypes that are amenable to SE- and *Agrobacterium*-mediated transformation, by empirically formulating and devising new optimized media towards achieving a genotype-independent transformation and regeneration system in cotton (Mishra et al., 2003; Han et al., 2009), including over forty regional commercial varieties (Reichert et al., 1999; Zhang et al., 2000; Sakhanokho et al., 2001; Aydin et al., 2004; Rajasekaran et al., 2004; Wu et al., 2004; Poon et al., 2005; Chen et al., 2008; Zhang et al., 2009; Khan et al., 2010) and about fourteen wild (Finer et al., 1987; Sun et al., 2003; Sun et al., 2004; Rao et al., 2006; Sun et al., 2006; Wang et al., 2007; Yang et al., 2007b, Yan et al., 2010; Zhang et al., 2010).

Similarly, the efficiency of the cotton transformation system is also hinged on the regeneration regime that consists in a prolonged culture period, and which frequently features: excretion of secondary metabolites from the explants into the medium, browning of callus after a short period of culture, high frequency of abnormal embryo development, low frequency of somatic embryo maturation, low conversion rate of somatic embryos into plants, and a lack of shoot elongation (Kumar and Pental, 1998; Wilkins et al., 2000; Wilkins et al., 2004; Sun et al., 2006). Yet again, the strategies being used to overcome some of these problems are aimed at developing broad-range highly optimized protocols. Some of these include manipulating the media component by using diverse combinations of PGRs (Yang et al., 2007b; Wang et al., 2008; Han et al., 2009), adding various kinds of addenda such as inorganic salts ($MgCl_2$, KNO_3) (Kumria et al., 2003a; Lashari et al., 2008) and amino acids (Wu et al., 2004; Han et al., 2009), using maltose alone or in combination with glucose or sucrose (Kumria et al., 2003a; Sun et al., 2003; Sun et al., 2006), introducing inorganic- (Kumria et al., 2003a) or organic stress-inducing agents (Ganesan et al., 2004), or applying physical stress (by dehydration on filter papers and by adjusting the humidity) (Firoozabady and DeBoer, 1993; Wu et al., 2008). Furthermore, the presence of GA in the medium has been proven to greatly enhance shoot elongation of the germinating embryos and eventual rooting of the matured shoot (Sun et al., 2006), sometimes in the presence of activated charcoal (Zhang et al., 2001).

This step might be especially important considering the stringent transformation selection pressure of the antibiotic- or herbicide-containing medium on which the EC are generated, which might influence the vigor of the resulting somatic embryos. The entire conversion cycle of a somatic embryo to plant usually consists in a process of developmental changes that involves the formation of primary roots, a shoot meristem with a leaf primordium and greening of hypocotyls and cotyledons (Redenbaugh et al., 1986). The challenge of rooting, in particular, is being circumvented by a technique that was developed to graft transgenic plants onto cotton seedlings to rescue events that did not root (Jin et al., 2006; Zhu et al., 2006). The problem of low conversion rate of SE to plant has been the single most important factor influencing the efficiency of the system generally, and as such, it has attracted several different strategies for overcoming it. These strategies include the addition of other remotely used PGRs like the absicisic acid (ABA), indole butyric acid (IBA) or cytokinins (Madakadze and Senaratna, 2000; Sarasan et al., 2001) and other treatments such as chilling (Reidiboym-Talleux et al., 2000) or dessication of the somatic embryos alone (Chaudhary et al., 2003) or in combination with ABA (Hansen, 2000). Although there are contrary views as to whether or not abnormal embryo morphology affects development into plant (Park and Facchini, 2000; Gaj, 2001; Hussain et al., 2009), nonetheless, Griga (1998) reported that the presence of 10 µM TDZ greatly enhanced the conversion rate (70%) of the somatic embryos of pea exhibiting various morphology.

CONCLUDING REMARKS

Plant biotechnology obviously holds the future for the cotton industry. As such, the need to make cotton regeneration and transformation more robust cannot be overemphasized. One of the limiting factors of the systems, which is the SE pathway of plant regeneration has been under intense research focus over the years. The much sought-after breakthrough from this biotechnological intervention, which is to dramatically increase the range of cotton species that are amenable to transformation approaches, promises to be the key for reversing the dwindling trend in production. This therefore calls for more research interest from different groups around the world, especially where research activities on this all-important crop is currently absent. It equally calls for more encouragement on the part of those presently working on it to continue to strive for all-inclusive genoytype-independent regeneration and transformation procedure.

ACKNOWLEDGEMENT

Olawole O. Obembe would like to thank the International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy for sponsoring his Post-doctoral training at the Plant Transformation Laboratory of ICGEB, New Delhi Component, India. He is also indebted to the Management of the Covenant University, Ota Nigeria, for granting him study leave during the training period.

REFERENCES

Agrawal DC, Banerjee AK, Kolala RR, Dhage AB, Kulkarni AV, Nalawade SM, Hazra S, Krishnamurthy KV (1997). *In vitro* induction of multiple shoots and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep., 16: 647-652.

Aragão FJL, Vianna GR, Carvalheira SBRC, Rech EL (2005). Germ line genetic transformation in cotton (*Gossypium hirsutum* L.) by selection of transgenic meristematic cells with a herbicide molecule. Plant Sci., 168: 1227-1233.

Aydin Y, Ipekci Z, Talas OT, Zehir A, Bajrovic K, Gozukirmizi N (2004). High Frequency Somatic Embryogenesis in Cotton. Biol. Plant., 48: 491-495.

Balasubramani G, Amudha J, Mayee CD (2005). Genetic transformation of cotton (*G. hirsutum* L.) by Agrobacterium tumefaciens and regeneration by direct shoot organogenesis. Adv. Plant Sci., 18: 497-504.

Benedict JH, Altman DW (2001). Commercialization of transgenic cotton expressing insecticidal crystal protein. In: Jenkins JN, Saha S (eds) Genetic improvement of cotton. Science Publishers, Inc. Enfield, pp. 136-201.

Beringer J, Palta AM, Baker LW, Petolino JF (2004). Transgenic cotton via WHISKERS™-mediated transformation. Recent Res. Dev. Crop Sci., 1: 335-347.

Boutilier K, Offringa R, Sharma VK, Kieft H, Ouellet T, Zhang L (2002). Ectopic Expression of BABY BOOM Triggers a Conversion from Vegetative to Embryonic Growth. Plant Cell, 14: 1737-1749.

Chaudhary B, Kumar S, Prasad KV, Oinam GS, Burma PK, Pental D (2003). Slow desiccation leads to high-frequency shoot recovery from transformed somatic embryos of cotton (*Gossypium hirsutum* L. cv. Coker 310 FR). Plant Cell Rep., 21: 955-960.

Chen TZ, WU SJ, Li FF, Guo WZ, Zhang TZ (2008). *In Vitro* Regeneration of Four Commercial Cotton Cultivars (*Gossypium hirsutum L.*) Grown in Xinjiang, China Acta Agron. Sin., 34: 1374-1380.

Chen TZ, Wu SJ, Zhao J, Guo WZ, Zhang TZ (2010). Pistil drip following pollination: a simple *in planta Agrobacterium*-mediated transformation in cotton. Biotechnol. Lett., 32: 547-555.

Chen ZJ, Scheffler BE, Dennis E, Triplett BA, Zhang T, Guo W, Chen X, Stelly DM, Rabinowicz PD, Town CD, Arioli T, Brubaker C, Cantrell RG, Lacape JM, Ulloa M, Chee P, Gingle AR, Haigler CH, Percy R, Saha S, Wilkins T, Wright RJ, Van DA, Zhu Y, Yu S, Abdurakhmonov I, Katageri I, Kumar PA, Mehboob UR, Zafar Y, Yu JZ, Kohel RJ, Wendel JF, Paterson AH (2007). Toward Sequencing Cotton (Gossypium) genomes. Plant Physiol., 145: 1303-1310.

Cheong EJ, Pooler MR (2004). Factors affecting somatic embryogenesis in *Prunus incisa* cv. February Pink. Plant Cell Rep., 22: 810-815.

Davidonis GH, Hamilton RH (1983). Plant regeneration from callus tissue of *Gossypium hirsutum* L. Plant Sci. Lett., 32: 89-93.

Dharmasiri N, Dharmasiri S, Estelle M (2005). The F-box protein TIR1 is an auxin receptor. Nature, 435: 441-445.

Divya K, Anuradha TS, Jami SK, Kirti PB (2008). Efficient regeneration from hypocotyls explants in three cotton cultivars. Biol. Plant, 52: 201–208.

Dudits D, Bogre L, Gyorgyey J (1991). Molecular and cellular approaches to the analysis of plant embryo development from somatic cells *in vitro*. J. Cell Sci., 99: 475-484.

Duncan DR (2010). Cotton Transformation. In Usha Barwale Zehr (ed.) Cotton Biotechnological Advances, In Widholm JM, Lorz H, Nagata T(eds.) Biotechnology in Agriculture and Forestry, Springer-Verlag

Berlin. 65:65-77. ISBN 978-3-642-04795-4 e-ISBN 978-3-642-04796-1.
Efe L (2005). Callus formation and plant regeneration from two cotton species (*Gossypium hirsutum* L., and *G. barbadense* L.). Pak. J. Bot., 37: 227-236.
Farahani F, Yahyazadeh F, Sheidai M (2010). Optimization of *in vitro* direct regeneration of multiple shoots in upland cotton (*Gossypium hirsutum* L.) cv. Iranian varieties. Afr. J. Agric. Res., 5: 1304-1309.
Fehér A, Pasternak TP, Dudits D (2003). Transition of somatic plant cells to an embryogenic state. Plant Cell Tiss. Org. Cult., 74: 201-228.
Filonova L, Bozhkov P, Von AS (2000). Developmental pathway of somatic embryogenesis in *Picea abies* as revealed by time-lapse tracking. J. Exp. Bot., 51: 249-264.
Finer JJ (1988). Plant regeneration from somatic embryogenesis suspension culture of cotton (*Gossypium hirsutum* L.). Plant Cell Rep., 7: 399-402.
Finer JJ, McMullen MD (1990). Transformation of cotton (Gossypium hirsutum L.) via particle bombardment. Plant Cell Rep., 8: 586-589.
Finer JJ, Reilley AA, Smith RH (1987). Establishment of embryogenic suspension cultures of a wild relative of cotton (*Gossypium klotzschianum* Anderss.). *In vitro* Cell Dev. Biol. Plant, 23: 717-722.
Firoozabady E, DeBoer DL (1993). Plant regeneration via somatic embryogenesis in many cultivars of cotton (*Gossypium hirsutum* L.). *In Vitro* Cell Dev. Biol. Plant, 29: 166–173.
Firoozabady E, DeBoer DL, Merlo DJ, Halk EL, Amerson LN, Rashka KE, Murray EE (1987). Transformation of cotton (*Gossypium hirsutum* L.) by *Agrobacterium tumefaciens* and regeneration of transgenic plants. Plant Mol. Biol., 10: 105-116.
Fu LL, Yang XY , Zhang XL, Wang Z, Feng C, Liu C, Jiang PY, Zhang JL (2009). Regeneration and identification of interspecific asymmetric somatic hybrids obtained by donor-recipient fusion in cotton. Chin. Sci. Bull., 54: 3035-3044.
Gaj MD (2001). Direct somatic embryogenesis as a rapid and efficient system for in vitro regeneration of *Arabidopsis thaliana* (L.) Heynh. Plant Cell Tiss. Org. Cult., 64: 39-46.
Gaj MD (2004). Factors influencing iomatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.) Heynh. Plant Growth Regul., 43: 27-47.
Gaj MD, Zhang SB, Harada JJ, Lemaux PG (2005). Leafy cotyledon genes are essential for induction of somatic embryogenesis of *Arabidopsis*. Planta, 222: 977-988.
Gamborg OL, Miller RA, Ojima K (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell. Res., 50: 151-158.
Ganesan M, Jayabalan N (2004). Evaluation of haemoglobin (erythrogen): for improved somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum* L. cv. SVPR 2). Plant Cell Rep., 23: 181-187.
Ganesan M, Jayabalan N (2005). *In vitro* plant regeneration from the callus of shoot tips in cotton (*Gossypium hirsutum* L. cv. SVPR 2). Iranian J. Biotechnol., 3: 144-151.
Ganesan M, Jayabalan N (2006). Isolation of disease-tolerant cotton (*Gossypium hirsutum* L. cv. SVPR 2) plants by screening somatic embryos with fungal culture filtrate. Plant Cell Tiss. Org. Cult., 87: 273-284.
Gayle HD, Hamilton HR (1983). Plant regeneration from callus tissue of *Gossypium hirsutum* L. Plant Sci. Lett., 3: 89-93.
Gazzarrini S, McCourt P (2003). Cross-talk in plant hormone signalling: what *Arabidopsis* mutants are telling us. Ann. Bot., 91: 605-612.
Griga M (1998). Direct somatic embryogenesis from shoot apical meristem of pea, and thidiazuran induced high conversion rate of somatic embryos. Biol. Plant, 41: 481-495.
Gutiérrez OA, Basu S, Saha S, Jenkins JN, Shoemaker DB, Cheatham CL, McCarty JC (2002). Genetic distance of cotton cultivars and germplasm lines based on SSR markers and its association with agronomic and fiber traits of their F2 hybrids. Crop Sci., 42: 1841-1847.
Han GY, Wang XF, Zhang GY, Ma ZY (2009). Somatic embryogenesis and plant regeneration of recalcitrant cottons (*Gossypium hirsutum*). Afr. J. Biotechnol., 8: 432-437.
Hansen M (2000). ABA treatment and desiccation of microsporederived embryos of cabbage (*Brassica oleracea* ssp. Capitata L.) improves plant development. J. Plant Physiol., 156: 164-167.
Hazra S, Agrawal DC, Banerjee AK, Krishnamurthy KV, Nalawade SM (2001). Induction of multiple shoots and plant regeneration from 'accessory buds' of nodal Segments from field-grown mature cotton plants (*Gossypium hirsutum* l.). *In vitro* Cell Dev. Biol. Plant, 37: 830-834.
Hecht V, Vielle CJP, Hartog MV, Schmidt EDL, Boutilier K, Grossniklaus U, De VSC (2001). The *Arabidopsis* SOMATIC Embryogenesis Receptor Kinase 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. Plant Physiol., 127: 803-816.
Hussain SS, Husnain T, Riazuddin S (2004). Somatic embryo germination and plant development from immature zygotic embryos in cotton. Pak. J. Biol. Sci., 7: 1946-1949.
Hussain SS, Rao AQ, Husnain T, Riazuddin S (2009). Cotton somatic embryo morphology affects its conversion to plant. Biol. Plant, 53: 307-311.
Ikeda IM, Umehara M, Satoh S, Kamada H (2003). Stress-induced somatic embryogenesis in vegetative tissues of *Arabidopsis thaliana*. Plant J., 34: 107-114.
Jiménez V, Thoma C (2006). Participation of plant hormones in determination and progression of somatic embryogenesis. In: Mujid A, Samaj J, (eds). Somatic embryogenesis. Plant Cell Monograps, vol 2, Robin-son DG series ed., Springer-Verlag, Berlin Heidelberg, Germany, pp. 103-118.
Jimenez VM (2001). Regulation of *in vitro* somatic embryogenesis with emphasis on to the role of endogenous hormones.Rev. Physiol. Bra. VEGLA, 13: 196-223.
Jin S, Liang S, Zhang X, Nie Y, Guo X (2006). An efficient grafting system for transgenic plant recovery in cotton (*Gossypium hirsutum* L.). Plant Cell Tiss. Org. Cult., 85: 181-185.
Jin S, Zhang X, Liang S, Nie Y, Guo X, Huang C (2005). Factors affecting transformation efficiency of embryogenic callus of upland cotton (*Gossypium hirsutum*) with *Agrobacterium tumefaciens*. Plant Cell Tiss. Org. Cult., 81: 229-237.
Karami O, Aghavaisi B, Pour AM (2009). Molecular aspects of somatic-to-embryogenic transition in plants. J. Chem. Biol., 2: 177-190.
Karami O, Saidi A (2010). The molecular basis for stress-induced acquisition of somatic Embryogenesis. Mol. Biol. Rep., 37: 2493-2507.
Katageri IS, Vamadevaiah HM, Udikeri SS, Khadi BM, Kumer PA (2007). Genetic transformation of an elite Indian genotype of cotton (*Gossypium hirsutum* L.) for insect resistance. Curr. Sci., 93: 1843-1847.
Kepinski S, Leyser O (2005). The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature, 435: 446-451.
Khan T, Reddy VS, Leelavathi S (2010). High-frequency regeneration via somatic embryogenesis of an elite recalcitrant cotton genotype (*Gossypium hirsutum* L.) and efficient *Agrobacterium*-mediated transformation. Plant Cell Tiss. Org. Cult., 101: 323-330.
Khan T, Singh AK, Pant RC (2006). Regeneration via somatic embryogenesis and organogenesis in different cultivars of cotton (*Gossypium* spp.). *In vitro* Cell Dev. Biol. Plant, 42: 498-501.
Kobayashi Y, Murata M, Minami H, Yamamoto S, Kagaya Y, Hobo T, Yamamoto A, Hattori T (2005). Abscisic acid-activated SNRK2 protein kinases function in the gene-regulation pathway of ABA signal transduction by phosphorylating ABA response element-binding factors. Plant J., 44: 939-949.
Kouakou TH, Waffo TP, Kouadio YJ, Valls J, Richard T, Decendit A, Mérillon JM (2007). Phenolic compounds and somatic embryogenesis in cotton (*Gossypium hirsutum* L.) Plant Cell Tiss. Org. Cult., 90: 25-29.
Kumar M, Tuli R (2004). Plant regeneration in cotton: A short-term inositol starvation promotes developmental synchrony in somatic embryogenesis. *In vitro* cell Dev. Biol. Plant, 40: 294-298.
Kumar S, Pental D (1998). Regeneration of Indian cotton variety MCU-5 through somatic embryogenesis. Curr. Sci., 74: 538-540.
Kumria R, Leelavathi S, Bhatnagar RK, Reddy VS (2003a). Regeneration and Genetic transformation of Cotton: Present Status and Future Perspectives. Plant Tissue Cult., 13: 211-225.
Kumria R, Sunnichan VG, Das DK, Gupta SK, Reddy VS, Bhatnagar

RK, Leelavathi S (2003b). High-frequency somatic embryo production and maturation into normal plants in cotton (Gossypium hirsutum) through metabolic stress. Plant Cell Rep., 21: 635-639.
Kwaaitaal MA, De VSC, Russinova E (2005). *Arabidopsis thaliana* Somatic Embryogenesis Receptor Kinase 1 protein is present in sporophytic and gametophytic cells and undergoes endocytosis. Protoplasma, 226: 55-65.
Kwong RW, Bui AQ, Lee H, Kwong LW, Fischer RL, Goldberg RB, Harada JJ (2003). LEAFY COTYLEDON1-LIKE defines a class of regulators essential for embryo development. Plant Cell, 15: 5-18.
Lashari MI, Arshad M, Zafar Y, Asad S (2008). Optimization of zeatin and explant types for efficient embryogenesis and plant regeneration of diploid cotton (*Gossypium arboreum* L.). J. Agric. Res., 46: 1-13.
Leelavathi S, Sunnichan VG, Kumria R, Vijaykanth GP, Bhatnagar RK, Reddy VS (2004). A simple and rapid Agrobacterium-mediated transformation protocol for cotton (Gossypium hirsutum L.): embryogenic calli as a source to generate large numbers of transgenic plants. Plant Cell Rep., 22: 465-470.
Legrand S, Hendriks T, Hilbert JL, Quillet MC (2007). Characterization of expressed sequence tags obtained by SSH during somatic embryogenesis in *Cichorium intybus* L. BMC Plant Biol., 7: 27 doi:10.1186/1471-2229-7-27.
Leibfried A, To JPC, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU (2005). WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature, 438: 1172-1175.
Li X, Wang XD, Zhao X, Dutt Y (2004). Improvement of cotton fiber quality by transforming the acsA and acsB genes into *Gossypium hirsutum* L. by means of vacuum infiltration. Plant Cell Rep., 22: 691-697.
Litz RE, Gray DJ (1995). Somatic embryogenesis for agricultural improvement (1995). World J. Microbiol. Biotechnol., 11: 416-425.
Madakadze RM, Senaratna T (2000). Effect of growth regulators on maturation of geranium (*Pelargonium_hortorum*) somatic embryos. Plant Growth Regul., 30: 55-60.
Meredith WRJ (2000). Continued Progress for Breeding for Yield in the USA. In Kechagia U, Ed., Proceedings of World Cotton Research Conference II. Athens, Greece, pp. 97-101.
Merkle SA, Parrot WA, Flinn BS (1995). Morphogenic aspects of somatic embryogenesis. In: Thorpe TA, Ed. *In vitro* embryogenesis in plants. Dordrecht, Kluwer Academic Publishers, pp. 155-203.
Mishra R, Wang HY, Yadav NR, Wilkins TA (2003). Development of a highly regenerable elite Acala cotton (*Gossypium hirsutum* cv. Maxxa) - a step towards genotype-independent regeneration. Plant Cell Tiss. Org. Cult., 73: 21-35.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant, 15: 473-497.
Nakano T, Suzuki K, Fujimura T, Shinshi H (2006). Genomewide analysis of the ERF gene family in Arabidopsis and rice. Plant Physiol., 140: 411-432.
Nawy T, Lukowitz W, Bayer M (2008). Talk global, act local- patterning the *Arabidopsis* embryo. Curr. Opin. Plant Biol., 11: 28-33.
Nitsch JP, Nitsch C (1969). Haploid plants from pollen grains. Science, 169: 85.
Obembe OO, Khan T, Popoola JO, Singh AK, Pant AC (2010). Endogenous levels of reducing sugars, free amino acids and phenols during various stages of *in vitro* culture of cotton *(Gossypium* spp.). Int. J. Biol. Chem. Sci., 4: 2371-2378.
Obembe OO, Khan T, Popoola JO (2011). Multiple shoots induction from Indian elite cotton (*Gossypium* spp.) cultivars. Canadian J. Pure Appl. Sci., 6: 1385-1389.
Ogas J, Kaufmann S, Henderson J, Somerville C (1999). PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. Proc. Natl. Acad. Sci. USA, 96: 13839-13844.
Ouma JP, Young MM, Reichert NA (2004). Optimization of in vitro regeneration of multiple shoots from hypocotyl sections of cotton (*Gossypium hirsutum* L.). Afr. J. Biotechnol., 3: 169-173.
Ozyigit II, Gozukirmizi N (2008). High efficiency shoot and root formation from cotyledonary nodes of cotton (*Gossypium hirsutum* L.). Pak. J. Bot., 40: 1665-1672.
Park SU, Facchini PJ (2000). High-efficiency somatic embryogenesis and plant regeneration in California poppy, *Eschscholzia californica* Cham. Plant Cell Rep., 19: 421-426.
Pasternak TP, Prinsen E, Ayaydin F, Miskolczi P, Potters G, Asard H, Van OHA, Dudits D, Fehér A (2002). The Role of auxin, pH, and stress in the activation of embryogenic cell division in leaf protoplast-derived cells of Alfalfa. Plant Physiol., 129: 1807-1819.
Peeters JM, Turnbull PCB, Gibson JR (1994). Protolast to plant regeneration in cotton (*Gossypium hirsutum* L. cv. Coker312), using feeder layers. Plant Cell Rep., 13: 208-211.
Poon S, Heath RL, Clarke AE (2005). Arabinogalactan Protein Compositions and Methods for Fostering Somatic Embryogenic Competence.- US Patent App. Patent WO/2005/095, 625, 2005.
Price HJ, Smith RH (1979). Somatic embryogenesis in suspension cultures of *Gossypium klotzschiaanum* Anderss. Planta, 145: 305-307.
Rajasekaran K (1996). Regeneration of plants from cryo-preserved embryogenic cell suspension and callus cultures of cotton. Plant Cell Rep., 15: 859-864.
Rajasekaran K, Chlan CA, Cleveland TE (2001). Tissue culture and genetic transformation of cotton. In: Jenkins JN, Saha S (eds) Genetic Improvement of Cotton. Science Publishers, Inc. Enfield, pp. 269-290.
Rajasekaran K, Hudspeth RL, Cary JW, Anderson DM, Cleveland TE (2000). High frequency stable transformation of cotton (*Gossypium hirsutum* L.) by particle bombardment of embryogenic cell suspension cultures. Plant Cell Rep., 19: 539-545.
Rajasekaran K, Sakhanokho HF, Zipf A, Saha S, Sharma GC, Peng WC (2004). Somatic embryo initiation and germination in diploid cotton (*Gossypium arboreum* L.). *In vitro* Cell Dev. Biol. Plant, 40: 177-181.
Rangan TS, Zavala T, Ip A (1984). Somatic embryogenesis in tissue culture of *Gossypium hirsutum* L. *In vitro*, 20: 256.
Rao AQ, Hussain SS, Shahzad MS, Bokhari SYA, Raza MH, Rakha A, Majeed A, Shahid AA, Saleem Z, Husnain T, Riazuddin S (2006). Somatic embryogenesis in wild relatives of cotton (*Gossypium* Spp.) J Zhejiang Univ. Sci. B, 7: 291-298.
Rech EL, Vianna GR, Aragão FJL (2008). High-efficiency transformation by biolistics of soybean, common bean and cotton transgenic plants. Nat. Protocols, 3: 410-418.
Redenbaugh K, Paasch BD, Nichol JW, Kossler ME, Viss PR, Walker KA (1986). Somatic seeds: encapsulation of sexual plant embryos. Bio/Technol., 4: 797-801.
Reichert NA, Lim TK, Young MM (1999). Method for transformation of cotton and kenaf and organogenic regeneration- US Patent, 5: 998, 207.
Reidiboym TL, Souridioux M, Grenier E, Grenier DMG (2000). Lipid composition of somatic and zygotic embryos from *Prunus avium*. Effect of cold treatment on somatic embryo quality. Physiol. Plant, 108: 194-201.
Saha S, Jenkins JN, Wu J, McCarty JC, Gutiérrez OA, Percy RG, Cantrell RG, Stelly DM (2006). Effects of Chromosome-Specific Introgression in Upland Cotton on Fiber and Agronomic Traits. Genetics, 172: 1927-1938.
Sakhanokho HF, Saha S, Sharna GC, Zipf A, Rajasekaran K (1998). Tissue culture potential of diverse diploid and tetraploid cotton genotypes. In 1998 Proc. Beltwide Cotton Conf., pp. 590-593, Jan 5-9. San Diego, CA USA.
Sakhanokho HF, Zipf A, Rajasekaran K, Saha S, Sharma GC (2001). Induction of Highly Embryogenic Calli and Plant Regeneration in Upland (*Gossypium hirsutum* L.) and Pima (*Gossypium barbadense* L.) Cottons. Crop Sci., 41: 1235-1240.
Sakhanokho HF, Zipf A, Rajasekaran K, Saha S, Sharma GC, Chee PW (2004). Somatic embryo initiation and germination in diploid cotton (*Gossypium arboreum* L.). *In vitro* Cell Dev. Biol., 40: 177-181.
Sarasan V, Roberts AV, Rout GR (2001). Methyl laurate and 6-benzyladenine promote the germination of somatic embryos of hybrid rose. Plant Cell Rep., 20: 183-186.
Satoh S, Kamada H, Harada H, Fujii T (1986). Auxin-controlled glycoprotein release into the medium of embryogenic carrot cells. Plant Physiol., 81: 931-933.
Satyavathi VV, Prasad V, Lakshmi BG, Sita GL (2002). High efficiency transformation protocol for three Indian cotton varieties via

Agrobacterium tumefaciens. Plant Sci., 162: 215-223.
Sawahel WA (2001). Stable genetic transformation of cooton plants using polybrene-spermidine treatment. Plant Mol. Biol. Rep., 19: 377a-377f.
Sharma SK, Millam S, Hedley PE, McNicol J, Bryan GJ (2008). Molecular regulation of somatic embryogenesis in potato: An auxin led perspective. Plant Mol. Biol., 68: 185-201.
Sharma M, Gill MS, Gosal SS, Sandhu JS (2007). Induction of multiple shoots and plant regeneration from apical tips of diploid cotton, *Gossypium arboreum* L. Indian J. Crop Sci., 2: 73-78.
Shen X, Guo W, Zhu X, Yuan Y, Yu JZ, Kohel RJ, Zhang T (2005). Molecular mapping of QTLs for fiber qualities in three diverse lines in Upland cotton using SSR markers. Mol. Breed, 15: 169-181.
Smith RH, Jamesprice H, Thaxton JB (1977). Defined conditions for the initiation and growth of cotton callus *in vitro I. Gossypium arboreum*. *In Vitro* Cell Dev. Biol., 13: 329-334.
Souter M, Lindsey K (2000). Polarity and signalling in plant embryogenesis. J. Exp. Bot., 51: 971-983.
Stewart JM, Hsu CL (1977). In-ovulo embryo culture and seedling development of cotton (*Gossypium hirsutum* L.). Planta, 137: 113-117.
Sun Y, Zhang X, Huang C, Guo X, Nie Y (2006). Somatic embryogenesis and plant regeneration from different wild diploid cotton (*Gossypium*) species. Plant Cell Rep., 25: 289-296.
Sun Y, Zhang X, Huang C, Nie Y, Guo X (2005a). Plant regeneration via somatic embryogenesis from protoplasts of six explants in Coker201 (*Gossypium hirsutum*). Plant Cell Tiss. Org. Cult., 82: 309-315.
Sun Y, Zhang X, Huang C, Nie Y, Guo X (2005c). Factors influencing *in vitro* regeneration from protoplasts of wild cotton (*G. klotzschianum A*) and PARD analysis of regenerated plantlets. Plant Growth Regul., 46: 79-86.
Sun YQ, Nie YC, Guo XP, Huang C, Zhang X (2006). Somatic hybrids between *Gossypium hirsutum* L. (4×) and *G. davidsonii* Kellog (2×) produced by protoplast fusion. Euphytica, 151: 393-400.
Sun YQ, Zhang XL, Jin SX, Guo X, Jin S, Liang S (2004). Production and characterization of somatic hybrids between upland cotton (*Gossypium hirsutum*) and wild cotton (*G. klotzschianum* Anderss) via electrofusion. Theor. Appl. Genet., 109: 472-479.
Sun YQ, Zhang XL, Jin SX, Liang S, Nie Y (2003). Somatic embryogenesis and plant regeneration in wild cotton (*Gossypium klotzschianum* Anderss). Plant Cell Tiss. Org. Cult., 75: 247-253.
Sun YQ, Zhang XL, Nie YC, Guo X (2005b). Production of fertile somatic hybrids of *Gossypium hirsutum* + *G. bickii* and *G. hirsutum* + *G. stockii* via protoplast fusion. Plant Cell Tiss. Org. Cult., 83: 303-310.
Sunilkumar G, Rathore KS (2001). Transgenic cotton: factors influencing Agrobacterium-mediated transformation and regeneration. Mol. Breed, 8: 37-52.
Thibaud NF, Shealy RT, Khanna A, Vodkin LO (2003). Clustering of microarray data reveals transcript patterns associated with somatic embryogenesis in soybean. Plant Physiol., 132: 118-136.
Tohidfar M, Mohammadi M, Ghareyazie B (2005). *Agrobacterium* - mediated transformation of cotton (*Gossypium hirsutum*) using a heterologous bean chitinase gene. Plant Cell Tiss. Org. Cult., 83: 83-96.
Trolinder NL, Goodin JR (1988b). Somatic embryogenesis in cotton (*Gossypium*) II. Requirements for embryo development and plant regeneration. Plant Cell Tiss. Org. Cult., 12: 43-53.
Trolinder NL, Xhixian C (1989). Genotype specificity of the somatic embryogenesis response in cotton. Plant Cell Rep., 8: 133-136.
Trolinder NL, Goodin JR (1998a). Somatic embryogenesis in cotton (*Gossypium*) I. Effects of source of explant and hormone. Plant Cell Tiss. Org. Cult., 12: 31-42.
Ueguchi TM, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I, Matsuoka M (2005). Gibberellin insensitive dwarf 1 encodes a soluble receptor for gibberellin. Nature, 437: 693-698.
Ulloa M, Meredith WR (2000). Genetic linkage map and QTL analysis of agronomic and fiber quality traits in an interspecific population. J. Cot. Sci., 4: 161-170.
Umbeck P, Johnson G, Barton K, Swain W (1987). Genetically transformed cotton (*Gossypium hirsutum* L.) plants. Bio/Technol., 5: 263-266.
Vogler H, Kuhlemeier C (2003). Simple hormones but complex signalling. Curr. Opin. Plant Biol., 6: 51-56.
Von AS, Sabala I, Bozhkov P, Dyachok J, Filonova L (2002). Developmental pathways of somatic embryogenesis. Plant Cell Tiss. Org. Cult., 69: 233-249.
Wang J, Sun Y, Yan S, Daud MK, Zhu S (2008). High frequency plant regeneration from protoplasts in cotton *via* somatic embryogenesis. Biol. Plant, 52: 616-620.
Wang JE, Sun YQ, Zhang FB, Shen XJ, Zhu SJ (2007). Somatic Embryogenesis and Plant Regeneration in Wild Cotton Species of G Genome. Acta Agronomica Sinica, DOI CNKI:SUN:XBZW.0.2007-08-011.
Wendel JF, Cronn RC (2003). Polyploidy and the evolutionary history of cotton. Adv. Agron., 78: 139-186.
Wilkins TA, Mishra R, Trolinder NL (2004). *Agrobacterium*-mediated transformation and regeneration of cotton. J. Food Agric. Environ., 2: 179-187.
Wilkins TA, Rajasekaran K, Anderson M (2000). Cotton biotechnology. Crit. Rev. Plant Sci., 19: 511-550.
Woodward AW, Bartel B (2005). A receptor for auxin. Plant Cell, 17: 2425-2429.
Wu J, Zhang X, Nie Y, Luo X (2005). High-efficiency transformation of Gossypium hirsutum embryogenic calli mediated by Agrobacterium tumefaciens and regeneration of insect-resistant plants. Plant Breed, 124: 142-146.
Wu JH, Zhang XL, Nie YC, Jin S, Liang S (2004). Factors affecting somatic embryogenesis and plant regeneration from a range of recalcitrant genotypes of Chinese cottons (*Gossypium hirsutum* L.). *In Vitro* Cell Dev. Biol. Plant, 40: 371-375.
Wu SJ, Wang HH, Li FF, Chen TZ, Zhang J, Jiang JJ, Ding Y, Guo WZ, Zhang TZ (2008). Enhanced Agrobacterium-mediated transformation of embryogenic calli of upland cotton via efficient selection and timely subculture of somatic embryos. Plant Mol. Biol. Rep., 26: 174-185.
Wu X, Li F, Zhang C, Liu C, Zhang X (2009). Differential gene expression of cotton cultivar CCRI24 during somatic embryogenesis. J. Plant Physiol., 166: 1275-1283.
Xiao W, Custard KD, Brown RC, Lemmon BE, Harada JJ, Goldberg RB, Fischer RL (2006). DNA methylation is critical for Arabidopsis embryogenesis and seed viability. Plant Cell, 18: 805-814.
Yan SF, Zhang Q, Wang JE, Sun YQ, Daud MK, Zhu SJ (2010). Somatic embryogenesis and plant regeneration in two wild cotton species belong to G genome. *In vitro* Cell Dev. Biol. Plant, 46: 298-305.
Yang X, Guo X, Zhang X, Nie Y, Jin S (2007b). Plant regeneration from *Gossypium davidsonii* protoplasts *via* somatic embryogenesis. Biol. Plant, 51: 533-537.
Yang XY, Zhang XL, Fu LL, Min L, Liu GZ (2010). Multiple shoots induction in wild cotton (*Gossypium bickii*) through organogenesis and the analysis of genetic homogeneity of the regenerated plants. Biologia, 65: 496-503.
Yang XY, Zhang XL, Jin SX, Fu L, Wang L (2007a). Production and characterization of asymmetric hybrids between upland cotton Coker 201 (*Gossypium hirsutum*) and wild cotton (*G. klozschianum* Anderss). Plant Cell Tiss. Org. Cult., 89: 225-235.
Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S (2000). Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. Plant Physiol., 123: 223-234.
Yuceer SU, Koc NK (2006). Agrobacterium-mediated transformation and regeneration of cotton plants. Russ. J. Plant Physiol., 53: 413-417.
Zeng F, Zhang X, Zhu L, Tu L, Guo X, Nie Y (2006). Isolation and characterization of genes associated to cotton somatic embryogenesis by suppression subtractive hybridization and macroarray. Plant Mol. Biol., 60: 167-183.
Zhang BH, Feng R, Fenglia L, Xiulan L (1996). Anther culture and plant regeneration of cotton (*Gossypium klotzchianum* anderss). Chin. Sci. Bull., 41: 145-148.
Zhang BH, Feng R, LIU F, Wang Q (2001). Direct somatic embryo genesis and plant regeneration from cotton (*Gossypium hirsutum* L.) explants. Isr. J. Plant Sci., 49: 193-196.

Zhang BH, Liu F, Yao CB, Wang KB (2000). Recent progress in cotton biotechnology and genetic engineering in China. Curr. Sci., 79: 37-44.

Zhang H, Zhao F, Zhao Y, Guo C, Li C, Xiao K (2009). Establishment of transgenic cotton lines with high efficiency via pollen-tube pathway. Frontiers Agric. China, 3: 359-365.

Zhang Q, Wang JE, Ye CL, Yan SF, Chen JH, Hong CX, Zhu SJ (2010). Somatic Embryogenesis and Plant Regeneration in Wild Cotton Species of G.tomentosum. Cotton Sci., 3 DOI: CNKI:SUN:MHXB.0.2010-03-005.

Zhu SW, Gao P, Sun JS, Wang HH, Luo XM, Jiao MY, Wang ZY, Xia GX (2006). Genetic transformation of green-colored cotton. *In vitro* Cell Dev. Biol., 42: 439-444.

Zimmerman JL (1993). Somatic embryogenesis: a model for early development in higher plants. Plant Cell, 5: 1411-1423.

Zuo JR, Niu QW, Frugis G, Chua NH (2002). The WUSCHEL gene promotes vegetative-to-embryonic transition in *Arabidopsis*. Plant J., 30: 349-359.