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Use of Arabinogalactan Proteins in Coconut (*Cocos Nucifera* L.) Tissue Culture: An Alternative Approach for Improved Tissue Response

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Abstract

Arabinogalactan proteins (AGPs) are extra-cellular proteins involved in plant growth and development. The effects of these proteins on *in vitro* response of different species are well documented. This study assessed for the first time the role of AGPs on tissue culture of coconut, a highly recalcitrant species *in vitro*. Coconut (Sri Lanka Tall) plumules were cultured in medium containing 2, 4-dichlorophenoxyaceticacid (2,4-D) for callogenesis. Calli were multiplied by subculturing onto freshly prepared callusing medium. Somatic embryos were induced by transferring of calli to medium with 2/3 concentration of initial 2,4-D and matured by subsequent transferring to medium without any hormones. Embryos were converted in the presence of gibberelic acid. Arabic gum (1-50 mgL^{-1}) was used as the main source of AGP. Alternatively, different AGP sources like Larch wood gum, carrot seeds and defatted coconut kernel were also assessed. Arabic gum in callusing medium resulted in early callogenesis from 20-40% plumules compared to 10% in the control. However, it did not have a significant effect on final callusing efficiency. Its presence in somatic embryogenesis medium showed a significantly higher mass of embryogenic structures per an embryogenic clump (33.3-50.5mg) when compared to the control (26.9mg).The best result was obtained at $10mgL^{-1}$ Arabic gum. All AGP sources except Larch wood gum had positive effects on somatic embryogenesis. Among them, defatted coconut kernel ($25-50mgL^{-1}$) showed the best results giving 70% cultures with embryogenic structures compared to 37% in control. However, so far, the positive effect of AGP did not reflect in plant regeneration efficiency.

Key words: arabinogalactan proteins, coconut, somatic embryogenesis, tissue culture

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Introduction

Arabinogalactan proteins (AGPs) are a heterogeneous group of proteoglycans present in cell walls, plasma membrane and plant secretions and commonly found in culture media (Fincher *et al*., 1983). Different plant organs contain specific sets of AGPs that are dependent on the developmental stage of the tissue (Pennell *et al*., 1992). The molecules are involved in the regulation of several important events related to plant growth and development (Wisniewska and Majewska-Sawka, 2007 and references therein). AGPs have also been proven to play an important role in the course of *in vitro* development of plant cells and tissues (Table 1). Facts summarized in the Table 1 imply that by changing the AGPs of the extracellular matrix of cells in culture, it is possible to alter the development fate of cells.

Clonal propagation of coconut is of importance for speeding up the coconut breeding programmes and true to type propagation of elite mother palms. Due to the limitations pertinent to coconut (Fernando, 2008), it is still considered as a highly recalcitrant plant to *in vitro* culture. Considering the potential of AGPs recorded in other plants, it can be hypothesized that application of AGPs is an alternative way to improve *in vitro* response of coconut. So far, this has not been tested in any palm. Thus, the aim of the study was to test whether AGPs improve response of coconut tissues to *in vitro* culture. This paper presents the preliminary results.

Materials and Methods

Plant material

Zygotic embryos were collected from mature (12 months after pollination) nuts of coconut (variety Sri Lanka Tall).

Callogenesis

Zygotic embryos were sterilized in 3% (v/v) Clorox for five minutes and rinsed five times with sterile distilled water. Plumules were excised under stereo binocular microscope, crushed and placed on basal medium CRI 72 (Karunaratne and Periyapperuma, 1989) supplemented with 215 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1% (w/v) activated charcoal (BDH) for 10 weeks in the dark at 28°C to induce callusing. Embryogenic calli were multiplied by subculturing onto the freshly prepared callusing medium.

Somatic embryogenesis

Embryogenic cultures were transferred to the CRI 72 medium supplemented with low (133 µM) 2,4-D and incubated for 6 weeks followed by transfer to the same basal medium without hormones for four weeks. Subsequently cultures were maintained in Y3 (Eeuwens, 1978) supplemented with 0.45 µM gibberelic acid by subculturing at 4-5 weekly intervals until shoots were developed. The cultures were maintained at 28 ºC in the dark until somatic embryos developed into shoots. Then they were transferred to 16 hr light (PAR; 25 $\mu molm^{-2}s^{-1}$).

Effect of AGPs

The effect of AGPs on callogenesis was studied by culturing of plumules in callusing medium supplemented with filter sterilized Arabic gum (AG) (1, 10 and 50 mgL^{-1}). Embryogenic cultures were transferred to media containing 133 µM 2,4-D and AG (1, 10 and 50 mg L^{-1}) to assess the effect of AGPs on induction of embryogenic structures. Finally, the effect of various sources of AGPs on somatic embryogenesis was evaluated by maintaining cultures in low 2,4-D and hormone-free media supplemented with filter sterilized AG (1 and 10 mgL^{-1}), Larch wood gum (LW) (1 and 10 mgL^{-1}), carrot seed powder (CS) (25 and 50 mgL^{-1}) and defatted coconut kernel (DCK) (25 and 50 mgL^{-1}).

Data Collection and analysis

Data was collected from 10-15 replicates per treatment. The experiments were repeated at least twice. The number of explants producing callus was counted at two weekly intervals for six weeks. Cultures producing embryogenic structures (Fig. 1a) and total weight of embryogenic structures were recorded to assess the effect of AG on early somatic embryogenesis. Finally, the number of cultures producing somatic embryos (Fig. 1b) was

Table 1. Role of AGPs on plant tissue culture

Species	AGP source	Effect shown	Reference
Daucus carota L.	Carrot seeds	- Re-induced embryogenesis in non-embryogenic cell lines - Increased embryogenesis in young cell lines	Kreuger and Van Holst, 1993
Daucus carota L.	Tomato seeds	- Increased embryogenesis in young cell lines	Kreuger and Van Holst, 1993
Cyclamen persicum L.	Carrot seeds	- Increased proembryogenic masses in cell cultures	Kreuger *et al*., 1995
Picea abies L.	Spruce seeds Conditioned medium	- Increased embryogenesis in low embryogenic cell lines	Egertsdotter and Van Arnold, 1995
Daucus carota L.	Carrot seeds Conditioned medium	- Increased somatic embryogenesis from protoplasts.	Van Hengel *et al*., 2001
Gossypium hirsutum L.	Conditioned medium	- Increased embryogenesis in explants of responsive genotypes. - Embryogenesis in explants of non-responsive genotypes.	Poon *et al*., 2004
Zea mays L.	Arabic gum	-Increased embryogenesis in low responsive genotypes. - No response in recalcitrant genotypes.	Boderies *et al*., 2004
Triticum aestivum L.	Arabic gum Larch wood gum	- Haploid plant production which was otherwise impossible without an ovary nurse culture. - Reduced mortality of cultured microspores.	Letarte *et al*., 2006
Beta vulgaris L.	Conditioned medium	- Organogenesis in protoplast-derived calli. - Increased plant regeneration.	Wisinewska and Majewska, 2007
Daucus carota L.	Cashew nut tree gum	- Increased somatic embryogenesis and plant regeneration.	Pereira-Netto *et al*., 2007
Vitis vinifera L.	Larch wood gum Conditioned medium of zucchini/ parsley	- Increased somatic embryogenesis and plant regeneration from cell suspensions.	Ben Amar *et al*., 2007
Cucurbita pepo L.	Conditioned medium	- Enhanced multiplication of slow growing cell lines.	Ben Amar *et al*., 2010

Figure 1. Induction of somatic embryogenesis in plumule-derived callus of coconut. (a) Cultures with embryogenic structures (es) (x 4). (b) Cultures with somatic embryos (se) (x 1.5)

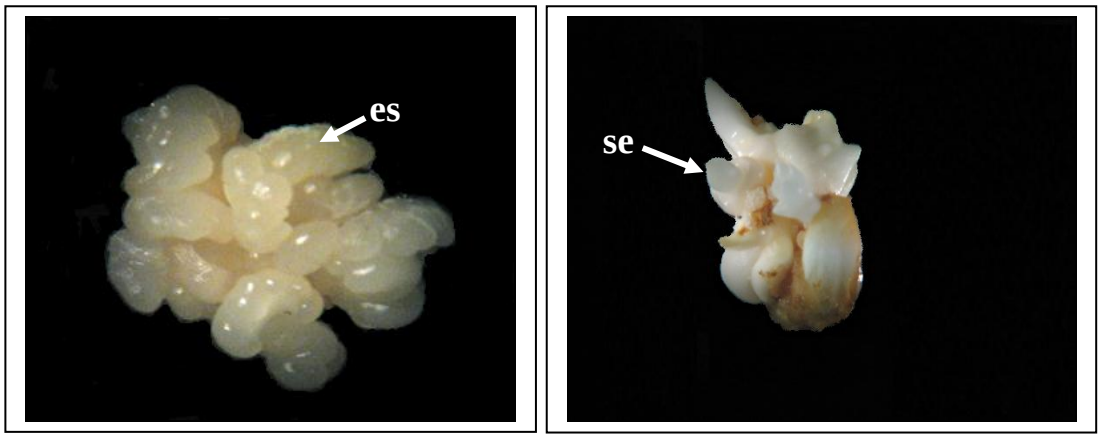
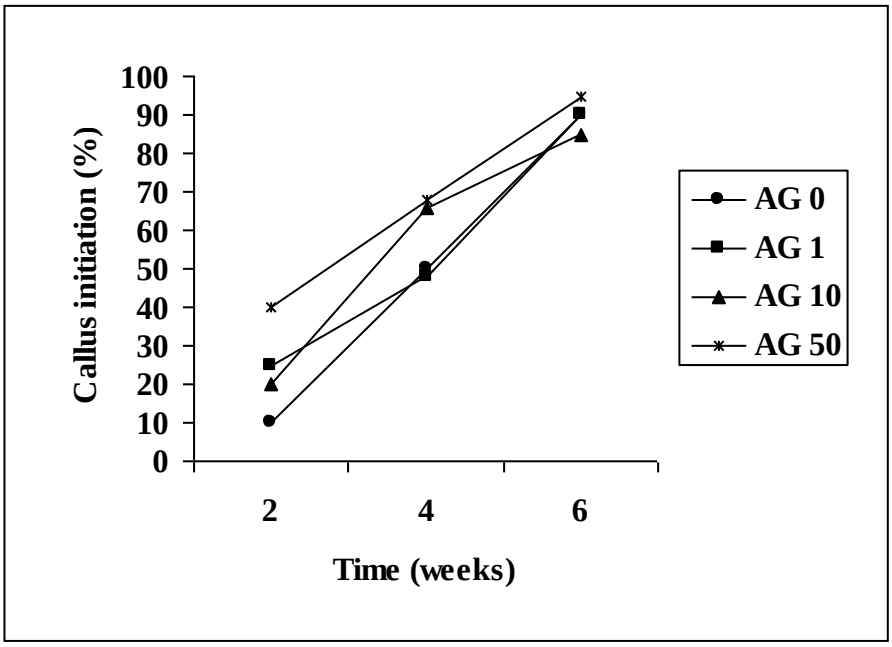


Figure 2. Effect of Arabic gum (AG) on initiation of callus from plumule explants of coconut



recorded to assess the effect of different AGP sources on somatic embryogenesis.

Difference between treatments was assessed using Logistic Regression Analysis in CatMod procedure in SAS software.

Results

The response of plumules cultured is shown in the Figure 2. The time taken to initiate callus varied among explants. The presence of AG in the medium resulted in early callogenesis in 20-40% plumules compared to 10% in the control. With the increase in AG concentration, the number of explants showing early callusing increased. However, the difference was statistically not significant. With time, the difference among callusing frequencies in media with varying levels of AG disappeared and all treatments including control resulted in above 85-95% callusing explants after six weeks of culturing.

In this study, the effect of AGPs on embryogenesis was studied at two stages. Initially, the effect of 1-50 mgL^{-1} AG on embryogenic structure formation from plumule callus was assessed. The results revealed that the addition of AG had no significant improvement over the control in efficiency of forming cultures with embryogenic structures. However, it showed a significant increase in the mass of embryogenic structures formed per embryogenic clump (Table 2). The best results (0.0505 g embryogenic structures per callus clump) could be seen at 10 mgL^{-1}AG.

At the second stage, the effect of various sources of AGPs on somatic embryogenesis was evaluated. As shown in the Figure 3, among the tested sources of AGPs, DCK at 25-50 mgL^{-1} significantly improved the number of cultures producing somatic embryos (70%) when compared to the control (37%). Somatic embryogenesis in media containing other AGP sources like CS (25 and 50 mgL^{-1}) and AG (10 mgL^{-1}) also showed an increase over the control but did not vary significantly. LW showed an inhibitory effect and the inhibition was significant at the level of 10 mgL^{-1} when compared to the results of control.

At the stage of plant regeneration, a few shoots were developed from cultures irrespective of their AGP treatments. In the present study, the positive impact of AGPs on somatic embryogenesis did not reflect in plant regeneration efficiency.

Discussion

Callogenesis

Coconut (Sri Lanka Tall) plumules excised from pre-germinated mature zygotic embryos have shown to be potential explants for clonal propagation of coconut. The average callusing recorded was 50-60%. The time taken to initiate callus varied from three to ten weeks. The variation in response of individual explants at least partially might be due to differences in maturity stage of individual explant (Fernando, 2001).

In the present study, overall performance of plumules was better and callusing recorded in six weeks of culturing was above 85-95% in all treatments. The improved response of plumules in the present study might be due to the use of plumules excised from freshly harvested embryos. In agreement with the study of Fernando (2001), in this study also explants needed varying time to initiate callus. However, application of AG resulted in early callusing in a higher number of explants compared to control.

AGPs are known to play an important role in *in vitro* development of tissues. Cells are surrounded by complex mixtures of AGP that are specific to tissue type and differentiation status (Kreuger and van Holst, 1995). Since the *in vitro* development of cells is influenced by the presence of specific AGP mixtures, explants with different degree of maturity might be under the influence of different AGP mixtures which might cause the variable response of explants. Under such circumstance, external application of AGP is an alternative approach to induce a controlled *in vitro* development.

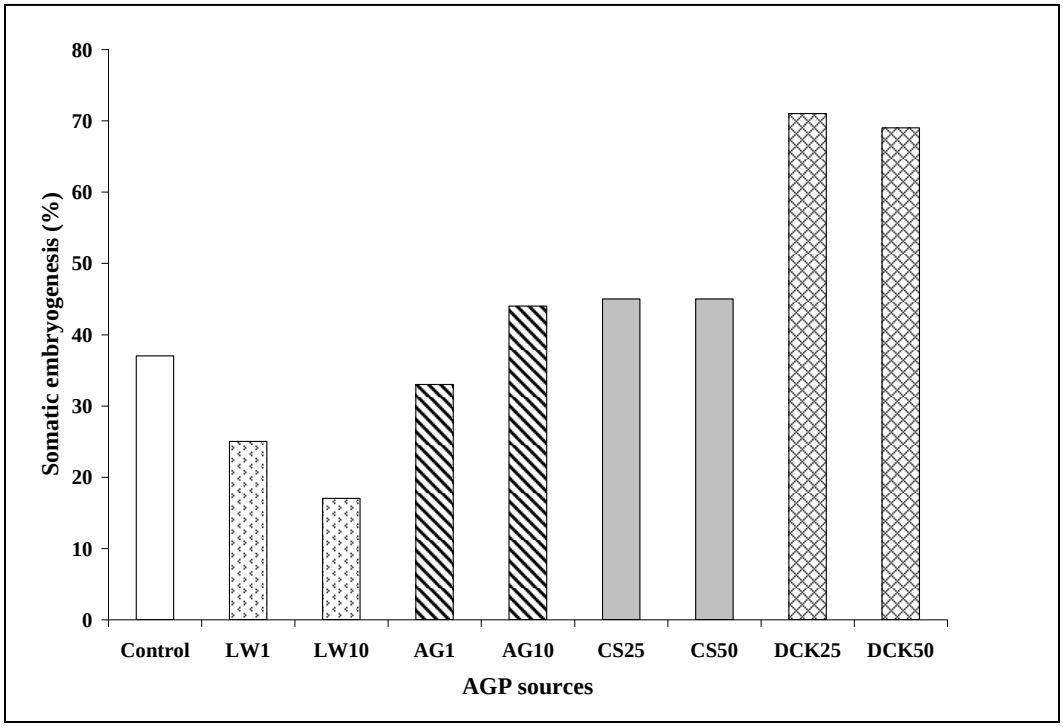
Based on the results, it can be assumed that external application of AG readily created an optimum environment for more explants to induce callusing. However, explants in the

Table 2. Effect of AG on initiation of cultures with embryogenic structures

AG LEVEL (mgL^{-1})	CULTURES WITH EMBRYOGENIC STRUCTURES (%)	EMBRYOGENIC STRUCTURES PER EMBRYOGENIC CLUMP (G)
0 (CONTROL)	76	0.0269^{B}
1	80	0.0333^{B}
10	72	0.0505^{A}
50	68	0.0396^{AB}
SIGNIFICANCE	NS	*

NS = Not significant; * = Significant at $p< 0.05$

Figure 3. Effect of different sources of AGPs on somatic embryogenesis in coconut plumule derived callus. AGP sources: control = Without AGP source, LW = Larch wood gum (1 and 10 mgL^{-1}), AG = Arabic gum (1 and 10 mgL^{-1}), CS = Carrot seed powder (25 and 50 mgL^{-1}) and DCK = Defatted coconut kernel (25 and 50 mgL^{-1})



medium free of AG (control) also with time developed suitable conditions for callusing.

Somatic embryogenesis

Specific AGPs are essential for somatic embryogenesis (Kreuger and van Holst, 1993) and their presence or absence differentiates embryogenic and non-embryogenic/ weakly embryogenic lines (McCabe *et al*., 1997). Furthermore, the amount of specific AGPs in the environment also decides the embryogenic capacity (Poon, 2004).

In agreement with the previous studies, in this study, application of AG has shown increased embryogenic structure formation and the response was concentration dependant.

In the literature, evidence is available to support that the activity of AGPs is not species specific (Kreuger *et al*., 1995; Boideries *et al*., 2004; Letarte *et al*., 2006; Ben Amar *et al*., 2007). However, improved effect of species specific AGPs was also reported in grape (Ben Amar *et al*., 2007). This might be due to AGPs collected from a particular species are more suitable for cultures of the same species.

In the present study, when the effect of several sources of AGPs on somatic embryogenesis in coconut was tested, AGP sources improving somatic embryogenesis at different levels as well as inhibiting could be identified. DCK was found to be the best source used in this study and it might be due to the use of a coconut based AGP source.

However, in this study AGP extracted from DCK was not used to confirm the results. As coconut endosperm contains AGP (White *et al*., 1989), the improved response of DCK treated tissues might be at least partially due to AGP.

In this study, expected effect of AGPs on plant regeneration could not be seen. The modification of type, concentration, time and duration of application of AGP might be useful in optimizing culture conditions for improving clonal plant regeneration efficiency in coconut.

Conclusion

This study showed for the first time that the sources of AGPs play a positive role in different stages of clonal propagation of coconut. Arabic gum has the potential to induce early callusing and increase embryogenic callus formation from coconut plumules. Defatted coconut kernel extract have significant impact on somatic embryogenesis in coconut.

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