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Comparative study on agar and cassava gelled media in *in-vitro* propagation of ginger

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Ginger is propagated vegetatively using underground rhizomes. Most farmers use planting materials saved from previous harvest. These materials could have been sold for cash. Contingent on this, many farmers are reluctant to use healthy succulent rhizomes for planting. These are rather sold thereby resulting in acute shortage of planting materials. Tissue culture technique can be applied to mass-produce seedlings for distribution to ginger farmers. This however, is not cost effective now due to lack of the necessary materials required for tissue culture. Most of the materials for tissue culture are not locally available and are therefore, procured at prohibitive cost. It is on the basis of these considerations that the present research was set up to develop a locally available and cheap protocol that is reproducible in ginger plant tissue culture work. Cassava flour possesses gelling properties with potentials for preparation of plant tissue culture medium. Agar gelled medium was compared with cassava gelled medium. Cassava gelled medium at four weeks after initiation, gave a higher mean value of 2.033 buds per plant and bud length of 1.411 cm compared to agar with a mean value of 1.9 buds and 1.192 cm, respectively (P>0.05). At eight weeks, cassava consistently gave a higher mean value of 2.611 buds per plant and bud length of 2.19 cm compared to agar with a mean value of 1.944 buds per plant and 1.42 cm respectively (P>0.05).

Key words: Ginger, tissue, culture, cassava gel, buds, initiation.

INTRODUCTION

Preparation of tissue culture media may require 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 a gelatinous complex polysaccharide obtained from marine algae such as Gelidiella and Gracilaria species (Nene and Sheila, 1994). It does not provide any nutrient 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). Due to high cost of consumables like agar, potential agar substitutes like cassava starch are now been investigated. Cassava starch is processed from roots of *Manihot esculenta*. It has a high content of starch (over 90%) (Onuweme, 1982). Pure cassava starch forms a gelatinous matrix that can be autoclaved and stored or 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. Kasanadze (2000) and Gerbe and Santhyanarayana (2001) have confirmed the gelling ability of cassava flour and reported it as a potential cheap substitute for agar. However, Mbanaso et al. (2001) observed that the stability of cassava starch gelled medium may be

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Table 1. Effect of auxin (NAA) at a blanket application of 0.25 mg/L of BAP on length of buds and number of buds produced by the explant at four weeks after initiation using agar and cassava gelled media.

Treatment NAA (mg/L)	Mean length of buds (agar)	Mean length of buds (cassava)	Mean number of buds (agar)	Mean number of buds (cassava)
0	0.9	1.6	2.6	2.7
0.1	1.5	1.5	2.2	2.5
0.2	2.7	1.8	1.5	1.8
0.3	0.1	1.2	1.1	2.2
0.4	0.1	0.1	1.7	1.7
0.5	1.0	1.0	1.1	1.6
0.6	1.9	2.7	1.8	2.0
0.7	2.0	2.0	2.8	1.9
0.8	1.7	0.9	2.3	1.9
$LSD_{0.05}$	1.6	0.6	1.3	0.6

affected by pH.

MATERIALS AND METHODS

Agar and cassava gelled media were prepared. The cassava gelled medium was prepared using 30 g/L of commercial cassava flour as a gelling agent in 4.5 L of MS medium (Murashige and Skoog, 1962) while the agar gelled medium was prepared with 8 g/L of agar as a gelling agent in 4.5 L of MS medium (Murashige and Skoog, 1962). 500 ml from each of the prepared media, was dispensed into beakers giving rise to nine beakers of agar gelled media and nine beakers of the cassava gelled media. Different levels of an auxin (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 mg/L NAA) and a blanket application of 0.25 mg/L cytokinine (BAP) were added to the different beakers to form the treatments. The beakers were labelled according to the treatment they contain. Each beaker's content was dispensed into 10 culture vessels forming replications. The culture vessels were labelled according to the media treatment they contain, thus: two different media, each having 9 treatments with 10 replications. The buds were excised by peeling off the cotyledons - layer by layer to expose the meristematic tissue - the ex-plant. The ex-plants were then transferred into culture vessels.

Statistical analysis

The experiment was laid out in completely randomised design (CRD). Statistical difference between agar gelled medium and cassava gelled medium was determined with t-test using SPSS Version 15.0.

RESULTS

Length of buds produced by the ex-plants at four weeks after initiation

The hormone variations significantly affected the length of buds produced by the explants. The explants cultured in agar gelled medium with 0.3 and 0.4 mg/L NAA in the presence of 0.25 mg/L BAP produced the least length of buds at four weeks after initiation. In the cassava gelled medium, the least length of buds was also observed with explants cultured in 0.4 mg/L NAA in the presence of 0.25 mg/L BAP. Explants cultured in 0.2 mg/L NAA in the presence of 0.25 mg/L BAP significantly gave highest mean bud length of 2.7 cm per plant in agar gelled medium while 0.6 mg/L NAA in the presence of 0.25 mg/L BAP gave the highest bud length in cassava gelled medium. Except for explants cultured in agar gelled medium with 0.2 and 0.8 mg/L NAA in the presence of 0.25 mg/L BAP, cassava gelled medium consistently produced bud lengths that were significantly higher than bud lengths produced in agar gelled medium (Table 1).

Number of buds produced by the ex-plants at four weeks after initiation

There were significant effects of the treatments on number of buds produced by the explants at four weeks. Explants cultured in 0 mg/L NAA in the presence of 0.25 mg/L BAP significantly gave the highest mean value of 2.7 buds per plant in cassava gelled medium while explants cultured in 0.7 mg/L NAA in the presence of 0.25 mg/L BAP significantly gave the highest number of 2.8 buds per plant in agar gelled medium. In agar gelled medium, explants cultured in 0.3 and 0.5 mg/L NAA significantly gave the lowest mean value of 1.1 beds per plant while in cassava gelled medium, explants cultured in 0.5 mg/L NAA in the presence of 0.25 mg/L BAP produced the lowest mean value of 1.6 buds per plant (Table 1).

Effect of media on length of buds and number of buds at four weeks after initiation

There was no significant media effect both on number of buds and on length of buds produced by the explants *in vitro*. For number of buds, cassava however gave a

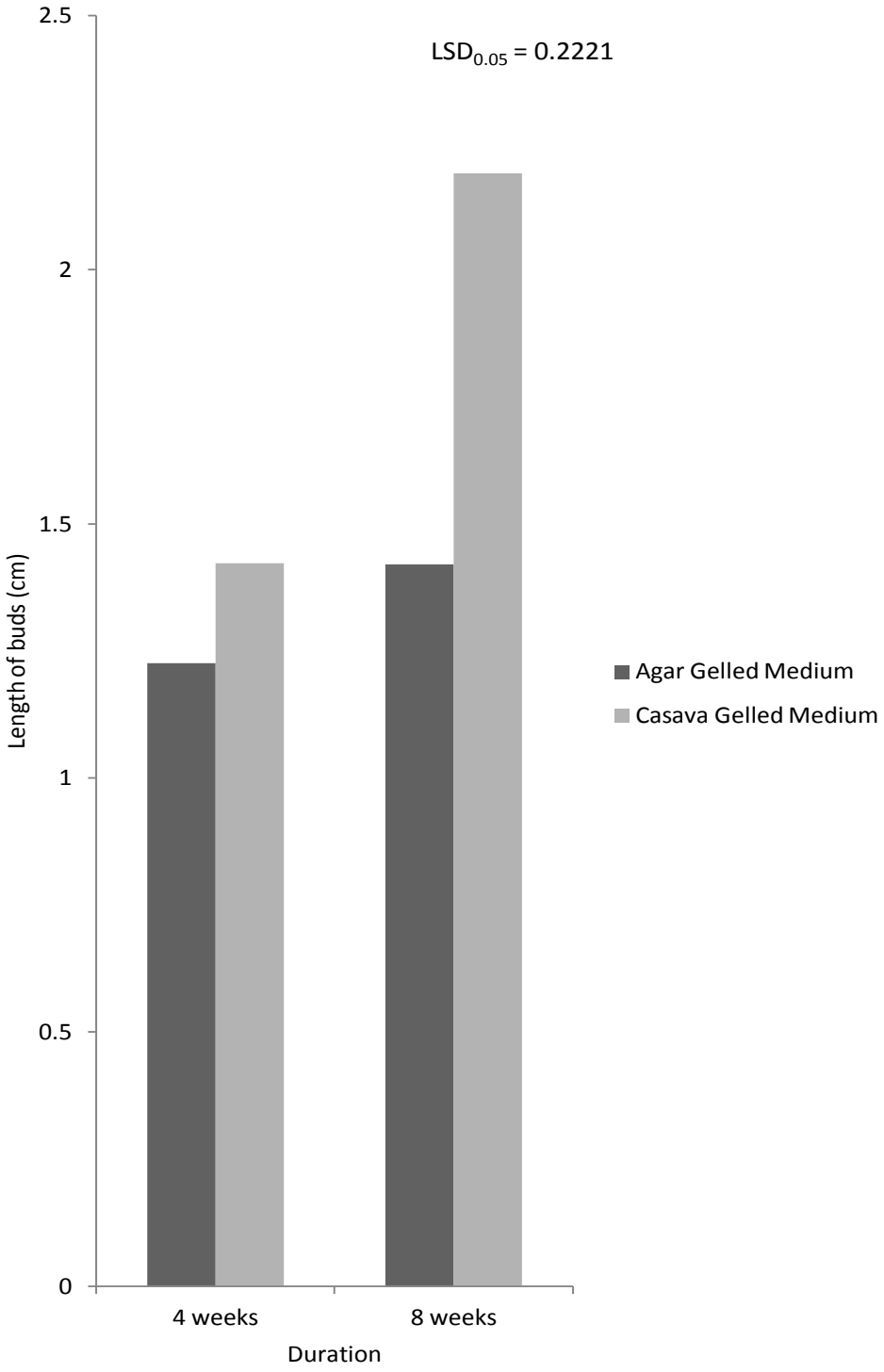

Figure 1. The effects of media (agar and cassava) on length of buds produced by the explants at four weeks and at eight weeks after initiation using t-test.

higher mean value of 2.033 buds per plant as compared to agar with a mean value of 1.9 buds per plant (Figure 2). Cassava also gave a higher mean length of buds value of 1.422 cm, higher than agar with 1.226 cm (Figure 1).

Length of buds produced by the ex-plants at eight weeks after initiation

The hormone variations significantly affected the length of buds produced by the explants. The explants cultured in

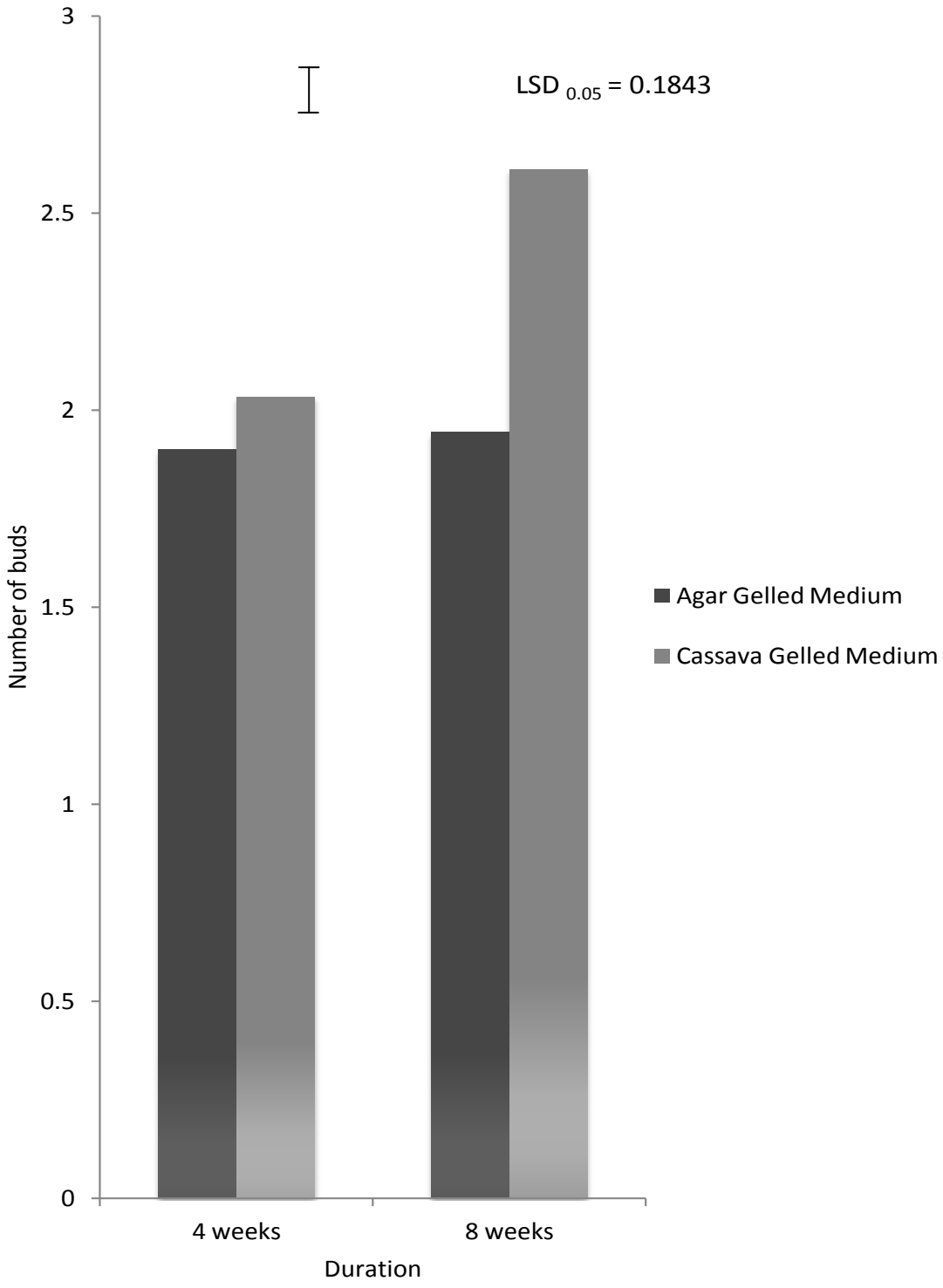

Figure 2. The effects of media (agar and cassava) on number of buds produced by the explant at four weeks and at eight weeks after initiation using Two-sample T-test.

0.3 mg/L NAA in the presence of 0.25 mg/L BAP on agar gelled medium produced the least length of buds at four weeks after initiation in agar gelled medium. They are significantly the hormone treatments with the lowest bud lengths of 0.1 cm. The explants cultured in 0.2 mg/L NAA in the presence of 0.25 mg/L BAP significantly produced the highest length of buds in both agar gelled and cassava gelled medium. With the exception of the

Table 2. Effect of auxin (NAA) and a blanket application of 0.25 mg/L of BAP on length of buds and number of buds produced by the explants at eight weeks after initiation using agar and cassava gelled medium.

Treatment NAA (mg/L)	Mean length of buds (agar)	Mean length of buds (cassava)	Mean number of buds (agar)	Mean number of buds (cassava)
0	1.0	1.5	3.0	1.6
0.1	1.7	2.4	2.4	3.1
0.2	2.8	3.4	1.3	2.5
0.3	0.1	3.0	0.9	2.3
0.4	0.8	2.4	1.7	2.0
0.5	0.8	1.3	1.3	2.5
0.6	1.9	1.7	1.8	2.9
0.7	2.0	1.6	2.8	3.2
0.8	1.7	2.4	2.3	3.4
$Lsd_{0.05}$	1.61	0.57	1.37	0.60

explants cultured in 0.6 and 0.7 mg/L NAA in the presence of 0.25 mg/L BAP, cassava gelled media consistently produced bud lengths that were longer than bud lengths produced in agar gelled medium (Table 2).

Number of buds produced by the ex-plants at eight weeks after initiation

Explants cultured in 0.3 mg/L NAA in the presence of 0.25 mg/L of BAP significantly gave the lowest mean value of 0.9 buds per plant in agar gelled medium while explants cultured in 0 mg/L NAA in the presence of 0.25 mg/L BAP gave the highest mean number of buds of 3.0 buds per plant. In cassava gelled medium, the highest mean number of buds per plant was observed from explants cultured in 0.8 mg/L NAA in the presence of 0.25 mg/L BAP while the lowest mean number of buds per plant came from explants cultured in 0 mg/L NAA in the presence of 0.25 mg/L BAP (Table 2).

Effect of media on length of buds and number of buds at eight weeks after initiation

The result shows a significant media effect on both number of buds and length of buds of explants at eight weeks after initiation. Cassava significantly gave a higher mean value of 2.6 buds per plant as compared to agar with a mean value of 1.9 buds per plant (Figure 2). For length of buds, cassava also gave a significantly higher value of 2.19 cm when compared to agar with 1.42 cm of bud length (Figure 1).

DISCUSSION

Bud proliferation ability of plant tissues and the formation of adventitious roots depend upon the interaction of several endogenous and exogenous factors (Ahmed, 2002). Ontogeny of explants and their position on mother plant greatly affects the *in vitro* development. According to Chern et al. (1993), different explant sources have different growth potential due to differences in age, endogenous metabolic status and differential genome. Physiological status of an explant, concentration of plant growth regulators in culture medium, type of media, media components and their mutual interaction are one of the major determinants among these factors (Palanisamy and Kumar, 1997; Litz et al., 2005). Sucrose has also been reported to have a stimulatory effect on root initiation up to a concentration of 8% (Zimmerman et al., 2007). At four weeks after initiation, explants cultured in 0.2 mg/L NAA supplemented with 0.25 mg/L BAP significantly gave the highest mean bud length of 2.7 cm per plant while 0.7 mg/L NAA supplemented with 0.25 mg/L BAP significantly gave the highest mean value of 2.8 buds per plant (Table 1). Cassava gelled medium gave a higher mean value of 2.033 buds per plant as compared to agar with a mean value of 1.9 buds per plant (Figure 1). Cassava also gave a higher mean length of buds value of 1.411 cm, higher than agar with 1.192 cm (Figure 2). At eight weeks, however, 0.2 mg/L NAA supplemented with 0.25 mg/L BAP gave highest mean bud length of 2.8 cm per plant while 0 mg/L NAA supplemented with 0.25 mg/L BAP significantly gave the highest mean value of 3.0 buds per plant in agar gelled medium.

Cassava significantly gave a higher mean value of 2.611 buds per plant as compared to agar with a mean value of 1.944 buds per plant. For length of buds, cassava also gave a significantly higher value of 2.19 cm when compared to agar with 1.42 cm of bud length. It was speculated that prolonged culturing might diminish the regenerative capacity of shoots due to altered sugar/nitrogen ratio in plantlets (Kuria et al., 2008). This was not observed in cassava medium at eight weeks as compared to bud lengths observed in agar gelled

medium. Since nutrient uptake is closely associated with the rate of water influx into tissues (George, 1993), an agar-solidified medium has lower water potential (more negative) than cassava gelled medium (Debergh and Zimmerman, 1991). Also, lower viscosity of cassava starch gelled medium may explain why plantlets preformed better in medium gelled with cassava starch as compared to plantlets on agar gelled medium. Similar studies have shown that cassava starch at 7% enhanced banana survival *in vitro* and produced consistent results in terms of shoots and roots proliferation as agar over four subcultures (Mbanaso et al., 2001). The better response on cassava starch gelled media could also be due to the absence of inhibitors which have been reported to be present in agar (Debergh, 1983; Singha, 1984; Pierik, 1997; Puchooa et al., 1999). Alternatively, the promontory effect of cassava starch may be because cassava starch can act as an additional carbon source and the beneficial compounds present which acts as ionic supplements (35% carbohydrates, 1% mineral matter) (Onuweme, 1982) to the medium, which resulted in improved cell growth and morphogenesis.

Better performance of plantlets on cassava starch gelled medium as compared to agar is an indication of potential of cassava starch as agar substitute in plant tissue culture. Gel strength is often regarded as an important criterion for agar quality (Debergh, 1983). The concentration of 14% has been reported by Gebre and Sathyanarayana (2001) for shoot regeneration. It is higher than the 8% reported by Nene and Sheila (1997) for tobacco and chickpea culture but less than 30% used in this experiment. Cassava starch is about 50 times cheaper than agar, therefore, it will find greater acceptability as a cheaper alternative to agar in Nigeria, reducing the need to import agar. Cassava starch is a product of plant origin. It is biodegradable and poses no threat to the environment on being properly disposed after use.

Conclusion

Cassava gelled medium at four weeks after initiation, gave a higher mean value of 2.033 buds per plant as compared to agar with a mean value of 1.9 buds per plant. Cassava also gave a higher mean length of buds value of 1.411 cm, higher than agar with 1.192 cm. These differences were however not statistically significant. At eight weeks however, cassava consistently gave a higher mean value of 2.611 buds per plant as compared to agar with a mean value of 1.944 buds per plant. For length of buds, cassava also gave a significantly higher value of 2.19 cm when compared to agar with 1.42 cm bud length.

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