Chapter 2

Coconut

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1. Introduction

Coconut (*Cocos nucifera* L.) is an important perennial palm crop, predominantly cultivated in about 12 million hectares of land in tropical and subtropical coastal lowlands (APCC, 2014). With about 2.1 million hectares of coconut plantations, India is the largest producer of coconut. About 10 million farmers and their families depend exclusively on coconut, while many others in rural and semi-urban locations depend on it marginally for their livelihood (Rethinam, 2006). This palm is primarily grown for its edible oil from dry kernel, but all other parts have one or the other uses which earned the name 'tree of life'. Products from coconut palm possess high nutritional and medicinal value, in addition to numerous industrial applications (Foale, 2003). Coconut production has declined slightly in the last few years owing to several factors. For stabilized yield in the traditional coconut-based farming systems, growing of high-yielding, disease-resistant cultivars has been advocated. Unfortunately, production and supply of homogeneous quality planting material to the farmers has been a major constraint in coconut productivity. Conventional methods can produce only about 3.5 million seedlings annually while there is a demand for 10 million seedlings (Karun *et al.*, 2015). Coconut, with cultivar specific vegetative phase, enters juvenile phase in about 4 to 7 years and has a single apical meristem, without branches or suckers. This restricts its propagation through vegetative means with sole dependence on seed propagation. With no known methods of propagating the coconut palm through vegetative methods, *in vitro* culture is the only option for rapid multiplication of elite, high yielding and disease-resistant germplasm. Successful protocol for clonal propagation has been reported in various palm species such as arecanut (Karun *et al.*, 2004), oil palm (Rabechault *et al.*,

1970), peach palm (Steinmacher *et al.*, 2007) and date palm (Tisserat and Demason, 1980). Regeneration in these palms has been achieved using several explants such as young leaf, immature inflorescence and immature zygotic embryo via both direct and indirect somatic embryogenesis.

Somatic embryogenesis (SE) is a process where somatic cells differentiate into somatic embryos which share morphological similarity with zygotic embryos. It is a kind of asexual embryogenesis considered as an evolved strategy to overcome various environmental and genetical factors (Arnold *et al.*, 2002). Indirect SE has an intermediate callus phase, whereas direct SE does not. SE results in high number of regenerates with a very limited level of somaclonal variation (Ahloowalia, 1991; Henry, 1998). SE is feasible because plants possess cellular totipotency, wherein individual somatic cells possess the capability of regenerating into a whole plant- this makes it an attractive option in comparison to organogenesis as a plant regeneration system.

Research on SE in coconut was initiated four decades ago at Wye College, UK (Eeuwens and Blake, 1977), and later at Office de la Recherche Scientifique et Technique Outre-mer (ORSTOM, France) (Pannetier and Buffard-Morel, 1982). These experiments made use of plant somatic tissues such as young leaves, meristem region of young seedlings, sections from rachillae of young inflorescences, as initial explants to generate embryogenic calli (Branton and Blake, 1983; Gupta *et al.*, 1984). Recent studies related to SE have utilized zygotic tissues apart from somatic tissues such as immature inflorescences and ovaries. Zygotic tissues *viz.*, immature or mature embryos and embryo-derived plumules, were found to be easier to manipulate for achieving SE. In spite of several concerted efforts, reproducible protocol for clonal propagation in coconut has not been achieved. Thus coconut has been considered as one of the most recalcitrant species for *in vitro* culturing. *In vitro* recalcitrance in coconut has been attributed to many factors which include influence of genotype and explant maturity, adsorption of nutrients and hormones by activated charcoal making culture conditions undefined, production of compact calli, less percentage of plantlet regeneration, underperformance of regenerated plantlets and very slow rate of growth during *in vitro* culturing (Fernando *et al.*, 2010). Optimization of culture media, type of explant, plant growth regulators and their concentrations, subculturing periods and other additives have paramount significance in developing a reproducible tissue culture protocol. Studies carried out till date in coconut tissue culture is reviewed briefly below.

2. Explants

The initial starter for tissue culture purpose is termed as explants with meristems generally used for this purpose. For *in vitro* propagation, choice of explants is considered as a key element for successful outcome; hence identification of suitable explants is of primary importance. The potential of various coconut tissues, such as leaves, inflorescence, ovary, anthers and zygotic embryos to undergo callo-genesis, has been tested.

2.1. Leaves

Juvenile leaves are an excellent source of explant in many plants. However, the recalcitrant nature of some plants, especially palms, limits the use of leaves as explants. Very few studies have reported the use of leaves as explants for callusing and SE in coconut. Pannetier and Buffard-Morel (1982) reported asexual embryogenesis from young leaf explants of juvenile coconut palms. Even though callus was induced in leaf explants obtained from mature palms, further development into embryoids was not observed. Plantlets were obtained using immature leaves as explants by Raju *et al.* (1984), but the procedure could not be repeated. Reports indicate that the embryogenic capacity of leaf explants lasts for very short durations which limit their use as explants in clonal propagation studies (Karunaratne *et al.*, 1991). Uncoupling of cell and nuclear size has been reported to interrupt cell co-ordination leading to the recalcitrant nature of leaf explants to *in vitro* culture (Jesty and Francis, 1992). Thus, there has not been much progress using leaves as a source of explants in coconut palm.

2.2. Immature Inflorescence

Immature inflorescence is a potential and promising source of explants to clonally propagate important crop plants since they contain numerous meristematic points. Success depends on the selection of inflorescence of correct maturity stage. Regeneration of plantlets from immature inflorescence explants has been successful in arecanut (Karun *et al.*, 2004), date palm (Fki *et al.*, 2003; Abul-Soad and Mahdi, 2010), peach palm (Steinmacher *et al.*, 2007), oil palm (Teixeira *et al.*, 1994) and juçara palm (Guerra and Handro, 1988). The first report on use of immature inflorescence of coconut as a source of explant dates back to the early 1980s when sections of inflorescence rachillae were observed to proliferate in the medium to form callus, termed as 'calloids' by Branton and Blake (1983).. Explants were cultured in a medium with a range of 2,4-D as auxin source and activated charcoal. Callogenesis of inflorescence tissue depended on its age and the concentration of auxins in the medium. Somatic embryo formation was achieved successfully with a functional bipolar organization and completely differentiated shoot meristem (Verdeil *et al.*, 1994; Sandoval-Cancino *et al.*, 2016). Callus induction in segments of explants depended on basal media used and the size of inflorescence explants. Among the tested media *viz.*, modified Eeuwens Y3 (Eeuwens, 1976), CRI72, anther culture medium and modified Blake medium, callus induction was observed only in CRI72 medium (Vidhanaarachchi and Weerakoon, 1997).

2.3. Unfertilized Ovary

Ovary culture has been used in many crops as a means of clonal propagation since it is a potential tissue for the induction of SE due to the juvenilizing influence of nearby meiotic tissues (Bonga, 1982). Studies by Griffis and Litz (1997) indicated callogenesis and formation of adventitious roots from unfertilized ovary tissues in coconut, but SE could not be induced. Increased efficiency of unfertilized ovaries of coconut for callogenesis was reported by Perera *et al.* (2007), suggesting the possibility of mass production of homogenous planting materials of improved coconut varieties. Consistent callogenesis of about 40 per cent was observed when

unfertilized ovaries were cultured in CRI72 medium containing 2,4-D. However plantlet regeneration was limited with a total of 83 plantlets being produced from 32 cultured ovaries (Perera *et al.*, 2007).

2.4. Anthers and Microspores

Anthers and microspores are used as basal explants to produce haploid plantlets or double haploids. Production of double haploids through anther and microspore culture has considerable potential for shortening the breeding cycle in coconut. Inflorescence growth and development in coconut is complex and requires careful observations for choosing anthers and microspores as an explant source. The developmental stage of inflorescence is very important at which collections are made. The stages of coconut inflorescence at which anthers and micropsores are to be samples have been standardized and well defined (Perera, 2003). Anthers consist of both diploid and haploid cells making selective cell division impossible- this may lead to the formation of both diploid and haploid plantlets. In coconut, ploidy analysis of anther derived plantlets indicated that half of the regenerated plantlets were haploid and the rest were diploid (Perera *et al.*, 2008). Auxin sources for culturing coconut anthers were standardized indicating 2,4-D in combination with NAA enhanced calli production, but picloram and IAA had repressive effects. Media supplemented with 2-iP and kinetin, as cytokinin sources, yielded good results as compared to zeatin (Perera *et al.*, 2009). Solid Eeuwens Y3 (Eeuwens, 1976) medium was superior when compared to liquid medium for culturing coconut anthers as indicated by the higher percentage of embryo formation and embryo conversion (Perera *et al.*, 2011). As a whole, even though anthers and microspores served as an excellent source of explants for *in vitro* culture in many crops with successful plantlet regeneration, the final conversion to plantlets was low in coconut.

2.5. Immature Zygotic Embryo

The level of maturity of zygotic embryos is a very important factor since differences in induction frequencies are often observed and immature zygotic embryos have been found to possess better potential and competence to respond *in vitro* than their mature counterparts in coconut. Callusing frequency depends on the developmental stage of the embryo. Under the best conditions, callusing was reported to be around 75 per cent in immature zygotic embryo (Diyasena, 1998).

Immature embryos of coconut (6-7 months post-anthesis) cultured in a medium supplemented with 2,4-D, produced calli with 50 per cent of them turning in to globular embryos. Around 22 per cent of germination was observed in these cultures. Age of the embryo was suggested as an important factor determining callus proliferation and subsequent embryogenesis (Karunaratne and Periyapperuma, 1990).

2.6. Plumular Tissues

In coconut, even though immature embryos were found to be responsive, the response from mature embryos could be improved by slicing the embryos (Adkins *et al.*, 1998; Samosir, 1999; Rajesh *et al.*, 2005, 2014) or by selective excision of plumular tissue from zygotic embryo (Figure 2.1) (Chan *et al.*, 1998; Lopez-

Figure 2.1: Excision of Plumules from Mature Zygotic Embryos of Coconut and Inoculation on to Culture Medium.

Villalobos, 2002; Perez-Nunez *et al.*, 2006). Bhavyashree *et al.* (2016a) compared three methods of isolation of plumule *viz.*, excision of shoot meristem aseptically from *in vitro* germinated embryo after 10-12 days, excision of shoot meristem from *in vitro* germinated embryo subjected to GA3 treatment for five days and excision of shoot meristem from fresh embryo. It was observed that initiation of callus and embryogenic calli was significantly high in plumular explants isolated from fresh embryo. More consistent results in induction of embryogenic calli, formation of somatic embryos and *in vitro* regeneration have been obtained with plumular explants (Chan *et al.*, 1998; Lopez-Villalobos, 2002; Fernando *et al.*, 2004; Perez- Nunez *et al.*, 2006; Saenz *et al.*, 2006; Rajesh *et al.*, 2005, 2014) (Figures 2.2–2.4).

3. Media Composition

3.1. Basal Media

Apart from explant selection, choosing a suitable medium for growing the tissues

Figure 2.3: Regeneration of Plantlet from Coconut Plumule.

Figure 2.2: Development of Embryogenic Calli from Plumular Explants of Coconut.

Figure 2.4: Different Growth Stages in Plumule Culture.

is decisive for successful micropropagation. Optimal growth and morphogenesis of tissues may vary for different palms according to their nutritional requirements. Moreover, tissues from different parts of palms may also have different requirements for satisfactory growth. In coconut, Y3 (Eeuwens, 1976) and CRI72 (Karunaratne and Periyapperuma, 1989) media have been used frequently and found to be better than MS (Murashige and Skoog, 1962) and B5 (Gamborg *et al.*, 1968) for callus induction (Branton and Blake, 1983; Bhalla-Sarin *et al.*, 1986).

3.2. Plant Growth Regulators and other Additives

Role of plant growth regulators in culture medium is very important in the induction of callus and further development into somatic embryos. Callus induction medium, in general, requires a strong auxin source and 2,4-D has been the auxin most commonly used in coconut, with the working concentration of 2,4-D differing for different cultivars and explants and also between laboratories (Perez- Nunez *et al.*, 2006; Saenz *et al.*, 2006; Rajesh *et al.*, 2005, 2014; Perera *et al.*, 2009; Bhavyashree *et al.*, 2016 a, b; Sandoval-Cancino *et al.*, 2016). In general, explants were inoculated initially in a higher concentration of 2,4-D and subsequently sub-cultured onto a medium supplemented with lower concentrations of 2,4-D at regular intervals. The uptake of 2,4-D, studied using radio-labeling technology, indicate maximum absorption by the explants was within 24 hours of inoculation (Oropeza and Taylor, 1994). Verdeil *et al.* (2001). reported the significance of 'gametophytic-like' conditions produced by 2, 4-D for the successful transition from the vegetative into the embryogenic state. Fate of 2, 4-D in coconut tissues indicated its conversion into fatty acid analogues and incorporation into triacylglycerol derivatives (Lo´pez-Villalobos *et al.*, 2004). Stable and stored form of 2, 4-D, incorporated into triacylglycerol, may influence SE even when 2, 4-D has been removed from the medium (Nguyen *et al.*, 2015).

Callus initiation has also been achieved using other auxins apart from 2, 4-D. NAA (27 µM), in combination with 2, 4-D (452 µM), has been reported to promote callogenesis in rachillae explants (Gupta *et al.*, 1984). Picloram has been used successfully as an auxin source for callogenesis in various palm species such as arecanut (Karun *et al.*, 2004) and in peach palm (Steinmacher *et al.*, 2007) and has shown promising results in coconut. Optimizing the type of auxin and its concentration for induction of callus and its subsequent multiplication would play a pivotal role in establishing a standard tissue culture protocol in coconut.

Reports have indicated the potential of thidiazuron (TDZ) to enhance callus production in woody plants (Huetteman and Preece, 1993). In coconut, supplementation of the callus induction medium with TDZ has been shown to enhance callus induction and formation of embryogenic calli from plumular explants (Rajesh *et al.*, 2005, 2014; Jayaraj *et al.*, 2014; Bhavyashree *et al.*, 2016a, b) A positive effect of TDZ was observed on coconut ovary culture which enhanced callus induction; however response was dependent on the concentration, with higher concentrations of TDZ (above 18 µM) reducing the frequency of callus formation (Perera *et al.*, 2009). Thus, a combination of auxin and TDZ could enhance callusing in a short period of time.

Perez-Nunez *et al.* (2006) and Chan *et al.* (1998) have reported promotion of SE by incorporating 6-benzylaminopurine (BAP; between 50 to 300 µM) in the medium, which also led to an enhanced number of viable plantlets at the end of the culture phase.

Supplementation of abscisic acid (ABA) in the tissue culture medium has also been reported to enhance induction of SE in coconut from immature embryo explants (Fernan-do, 2001) and plumular tissues (Samosir *et al.*, 1999; Fernando and Gamage, 2000; Fernando *et al.*, 2004). However, the plantlet regeneration was low, around 10 per cent. (Weerakoon, 2004). Adkins *et al.* (1998) and Rajesh *et al.* (2005, 2014) have shown that exogenous supply of polyamines, such as spermine and putrescine, could enhance induction of SE in coconut from plumular explants. Adkins *et al.* (1998) demonstrated the beneficial effects of aminoethoxyvinylglycine (AVG) an ethylene production inhibitor and silver thiosulphate (STS) an ethylene action inhibitor in callus multiplication and SE.

Incorporation of osmotically active agents such as polyethyleneglycol (PEG 3 per cent) along with ABA (45 µM) in tissue culture medium has yielded positive results on formation, maturation and germination of somatic embryos (Samosir *et al.*, 1998). Significant beneficial effect of growth retardant ancymidol (30 µM) was demonstrated by Antonova (2009) on somatic embryo germination using immature inflorescence as explants. SE in coconut has also been reported to be positively influenced by supplementation of polyvinylpolypyrrolidone (PVPP) in the medium (Samosir, 1999).

3.3. Activated Charcoal

Activated charcoal (AC) has been an integral part of the culture medium especially for crops such as coconut. AC adsorbs the unwanted phenols and other growth inhibitory compounds and in turn reduces tissue browning under *in vitro* conditions. However, AC can also adsorb exogenously supplied plant growth regulators (hormones, vitamins) and minerals (Pan and Staden, 1998) which can lead to ambiguities in the precise functional concentrations of these additives in the culture medium. Therefore, AC can be considered a 'necessary evil' as far as coconut is considered. Ebert *et al.* (1993) reported that AC adsorbs auxins and cytokinins to the tune of 99 and 98 per cent, respectively after five days of culture media preparation.

4. Callus Multiplication and Maintenance

In general, coconut explants give rise to highly heterogeneous compact callus, which is not of friable nature (Fernando, 2001). Callus multiplication is important in scaling up protocols for *in vitro* regeneration in coconut and this was achieved by Perez-Nunez *et al.* (2006). Studies have suggested that multiplication of embryogenic callus could be achieved by subdividing the callus and repeated subculturing into media supplemented with lower levels of auxin, which has yielded promising results. Callus maintenance is important as it can provide year round embryogenic calli for further conversion. A recent report indicates that coconut embryogenic

callus, obtained from plumular tissues, could be maintained for 21 weeks without compromising on the embryogenic potential (Bhavyashree *et al.*, 2015).

5. Studies of Gene Expression Patterns during Somatic Embryogenesis

Molecular approaches, such as gene expression studies, can help to decipher molecular mechanisms underlying SE in coconut (Perez-Nunez *et al.*, 2009). Efficiency of existing clonal propagation protocol in coconut could be enhanced if a thorough knowledge of molecular events during SE is available. A major breakthrough was achieved with the isolation of a coconut gene (*CnANT*) homologous to the *Arabidopsis AINTEGUMENTA*-like gene, encoding two APETALA2 domains and a linker region (Bandupriya *et al.*, 2013). Analysis of *CnANT* has demonstrated its role in SE as indicated by enhanced expression levels during the callus induction phase (Bandupriya *et al.*, 2013, 2014). The upregulation of *CnANT* gene resulted in increased shoot growth in absence of growth regulators (Bandupriya and Dunwell, 2012). However, *CnANT* did not aid in spontaneous formation of somatic embryos as observed earlier with other *PL/AIL* genes (Bandupriya and Dunwell, 2012; Boutilier *et al.*, 2002; Tsuwamoto *et al.*, 2010). Perez-Nunez *et al.* (2009) isolated *CnCDKA* and *CnSERK* homologs from coconut which are reported to be associated with the induction of SE. A cyclin-dependent kinase, encoded by *CnCDKA*, regulates cell division (Montero-Cortes *et al.*, 2010a) while *CnSERK* encodes a protein receptor (Perez-Nunez *et al.*, 2009), which may be a component of a signaling cascade involved in regulating the rate of SE (Hecht *et al.*, 2001; Schmidt *et al.*, 1997). Similar to *KNOX* class I gene, which is associated exclusively in tissue with meristematic activity, *CnKNOX1* gene was isolated in coconut and its role in increased rate of somatic embryo formation and germination, through the addition of gibberellin during coconut SE, has been demonstrated (Montero-Cortes *et al.*, 2010b).

Rajesh *et al.* (2016) undertook transcriptome analysis of coconut embryogenic calli using Next Generation Sequencing (NGS) which resulted in the identification of 14 genes known to be involved in SE in other plants. Quantitative real-time PCR (qRT-PCR) analyses of these 14 genes were carried in six developmental stages. The results revealed differential gene expression of these 14 genes: *CLV* was upregulated in the initial stage of callogenesis; *GLP, GST, PKL, WUS* and *WRKY* were upregulated during SE, whereas expression of *SERK, MAPK, AP2, SAUR, ECP, AGP, LEA* and *ANT* were higher in the embryogenic callus stage compared to initial culture and somatic embryo stages.

Bhavyashree *et al.* (2016b) carried out comparative studies of gene expression patterns, using RT-qPCR, of eight genes during various *in vitro* developmental stages of calli obtained from shoot meristem explants in WCT (West Coast Tall) and COD (Chowghat Orange Dwarf) cultivars of coconut. Enhanced expression of *PKL, SERK* and *WUS* was observed in embryogenic calli compared to non-embryogenic calli. Expression of *GLP, ECP* and *GST* was observed to be higher in normal somatic embryos compared to abnormal somatic embryos, whereas expression of *ECP, LEAFY, GLP* and *WRKY* was higher in normal meristemoids compared to aberrant meristemoids. Higher expression of *SERK, PKL* and *WUS* was reported in

embryogenic calli of WCT compared to COD. Somatic embryos of COD showed high expression of *GLP* and *GST* compared to WCT, where as in case of *ECP* gene, higher expression was observed in WCT compared to COD. Higher levels of expression *WRKY* and *LEC* were observed in WCT meristemoids compared to COD. These results revealed existence of genotypic differences of cultivars to *in vitro* culture and the use of these genes as markers for coconut SE was also suggested. Despite several studies, understanding of the molecular mechanisms that underlies coconut SE is limited warranting more prioritized research in this area for refining SE protocol.

6. Future Prospects

6.1. Establishment of Cell Suspension Cultures

The well established fact in plant tissue culture is that most plants grow better in liquid than on solid media. Several approaches have been proposed in order to enhance the productivity of *in vitro* culture depending on the final product desired and the species investigated. One such innovative approach is the use of cell suspensions (Figure 2.5) and bioreactors for plant culture. Cell suspensions in specific medium would be ideal for producing large number of somatic embryos and to extract commercially important plant metabolites. Culturing and maintaining of the embryogenic calli obtained from the explants in a liquid medium with appropriate nutrients, auxin source and additives under stable microclimatic condition is referred to as suspension culture. However, several factors such as aeration, agitation, light, temperature would influence the process as suspensions are maintained in flask culture. The embryogenic cells produced in cell suspension culture could be used in bioreactors to enhance SE. Highly recalcitrant nature of coconut to *in vitro* culture necessitates alteration in conventional tissue culture approaches. Some of the factors such as pH, temperature, dissolved oxygen; CO_2 concentrations could play a major role in SE. Keeping several of these factors constant could lead to an effective protocol with enhanced SE. The adaption of bioreactors in plant tissue culture is considered a major milestone since they offer several advantages *viz.*, time saving, labour-saving, relatively easy to scale-up, allow enhanced growth and

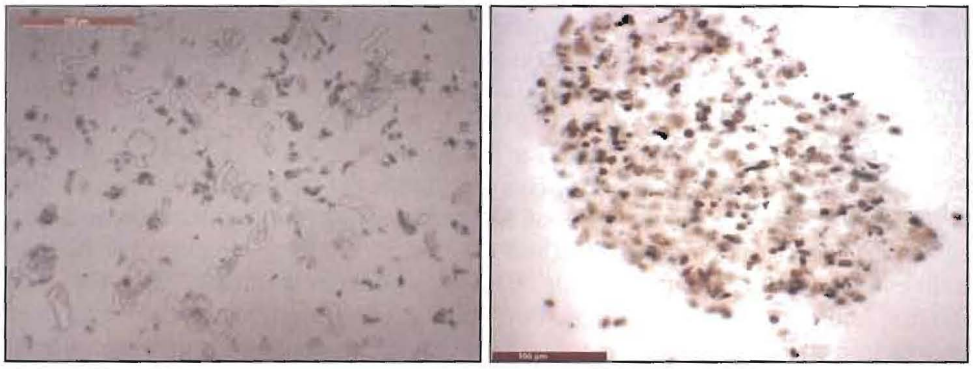

Figure 2.5: Initiation of Cell Suspension from Coconut Embryogenic Calli Derived from Plumular Explants.

multiplication and improved nutrient availability due to the use of liquid medium over traditional tissue culture techniques. Different kinds of bioreactor designs are in use such as aeration-agitation bioreactor, spin filter bioreactor, gaseous phase bioreactor, rotating drum bioreactor and air-driven bioreactor.

Many crop plants have been mass multiplied using bioreactors ever since its inception into plant tissue culture practices (Peak *et al.*, 2001; Ziv, 2005). As a plant production technique, bioreactors are far superior to traditional *in vitro* methods for all the species thus far tested. It is worth noting that with bioreactors, even the difficult-to-propagate woody and tree species can be produced relatively easily at high frequency. For instance, an efficient, somatic embryo-based mass propagation system for the recalcitrant species *Coffea arabica* was developed using a bioreactor (Barry-Etienne *et al.*, 1999). It is evident from several peer reviewed research papers that SE process could be scaled up many folds using bioreactors. A hybrid reactor would be ideal to reduce the *in vitro* culture duration in coconut and also to enhance the rate of SE and conversion of somatic embryos into plantlets.

6.2. Use of Temporary Immersion Systems

A system wherein explants are flooded with nutrient medium containing growth regulators at regular time intervals has been successfully used in scaling up of SE. The system named as temporary immersion systems (TIS) which offer the possibility of automating some culture stages. First of its kind was developed for pineapple (Escalona *et al.*, 1999), and use of similar system is shown to improve the regeneration rate and plantlet quality for other plant species (Etienne and Berthouly, 2002). Since then, the use of TIS in regeneration protocols for plant species has increasing continually (Niemenak *et al.*, 2008; Sankar-Thomas *et al.*, 2009). Culture containers provide additional head space which would improve the efficiency of regenerated plantlets. For coconut, which is classified as one of the toughest crops to clonally propagate, an improved protocol involving temporary immersion system needs to be developed (Figure 2.6). This would be an interesting strategy for up-scaling plantlet regeneration potential in coconut tissues under *in vitro* conditions.

6.3. Transformation Studies

A number of genes have been identified which govern SE. Several transcription factors control the transition from vegetative to embryogenic growth. Among them, members of APETALA2/Ethylene-responsive element binding protein domain family play an important role in promoting embryo development. In coconut, AINTEGUMENTA-like gene (*CnANT*), which codes for two AP2 domains, has been identified. Over-expression of *CnANT* in transgenic *Arabidopsis* yielded regeneration in hormone-free conditions (Bandupriya *et al.*, 2013). Genetic transformation studies could be a tool in developing a viable clonal propagation protocol in coconut. Micro-projectile bombardment method was used initially for inserting *GUS* gene in to embryogenic calli and young leaf tissues of coconut (Samosir, 1999). This was the first reported genetic transformation effort in coconut. *Agrobacterium*-mediated genetic transformation studies in various tissue of coconut such as immature anthers, excised zygotic embryos, plumule-derived embryogenic calli and SE-derived roots

Figure 2.6: Use of Temporary Immersion Systems in Coconut Plumule Culture Experiments.

and leaves were reported by Andrade-Torres *et al.* (2011). Genetic modification in coconut is still a long way away from becoming a reality. This could be useful for the improvement of coconut SE by introducing genes or by over expressing these genes which are known to regulate SE in other plant species.

6. Conclusion

It is evident from literature and from our laboratory experience that progress of coconut tissue culture work is slow. To achieve a commercially viable protocol, it warrants the requirement of explant, media and PGR optimization. SE obtained using plumular explant is promising; however, more efforts are required towards refining and developing a protocol which could be upscaled. Inclusion of automatic systems such as temporary immersion systems and bioreactors would reduce the cost and increase the efficiency of SE. Recent advancement in biotechnological tools will open up avenues to scale-up SE in coconut.

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