African Journal of Biotechnology Vol. 4 (13), pp. 1485-1489, December 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Review

Application of tissue culture to cashew (*Anacardium occidentale* L.) breeding: An appraisal

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Accepted 4 November, 2005

Summary of the previous works on the *in vitro* culture of cashew is highlighted with emphasis on the critical factors that influence the explants response and plantlet regeneration. The recalcitrant nature of cashew has been attributed to the limited success recorded so far in the *in vitro* culture of the crop and abnormal development has been reported in the calli derived from its explants.Browning of explants in cashew was found to be due to the presence of high secondary metabolites and it has been reduced through frequent transfer of explants, addition of activated charcoal and dark treatment. Explants necrosis has also been traced to the effect of strong sterilization. Meanwhile, the use of explants from *in vitro* germinated seedlings or fungicidal treated young flush has been found to improve the success rate significantly. The use of MS base salt supplemented with two-step treatment of cytokinins enhances the response of cashew explants and development of derived plantlets.

Key words: Cashew, *Invitro* Culture, Breeding, Explant Browning, Growth Regulators.

INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a tropical nut crop that belongs to the family *Anacardiaceae*, which consist about 75 genera and 700 species (Nakasone and Paull, 1998). Behrens (1998) described cashew as a tropical tree species cultivated in many tropical countries of the world from its centre of origin in South and Central America to Africa, Asia and Tropical Australia. The crop was introduced to India, Asia, and Africa in the 15th and 16th centuries (Woodroof, 1967; Ohler, 1979)., Asia, and Africa in the 15th and 16th centuries (Woodroof, 1967; Ohler, 1979). It is believed, that cashew nut was brought into Africa from the northern part of South America by Portuguese missionaries in 1400 (Mitchell and Mori, 1987). The crop has spread for over 500 years either naturally or through smallholder's cultivation. As smallholder crop, cashew in Africa is usually grown as a mono-crop or intercropped with food crops such as cassava, groundnuts, sesame, beans, maize, melon and sorghum. It is estimated that about 3 millions households in Africa are involved in cashew nut production with average of 3 hectares under cashew per household and this is contributing significantly to the economy and livelihood of the smallholders in the industry.

In the continent of Africa, this crop gained economic importance after 1920s. Thereafter, cashew nut production in Africa increased substantially and became one of the major export crops in some of the African countries. In the recent years, however, cashew cultivation in Africa has extended to other countries including Benin, Ghana, Guinea Bissau, Kenya, Madagascar, Nigeria and Togo. In the period between 1950 and 1970, the African continent as a whole supplied 70% of cashew nut production annually (Afrol News, 2000). The scenario, however, after mid 1970's was

Abbreviations: IBA, indole-3-butyric acid; BA, 6-benzyladenine; IAA, indole-3-acetic acid; BAP, 6-benzylaminopurine; Kin, kinetin; 2-ip, isopentenyladenine; TDZ, thidiazuron; NAA, 1-naphthalene-3-acetic acid; GA, gibberelic acid.

apparently different with cashew nut production gradually declined. The world cashew production has continued to increase steadily over the years, the Africa's share drastically diminished and this has been principally attributed to low tree yields. Cashew tree population in Africa mostly comprises of low yielding trees. Little has been done to improve the yield potential of African cashew germplasm. Tree yields in farmers' field have been found to be highly varied such that, some trees may yield nothing whilst the best usually produce over 20 kg of nuts (Martin and Kasuga, 1995; Aliyu, 2004). Martin et al. (1998) found that in most fields, the 50% highest yielding trees produced at least 70% of plot yields, while the remaining 30% of the yields are produced by 50% lowest yielding trees in the plot. This has led to the concept of establishing new cashew field with clonally propagated improved planting materials. And to achieve mass propagation of such elite materials, application of tissue culture becomes an important tool.

Bioengineering methods such as microclonal propagation of valuable elite plants, embryo and meristem cultures, anther culture, cell culture breeding based on somaclonal variability and somatic hybridization of protoplasts had been applied in the breeding and improvement of many tropical crops but with very little success in cashew. The use of isolated tissue and organ cultures have always fascinated investigators ever since their concept emerged at the turn of the last century, when G. Haberlant (1902) was the first to propose cultivation of plant cells as tool for improving their totipotency. It was opined that the cultivation of isolated cells in nutrient media would at least provide the researchers with a possibility to approach many problems from an entirely new angle. Currently, these methods are not only used in research but have also gained wide practical application in plant breeding, vegetable gardening, fruit growing, and especially, floriculture, more specifically in producing virus-free planting stock.

Cashew is propagated mainly by seeds, resulting in high levels of genotypic and phenotypic variability (Philip and Unni, 1984). However, progress in the improvement of this crop species through conventional breeding methods has been hampered because of its out-breeding nature thus making perpetuation of desirable characters difficult. Apart from low success of conventional vegetative propagation methods, e.g. air layering, mound strolling, grafting or cutting in cashew, they are not sufficiently rapid techniques like micropropagation and embryo cultures for faster multiplication of elite genotypes in evaluation and enhancement programmes. Cashew like other *Anacardiaceae* is strongly recalcitrant to *in vitro* culture techniques and only limited success has been achieved (Mantell et al., 1998). Calli from cashew explants have been reported to produce roots (Falcone and Leva, 1987; Leva and Falcone, 1990; Sy et al., 1991) and globular protuberances which developed into embryo-like structures but had many aberrations and misshaped plantlet forms (Jha, 1988). Das et al. (1996), Leva and Falcone (1990) and D' Silva and D'Souza (1992) opined that *in vitro* rooting and survival of *in vitro*-produced plants remain severe constraints to more widespread application of micropropagation techniques to cashew. Although direct somatic embryogenesis from mature and immature cotyledon sections (Hedge et al., 1990, 1991) and embryo axis (Aliyu and Awopetu, 2005) has been respectively reported, but a protocol for large scale somatic embryo production for this tree crop is however yet to be established.

However, to achieve any meaningful and significant improvement in cashew breeding through advanced biotechnological techniques, a dependable *in vitro* methodology for plant regeneration of cashew must be devised and perfected. *In vitro* culture has been successful for many horticultural fruit species (Ammirato et al., 1984). Even though species closely related to cashew have been propagated *in vitro* (Litz et al., 1984; Barghchi and Alderson, 1983; Martinelli, 1988), satisfactory conditions have not been achieved in cashew. This effort is to give a summary of progress made on the *in vitro* culture of cashew and highlight some critical factors influencing it.

CONSTRAINTS TO CASHEW MICROPROPAGATION

Explants browning

One of the serious constraints of micropropagation of cashew has been attributed to the presence of secondary metabolites (Mantell et al., 1998) which are oxidized after wounding and cause subsequent browning and necrosis of cashew explants (Jha 1988; Das et al., 1996). Leva and Falcone (1990) reported that pale-green calli exhibited morphogenic activity while the brown ones grew in an unorganized form. Mantell et al. (1998) reported the release of secondary metabolites from the duct of primary phloem elements of all organs of cashew, which has resulted into serious browning. Meanwhile several steps and approaches have been tried in attempts to solve browning problem of cashew explants e.g. antioxidants or activated charcoal have been added routinely to culture (Jha 1988; Lievens et al., 1989; Sy et al., 1991; Sardinha et al., 1993; Bessa and Sardinha 1994a,b; Das et al., 1996). Das et al. (1996) have reported that while only 15% of cotyledonary nodes survived culture establishment, daily transfer of explants into fresh medium with activated charcoal for a total period of 7 days increased explants viability of 60%. In addition to frequent transfer of explants and addition of activated charcoal, cultivation in darkness for one week has increased survival of explants to 90%. Mantell et al. (1998) remarked that the use of activated charcoal (AC) or cultivation in darkness for initial period of 7 days also had positive effects on shoot elongation, but sprouting

was significantly decreased. Sardinha et al. (1993) had reported that the use 280 μM of ascorbic acid also reduces browning of the medium and the subsequent necrosis of explants without inhibiting *in vitro* bud development.

Explants contamination and sterilization

Rodrigues Jr. (1995) and Das et al. (1996) reported that the difficulty to obtain survival of shoot explants from field-grow stock mature plants is due to the inability of the explants to survive the strong levels of surface sterilization required to decontaminate such material. The authors' initially recorded 3% and 25 % survival for shoot tips and nodal explants of field-grown twigs, respectively, subjected to thorough sterilization. Thereafter, few explants that survived sterilization turned brown and died after 20 days of culturing. It was however recommended that explants from seedlings germinated and raised *in vitro* will be most suitable for micropropagation of elite cashew. Mantell et al (1998) also suggested severe pruning of adult trees to be used as explants source, then treat the trunk with a cupric fungicide and allow the epicormic growth (young flush) to grow under protected conditions in isolation from rain and wind-blown dust.

Explants age

Another important cultural factor that affects axillary *in vitro* culture of cashew is the age of stock plants (Mantell et al., 1998). Bessa and Sardinha (1994a) and Sardinha et al. (1993) reported that in medium with 1 mg/l BA and 0.5 mg/l IAA, the percentage of bud break reached 100% in explants from 3-5 month old plants while it decreases to 57% in explants from 1-2 year old plants. Meanwhile Pierik (1990) remarked that responses of adult plant can be enhanced through partial rejuvenation of the shoots. This has been achieved by micrografting adult phase meristems onto seedlings rootstocks. Cashew shoots of mature plants have not been used as explant sources due to the recalcitrance problems mentioned earlier. Lievens et al. (1989) and Leva and Falcone (1990) reported promising results on the trials involving 6-15 month and 3-year old seedlings, respectively. For *in vitro* germinated seedlings, Das et al. (1996) observed decline in response at 30 days after germination, therefore recommends 20 day old *in vitro* germinated seedlings as best material to achieve highest morphogenic response.

Growth medium

Cashew explants have been generally cultured on the solidified media, but Lievens et al. (1989) found that a shoot induction period on liquid medium with BAP improved axillary development. In recalcitrant species like cashew, agar impurities have been noticed to cause problems of browning of explants through the presence of contaminating salts. Das et al. (1996) and Aliyu and Awopetu (2005) reported that MS salt formula was the most efficient for bud development and plantlet regeneration, respectively, in cashew. Mantell et al. (1998) recorded optimal shoot development *in vitro* through MS with reduced salts. Although D'Silva and D'Souza (1992) earlier reported significant effect of sucrose concentrations on the number of developing buds *in vitro*, no significant results was obtained by Mantell et al. (1998) with 40 g/l of sucrose. Combination of oligosaccharides such as glucose or maltose appeared to enhance both bud sprouting and number of nodes per explant (Mantell et al., 1998; D'Silva and D'Souza 1992).

GROWTH REGULATORS

Types and concentrations of plant growth regulators used in culture media play crucial roles in the induction of *in vitro* organogenesis, because cashew is a hard-rooter (D'Silva and D'Souza, 1992). Sardinha et al. (1993) and Bessa and Sardinha (1994a) reported 65 - 94% bud break in a growth-regulator-free-medium. Relatively high dose of cytokinins have been recommended for *in vitro* cashew bud development (Lievens et al., 1989; Leva and Falcone 1990; D'Silva and D'Souza 1992; Das et al., 1996) However, Leva and Falcone (1990) reported that only Zea riboside at concentration of 5 mg/l stimulated axillary bud development. Lievens et al. (1989) also found positive effect of Kin or 2-iP, in combination with 0.1 mg/l NAA, but the continuos presence of TDZ and BAP resulted in total necrosis of explants. Matell et al. (1998) observed that continual presence of strong cytokinin like TDZ, BAP or Kin decreased bud sprouting and shoot elongation, which are factors that are unsuitable for achieving shoot multiplication. Several studies on cotyledonary nodes led to the conclusion that shoot development from axillary buds of cashew would requires two-step procedures; the first in which high doses of cytokinin induce well-developed buds followed by a second in which low levels of cytokinin are deployed for shoot elongation. This situation is analogous to the rooting process that consists of several phases characterized by different, often opposite, sensitivities to growth regulators and the active substances (De Klerk, 1995). Meanwhile attempts on the use of GA to enhance bud sprouting and shoot elongation in cashew had been hindered by poor rooting of the derived plantlets (Leva and Falcone, 1990; Lievens et al., 1989; Mantell et al., 1998). Boggetti (1997) however reported a reduction in the rooting abilities of cashew leaf explants cultured in rooting medium supplemented with GA. Similar results had been published in apple (Pawlicki and Welander, 1992) and *Solanium aviculare* (Jasik et al., 1996). The negative rooting effect of GA was however not noticed

when embryo axis explants was used (Aliyu and Awopetu, 2005).

Temperature

Temperature has also been found to be critical to bud sprouting and shoot elongation of cashew. Studies have shown that bud sprouting was strongly suppressed at 35°C, although this temperature supported good shoot growth and nodes development. A two-step temperature treatment/procedure i.e. first step in which lower temperature could be used for induction of bud sprouting and followed by higher temperatures for obtaining optimal shoot elongation, are now being used.

Explants source

Rooting of microshoots derived from nodal explants appeared to be more difficult than those of cotyledonary node origin (Aliyu and Awopetu, 2005). This is because the latter materials are more meristematic/juvenile in character and at active morphogenic stage. D'Silva and D'Souza (1992), using appropriate combination of 2.9 µM IAA and 4.9 µM IBA and Das et al. (1996) using a 2-hour pulse of 2460 µM IBA, induced 80.3% and 40% rooting in cotyledonary node-derived shoots, respectively. By contrast, microshoots exposed to 5 mg/l NAA induced only 25% rooting response (Leva and Falcone, 1990) and 2 mg/l IBA gave only 30% (Lievens et al., 1989). Mantell et al. (1998) however recorded 42% rooting success in microshoots derived from nodal explants of 1-year old stock exposed to an IBA concentration of 100 µM for 5 days and about 30% of microshoots from 4- and 5-year old stock rooted after 24 h treatment in a 10^{-3} M solution of either IAA or IBA. Das et al. (1996) increased dramatically the rooting of cotyledonary node-derived microshoots of cashew using *Agrobacterium rhizogenes* transfection. Currently, efforts are on the way to increase the rooting of shoot node-derived microshoots using various wild and disarmed strains with proven root promoting activity as being done for *Solanum aviculare* (Jasik et al., 1996; Boggetti, 1997).

CONCLUSION

The importance of *in vitro* techniques in mass and rapid multiplication of improved planting materials, exchange of elite cashew genotypes and improvement of the crop species cannot be overemphasized. However, to effectively utilize the technique in achieving desired objectives, there is the need to understand the critical factors affecting it, which was attempted in this write-up. The factors are, however, interrelated and must be employed in appropriate combinations to achieve the desirable goal of producing an improved high yielding, disease and pest-tolerant and high quality cashew nuts for the sustainability of the industry.

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