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Full Length Research Paper

Potential of cassava flour as a gelling agent in media for plant tissue cultures

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The potential of a tissue culture medium gelled with cassava flour to support shoot proliferation of stem nodal sections of *Uapaca kirkiana* and *Faidherbia albida* was studied. A two factorial experiment was conducted with the first factor as type of gelling agent (80 g/L cassava flour; 80 g/L cassava flour mixed with 3.5 g/L agar; and 7 g/L agar) while the second factor was two plant species. Explants were obtained from 8-months old *U. kirkiana* and *F. albida in vitro* raised seedlings. Data included number of shoots and height and analysis of variance was performed. Number of shoots proliferating and shoot height from cultures on medium gelled with cassava flour was the lowest. This was attributed to the degradation of the cassava flour gel after two weeks. Performance of the cassava flour mixed with 3.5 g/L agar gel was better than agar alone. The stability of cassava flour as a gelling agent can therefore be improved by mixing with some agar.

Key words: Gelling agent, cassava flour, agar, shoot proliferation.

INTRODUCTION

Plant tissue culture, done under aseptic environments, has important applications in plant biotechnology. The technology is widely applied in both research and development of improved crops. The technologies include micropropagation, virus elimination, plant transformation, embryo rescue, anther culture across a range of crops from vegetables, roots and tubers, fruit trees, ornamental herbaceous plants and woody species (Bonga and Durzan, 1985; Maliro, 1997; Reinert and Bajaj, 1977). Well prepared and solidified artificial media provide suitable conditions for the growth of microbes and plant tissue. The media allows the cultures to maintain their normal biochemical and physiological processes during use of the technologies listed.

Creation of the growth media frequently requires the use of gelling agents for solidification. Agar is one of the consumables used in large quantities in plant tissue cultures especially where solid media are required. Agar is gelatinous complex polysaccharide obtained from marine algae such as *Gelidiella* and *Gracilaria* species (Nene and Sheila, 1994). It does not provide any nutrients for growth of microbes and plant tissues and hence, after purification, it is used extensively in microbial and biochemical studies. It is also used in large quantities as a gelling agent for plant tissue culture media (Murashige, 1974).

While plant tissue culture has been in application for decades most developing countries have not significantly benefited from it, partly due to high cost of consumables like agar. It is against this economic problem background that potential agar substitutes like cassava flour have been investigated.

Cassava flour, processed from roots of *Manihot esculenta*, has a high content of starch (over 90%) (Onuweme, 1982). Pure cassava starch is used in industries for making adhesives and latex. It forms a gelatinous matrix that can be autoclaved and stored or

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thereafter melted by heating (Kasanadze, 2000; Nene and Sheila, 1994). Cassava flour therefore possesses gelling properties with a potential use in plant tissue culture medium. Studies by Kasanadze (2000) and Gerbe and Santhyanarayana (2001) have confirmed this gelling ability and suggested it as a potential cheap substitute for agar.

Kasanadze (2000) worked with cassava flour (not pure starch) and found an optimum quantity of 80 g/L at particle size of 63 ∝m and gelling pH 5.7 to 5.8. He also found that quality of the gel was improved by mixing the cassava flour with some agar (80 g/L cassava flour + 3.5 g/L agar). Although the cassava flour has been proven to have gelling ability, its ability to support plant tissues or microbial cultures has not been evaluated. If proven effective to support plant tissue cultures, cassava flour will be a cheap alternative gelling agent due to its local availability. This study was therefore conducted to evaluate the potential of cassava flour as a media gelling agent to support plant tissue cultures. Specifically the study evaluated the performance of woody plant species (*U. kirkiana* and *F. albida*), *in vitro*, on Woody Plant Media (WPM) formulation when gelled with cassava flour, agar and a mixture of the two.

MATERIALS AND METHODS

The study was carried out in a plant tissue culture laboratory at Bunda College of Agriculture, Malawi, from March to December 2002.

Experimental Design

A two factorial experiment with Completely Randomised Design (CRD) was conducted. The first factor was three gelling agent types including 80g/L cassava flour, 80 g/L cassava flour + 3.5 g/L agar, and 7 g/L agar. The second factor was two woody plant species including *U. kirkiana* and *F. albida*. The experiment was repeated three times.

Cassava flour and source of explants

Cassava flour was prepared from roots of a commonly grown cassava cultivar -‘Mbundumale’. The roots were harvested from Bunda College students’ research farm and were washed with tap water, peeled, sliced into small pieces and sun-dried. The slices were ground using a hammer mill and sieved into fine flour using a sieve of 63 ∝m particle size. The flour was then ready for gelling culture media. Commercial ‘Bio Lab’ agar-agar was used as a commercial gelling agent. Stem nodal sections of *F. albida* (‘Nsangu’) and *U. kirkiana* (‘Masuku’), were obtained from 8-month old *in vitro* cultured seedlings.

Media preparation

Woody Plant Medium (WPM) (McCown and Lloyd, 1981) basal salt formulation was provided by ready prepared stock solutions and supplemented with growth regulators (cytokinins and auxins, at 1mg/L). Sucrose was then added to the media at 30 g/L (3%). The media were then adjusted to pH 5.8 before adding the gelling agents. Depending on treatment, gelling agents were added as described in the experimental design. They were dissolved by heating the solution to almost boiling point and then dispensed into bellico tubes at 20 ml/tube. The tubes with media were autoclaved at 121°C temperature for 15 min and allowed to cool in a sterile environment provided by an air flow laminar hood.

Inoculation and incubation

Under the air laminar flow hood 2 cm stem nodal sections of explants were inoculated into the tubes using surgical blades and foreceps. Ten culture tubes per treatment were inoculated and incubated under 16 h of light at a temperature range of 25 to 28°C in the plant tissue culture laboratory.

Data collection and Analysis

Data, which included number of shoots per culture and height of shoot, was collected weekly for five weeks. The data were subjected to analysis of variance (ANOVA) using GENSTAT statistical package. The statistical model used was $Y_{ij} = \propto + A_i + B_j + (AB)_{ij} + \varepsilon_{ij}$ where Y_{ij} is the jth observation in treatment I, $\propto$ is the overall mean, A_i is the ith treatment effect, (gel. agents), B_j is the jth treatment effect, (plant species), $(AB)_{ij}$ is the interaction of gelling agent and plant species, and ε_{ij} is random error component.

RESULTS AND DISCUSSION

Shoot proliferation

The number of shoots proliferating from nodal sections of *U. kirkiana* and *F. albida* from weeks 1 to 5 (Table 1, Figures 1 and 2) showed that there were no significant differences in the number of shoots of plant species on media gelled with cassava flour and agar for the first two weeks. Relatively, the media gelled with cassava flour or with a mixture of agar had more shoot proliferation during the 1st week of incubation than where agar only was used in the case of *F. albida* (Figure 1). The differences became significant ($p \leq 0.01$) in the 4th to 5th weeks of incubation with the cassava + agar gelled medium as the best.

Table 1. Number of shoots of *F. albida* and *U. kirkiana* that regenerated, *in vitro*, after 5 weeks of incubation period.

Gelling agent	Plant species	
	F. albida	***U. kirkiana***
Cassava flour (80 g/L)	0.6	0.1
Cassava + agar (80 g+3.5 g)/L	4.1	0.5
Agar (7 g/L)	2.9	0.7

After two weeks of incubation, the media gelled with cassava flour only, lost its gel and the solid particles of flour deposited to the bottom of the tubes. As a result the

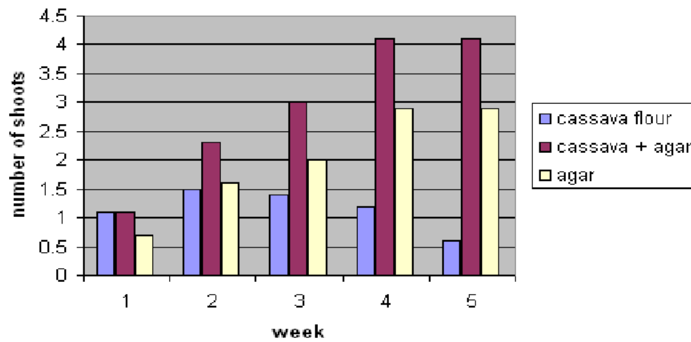

Figure 1. Number of shoots of *F. albida* regenerated from stem nodal sections, *in vitro*, during a five weeks incubation period.

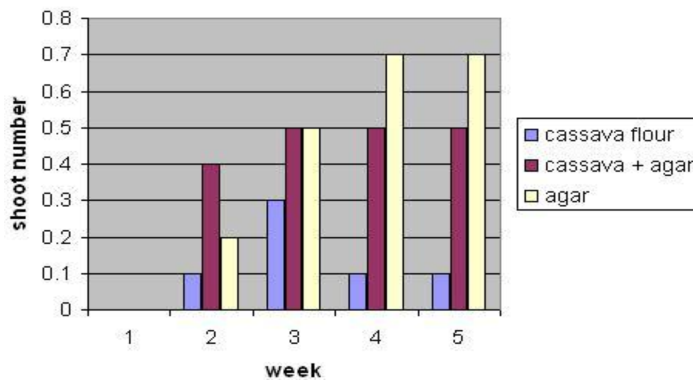

Figure 2. Number of shoots of *U. Kirkiana* regenerated from stem nodal sections, *in vitro*, during a five weeks incubation period.

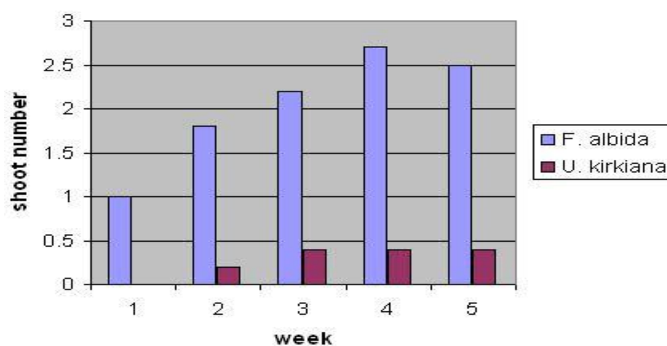

Figure 3. Average number of shoots of *F. albida* and *U. kirkiana* regenerated from stem nodal sections on the three media gelling agents.

tissues sank and disturbed the normal shoot proliferation. This is probably due to drop in pH which often occurs after autoclaving the media. Lupano and Gonzalez (1999) reported that gels with starch have an increased water holding capacity because of the ability of the starch to

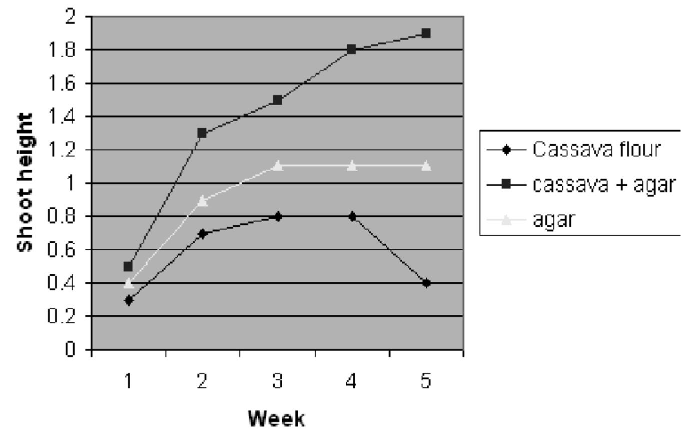

Figure 4. Height of shoots (cm) of *F. albida* regenerated from stem nodal sections, *in vitro*, after five weeks of incubation.

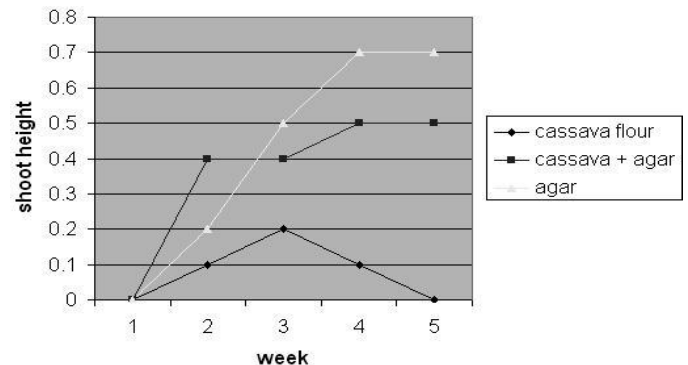

Figure 5. Height of shoots (cm) of *U. kirkiana* regenerated from stem nodal sections, *in vitro*, after five weeks of incubation.

form hydrogen bonds. Thus a drop in pH reduces its gel stability. Mbanaso et al. (2001) observed a similar 7-days stability in pH with cassava starch gelled medium although in their case its support for the banana shoot tips was not affected. The media gelled with cassava flour + agar, and agar only maintained their solid (gel) state and supported the cultures normally for up to five weeks. The cassava gelled medium was likely to produce more shoots if its gel was stable. This is supported by the fact that shoot proliferation was higher in media gelled with cassava flour + agar throughout the incubation period for *F. albida* and up to the 3rd week for *U. kirkiana*. This is most probably because cassava flour acts as an additional carbon source and adds other ionic supplements (35% carbohydrates, 1% mineral matter) (Onuweme, 1982) to the medium which most probably resulted in improved cell growth and morphogenesis.

Shoot height

The shoot height results (Figures 4 and 5) were significantly different at p≤0.05 in weeks 2 to 4 and at

p≤0.01 in week 5. The trend of shoot height results was similar to that of number of shoots such that there were no significant differences in shoot height of shoots from tissues on medium gelled with cassava flour for the first two weeks of incubation. When the cassava flour gel degraded some tissues were submerged by the medium thereby disturbing the normal growth of the shoots.

Plant species

Plant species showed significant differences at p≤0.01 throughout the incubation period. *F. albida* proliferated more than *U. kirkiana* (Figure 3). The latter is reported to be difficult to grow and takes a longer time to proliferate even under *in vivo* conditions (Nkanaunena, 2002). The species also performed differently across the three media gelling agents. Figures 4 and 5 show that medium gelled with cassava flour+agar supported fastest and steady height increase in *F. albida* while medium gelled with agar only was the best for in *U. kirkiana.* Although the differences between species were obviously expected, the results further suggest that the degree of different gelling agents to support tissues will vary with species.

The results showed that cassava flour (even without processing into pure starch) can be a substitute to agar and improve the growth of plant shoots. Where subculturing is done within two weeks of incubation, there is no need of mixing it with some agar. If the cassava flour can provide both the gelling and carbon source requirements in the medium then it can substantially reduce the medium cost.

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REFERENCES

Bonga JM, Durzan DJ (1985). Tissue Culture in Forest Trees. University of Califonia, Davis, U.S.A., 37-38.

Dodds JH, Roberts LW (1995). Experiments in plant tissue culture. Cambridge University Press.

Gerbe E, Sathyanarayana BN (2001). Cassava- A new and cheaper alternative to agar for direct in vitro regeneration and microtuber production from nodal cultures of potato. Ethiopian Agricultural research Organisation (EARO); Afr. Crop Sci. J. 9: 1-8.

Kasanadze AK (2000). Replacement of Agar by cassava flour in microbial media. Bsc. dissertation University of Malawi, Bunda College of Agriculture, Lilongwe.

Lupano CE, Gonzalez S (1999). Gelatination of whey protein concentrate -cassava starch in acidic conditions. Journal of Agricultural Food Chemistry; La Plata, Argentina 47: 918-923.

Maliro MFA (1997). Propagation of *Uapaca kirkiana* using tissue culture techniques. Msc Thesis, University of Malawi, Bunda College of Agriculture, Lilongwe.

Mbanaso ENA, Crouch J, Onofeghara FA, Pillay M (2001). Cassava Starch as Alternative to Agar for GellingTissue Culture Media. A paper presented at a conference on Cassava , an acient crop for modern times held at Donald Danforth Plant Science Center, St. Louis, Missouri, Nov. 4-9, 2001.

McCown BH, Lloyd G (1981). Woody Plant Medium (WPM) – a mineral nutrient formulation for microculture for woody plant species. Hort. Sci. 16: 453.

Murashige T (1974). Plant propagation through tissue culture. Ann. Rev. Plant Physiol. 25: 135 -166.

Murashige T, Skoog I (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 15: 473-474.

Nene Y, Sheila I (1994). A potential substitute for agar in routine cultural work on fungi, bacteria, and plant tissue culture. Indian J. Mycol. 17: 511-512.

Nkanaunena GA (2002). Rejuvination of *Uapaca kirkiana* (Muell.Arg) trees through in vitro micrografting. Msc Thesis, University of Malawi, Bunda College of Agriculture, Lilongwe.

Onuweme IC (1982). The Tropical Crops, Yams, Cassava, Sweetpotatoes and Cocoyams; University of Ife; Ile-Ife, Nigeria, p. 145.

Reinert J, Bajaj YPS (eds) (1977). Applied and fundamental aspects of plant cell, tissue and organ cultures; Springer-Verlag Berlin Heidelberg, New York.