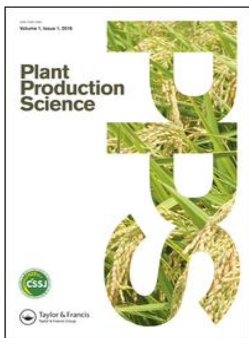

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Tissue Culture

Effects of Different Types and Concentrations of Gelling Agents on the Physical and Chemical Properties of Media and the Growth of Banana (*Musa* spp.) *in Vitro*

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Abstract : Banana shoots were cultured on the medium containing either one of the three gelling agents agar, gelrite and gellan gum for 13 weeks. The concentrations of the nutrient elements especially macro-elements, were higher in gelrite than in agar and gellan gum. Each of the three gelling agents had an optimal concentration for plant growth, but as a whole, shoot growth and multiplication was higher on the medium solidified with 0.9 g L^{-1} gelrite as compared with those on the medium solidified with 4−8 g L^{-1} agar or 2−6 g L^{-1} gellan gum. This may be attributed to the availability and uptake of water and mineral nutrients. Most of the shoots cultured on 0.7 g L^{-1} gelrite or 4 g L^{-1} agar and on the liquid medium showed poor growth and multiplication due to vitrification. The higher concentrations of gellan gum (6 g L^{-1}) and agar (8 g L^{-1}) did not support shoot growth and this was explained by reduced water and mineral salt uptake. At the concentration of each gelling agent that gave the best shoot growth and proliferation (gelrite 0.9, agar 6 and gellan gum 2 g L^{-1}) the water potential of the medium was about −0.5 MPa and the medium with a water potential of about −0.6 MPa did not support plant growth. We concluded that the growth of banana plants *in vitro* was affected by the type and concentration of gelling agent mainly due to the difference in the physical properties of the medium.

Key words : Banana, Elements, Gelling agents, *in vitro, Musa* spp., Water potential.

One of the advantages of *in vitro* micro propagation is the higher rates of multiplication and growth. These advantages are particularly relevant to vegetatively propagated crops (Dirk and Rhodomiro, 1996). The growth and multiplication of shoots *in vitro* are affected by many factors (Israeli et al., 1996), including the chemical and physical properties of the culture medium. One of the most important factors, which affect the chemical and physical characteristics of the culture medium *in vitro*, is the type and concentration of gelling agent. Three types of gelling agents are mainly used : agar, gelrite and gellan gum. Gelrite and gellan gum, the same type of gelling agent, are polysaccharide complex obtained by the same production system. Limited attention has however been paid to the effects of these agents on the chemical and physical state of the medium which may affect the growth and multiplication of shoots *in vitro* (Zimmerman and Broome, 1980). Gelling agents have a strong effect on the growth and development of various explants (Romberger and Tabor, 1971 ; Stoltz, 1971 ; Werner and Boe, 1980). For example, the type and concentration of gelling agent in the culture medium influence the *in vitro* shoot proliferation of many plant species. Singha (1982) reported that the explants of Seckel pear, cultured on the medium solidified with 3 g L^{-1} Difco Bacto Agar showed poor shoot proliferation developing large translucent leaves and woody stems, whereas they grow and proliferate well on the medium solidified with 6 g L^{-1} agar. In tobacco, the agar concentration has been reported to strongly influence morphogenesis and the development of plantlets (Kohlenbach and Wernicke, 1978 ; Than and Trinh, 1978). Singha et al. (1985) reported that the addition of 3, 6 and 12 g L^{-1} Difco Bactor Agar to one liter of MS medium increased the Na concentration of the medium from 202 to 1141, 2080 and 3958 μM, respectively. On the other hand, the addition of the same amounts of Phytar Agar increased Na content from 202 to 280, 357 and 512 μM, respectively.

In addition to the mineral nutrient concentration, the physical properties of the medium, such as water potential and nutrient availability are also affected by the gelling agent (Singha et al., 1985 ; Kusumoto, 1980). The *in vitro* response of plantlets to gelling agent has been reported to depend on plant species (Singha, 1982). Several studies have been done on the effects of gelling agents on the chemical composition of the growth medium with some plant species (Stoltz, 1971 ; Werner and Boe, 1980).

Banana (*Musa* spp), a vegetatively propagated plant, is one of the world's most important crops . Its *in vitro* micropropagation is spreading throughout the world due

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to the bulky nature of the planting material and the prevalence of fungal diseases with the conventional planting material (suckers) (Dirk 1996). However, no work has been done concerning *Musa* with regards to the effects of these gelling agents on the chemical and physical characteristics of the medium or on the growth and multiplication of plantlets *in vitro*. Rather, researchers have used these agents to solidify the media, to support the explant and to control vitrification of the plantlets without considering the type and concentration of the gelling agent. Jarret et al. (1985), Vessey and Rivera (1991), Panis et al. (1993), Cote et al. (1996), and Hirimburegama and Gamage (1997) have used MS media containing 0.64 to 8 g L^{-1} agar for the *in vitro* culture of *Musa*, whilst Novak et al. (1989) and Dhed'a et al. (1991) used gelrite at concentrations ranging from 0.7 to 2 g L^{-1}. On the other hand, Dore et al. (1989) and Vuylskete and Ortiz (1996) used gellan gum at 0.25 and 2 g L^{-1}, but none of these researchers examined the concentration effects of these agents on the medium properties.

Thus in this study, we examined the effects of different gelling agents on the water potential of the medium and the growth and multiplication of banana shoots cultured on it.

Materials and Methods

1. Plant materials

Explants were taken from sword suckers of healthy parents of Shima Banana (AAA), local variety from Okinawa in May 1997. They were grown in the open field with a good watering regime.

2. Preparation of explant

The plant materials were taken early in the morning using an earth chisel to separate the sucker from the parent at the point of attachment. The roots and the tops of the shoots were trimmed off before being washed with running tap water to clean the materials (Buah et al., 1998). The sheaths that form the pseudostem were carefully removed to reduce the size of the material to about 4 leaf sheaths. They were then sterilized with 70% ethanol for three minutes and washed three times in sterilized distilled water.

More of the leaf sheathings were then removed aseptically on a clean bench until about two leaf sheaths covered the shoot meristem. This process was followed by sterilization with sodium hypochlorite solution (1% active chlorine) containing a drop of polyoxyethylene (20) sorbitan monolaurate (Tween 20) for five minutes with occasional shaking, and thereafter washed three times with sterilized distilled water (Cronauer-Mitra and Krikorian, 1987). Prior to their implantation into the media, each shoot tip (about 1 cm) was divided longitudinally into two halves and again sterilized with 1% NaClO as above, for one minute.

3. Media composition

The medium (Murashige and Skoog, 1962) containing 5 μM 6-benzylaminopurine (BA) and 30g sucrose per liter was used throughout the experimental period. The medium was adjusted to pH 5.8 prior to autoclaving at 121°C for 15 minutes. The following gelling agents from WAKO Pure Chemical Ind. Ltd. Japan, were used; agar (a lot number LEP 7877) : 4, 6, 8 g L^{-1}, gelrite (a lot number LEN 7994) : 0.7, 0.9, 1.1 g L^{-1} and gellan gum (lot number APK 7609) : 2, 4, 6 g L^{-1}. The concentrations used in this experiment were 4, 6, and 8 g L^{-1} for agar, 0.7, 0.9, and 1.1 g L^{-1} for gelrite and 2, 4 and 6 g L^{-1} for gellan gum which were chosen based on our previous research and data reported previously. We set a control treatment (liquid MS medium) with the same composition but without any gelling agent. For the culture in the control medium, the Heller filter paper method (Singha, 1982; Singha et al., 1985) was used to support the explants. In each bottle, two filter papers previously autoclaved were folded with an opening at the center where the explant will be placed. The medium was then poured into the bottles for autoclaving and ten replicate cultures were conducted for each treatment.

4. Culture conditions

The culture bottles were placed in a growth chamber under conditions of 26°C, 16 hr photoperiod and a photon flux density of 71 μmol photons m^{-2} s^{-1}, in a randomized fashion. Initial subculturing onto fresh medium was done after three weeks and thereafter, at every two weeks.

5. Measurement of the water potential of medium and plant

An isopiestic psychrometer (Boyer, 1995) was used to measure the water potential of the various media and that of the leaves of the banana plants cultured on these media for 13 weeks. Prior to the measurements, the jointed parts of the psychrometer were made airtight by applying Vaseline to avoid the escape of water vapor from the samples. The thermocouples were then cleaned in 90% ethanol and rinsed in sterilized distilled water after which they were dried with an air stream. Leaf discs of about 2.0 cm in diameter were taken from the shoots with a cork borer. The leaf discs were then blotted with a wipe to remove any water adhering to the surfaces. They were then placed in the vapor chamber and covered with the cylinder containing the thermocouple. A drop of sucrose at a chosen concentration was placed at the tip of the thermocouple before inserting it into the cylinder that covers the chamber. For the media, samples of about 0.4 g were taken for the measurements (Boyer, 1988, 1995).

6. Elemental analysis of shoots and gelling agents

Elemental analysis was done on the banana shoots after 13 weeks of culture. The wet ashing method as

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described by Rechcigl and Payne (1990), was used for the plant analysis, while the dry ashing method (Greweling, 1976) was used for the analysis of the gelling agents. Prior to the analysis, the shoots were washed of all media with water and dried in an oven at 80°C for 72hr. Ten dried shoots from each treatment were ground and 1g of sample was used for the analysis. The same weight of the gelling agents was also used. Each treatment was replicated three times.

Wet ashing method

Five milliliter of concentrated sulfuric acid was carefully added to the sample and digested for 2h at 330°C. After cooling for 15 min, 4−5 drops of H_2O_2 was added and further digested for 5min to obtain a clear solution. It was then digested again for 1hr. Distilled water was added to the cooled solution to top it up to 50 mL. This was allowed to stand over night to settle out the silica before decanting the supernatant into tubes for spectrometric analysis with an inductively coupled plasma spectrometer (ICPS-2000, Shimadzu).

Dry ashing method

One gram of the sample was ashed in a muffle furnace at 550°C for 4hr. The ash was then moistened with a few drops of distilled water and then 2 mL of concentrated hydrochloric acid was added. This was evaporated to dryness on a hot plate and baked for an additional 1hr. After removing sample from the hot plate, 2.5 mL of 2 M nitric acid was added. The solution was then transferred to a 20 mL volumetric flask and brought to volume with distilled water. It was left overnight for silica to settle before removing aliquots for analysis.

Before the analysis, the samples were filtered twice by passing the solution through a filter paper (No. 6, 150 mm in diameter) to remove any particles. They were then injected into the ICPS for the analysis of their elemental composition and concentrations.

Total nitrogen and carbon contents for the various gelling agents were measured with an analyzer (NC-90A, Shimadzu).

Results

1. Shoot growth

Shoot growth was greatly affected by the different concentration of the gelling agents applied. The optimal concentration for shoot growth (fresh weight) was 0.9 g L^{-1} for gelrite, 6 g L^{-1} for agar and 2 g L^{-1} for gellan gum (Fig. 1). At each optimum concentration, shoots cultured on gelrite (0.9 g L^{-1}) had a higher fresh weight than those on agar (6 g L^{-1}), gellan gum (2 g L^{-1}) or the liquid medium. The higher concentrations of agar (8 g L^{-1}) and gellan gum (6 g L^{-1}) did not support plant growth and this led to wide variations among those treatments. Gelrite at 0.9 g L^{-1} was far more favorable for the increase in fresh weight than that at 0.7 and 1.1 g L^{-1} (Fig. 1). This pattern was also observed for the increase in plant height and shoot dry weight (Figs. 2 and 3). There was a large difference between the dry weight of shoots cultured on 4 and 6 g L^{-1} agar. The optimal concentration for the increase in the number of shoots per culture was similar in all gelling agents (Fig. 4). However, the number of shoots was greatly influenced by the concentration of each gelling agent. The number of leaves per shoot was influenced by gelling agents similarly to the number of shoots (compare Figs. 4 and 5).

2. Elemental analysis

The spectrometry results indicated that wide variations existed in the elemental concentration of the three gelling agents (Table 1). The major component of all the agents was carbon. Gelrite contained relatively high amounts of the elements as compared with the other gelling agents, especially K, Na, S, P and Mg. Agar contained higher amounts of most of the elements than gellan gum. On the contrary, gellan gum contained higher amounts of Al and B than gelrite and agar. It also contained higher amounts of S than agar.

The shoots cultured on gelrite (0.9 g L^{-1}) had higher concentrations of most of the macro- elements, especially K and N, than those cultured on gellan gum and agar, with the exception of P which was contained at higher concentrations in the shoots cultured on 2 g L^{-1} gellan gum than in those cultured on gelrite or agar (Table 2). Shoots cultured on 4 g L^{-1} gellan gum had the least concentration of macro elements but the concentration was higher than in liquid medium. The situation was different for the concentration of the micro elements. Shoots cultured on agar, gellan gum and liquid media had higher concentrations of most of the micro elements than those on gelrite (Table 2). There was no definite correlation between the concentration of elements in the shoots and that of the gelling agent in the medium. The concentration of some elements in the shoots increased but that of some other elements decreased with increasing concentration of gelling agent. For example, the concentration of Mg, P, Na and N in the shoots increased with increasing gelrite concentration up to 0.9 g L^{-1} and then decreased at the concentration of 1.1 g L^{-1}, whilst the concentration decreased with increasing concentration of gellan gum from 2 to 4 g L^{-1}. The concentration Al, Mn, Cu and Zn in the shoot increased with increasing concentration of gelling agent, with a few exceptions.

3. Water potential

The increase in the concentration of gelling agent resulted in a decrease in the water potential of the medium (Table 3). At the concentration of each gelling agent that gave the best shoot growth and proliferation (gelrite 0.9, agar 6 and gellan gum 2 g L^{-1}) the water potential of the medium was about −0.5 MPa and the medium with a water potential of about −0.6 MPa did not support plant growth. The decrease in water potential of the medium was reflected in the water potential of

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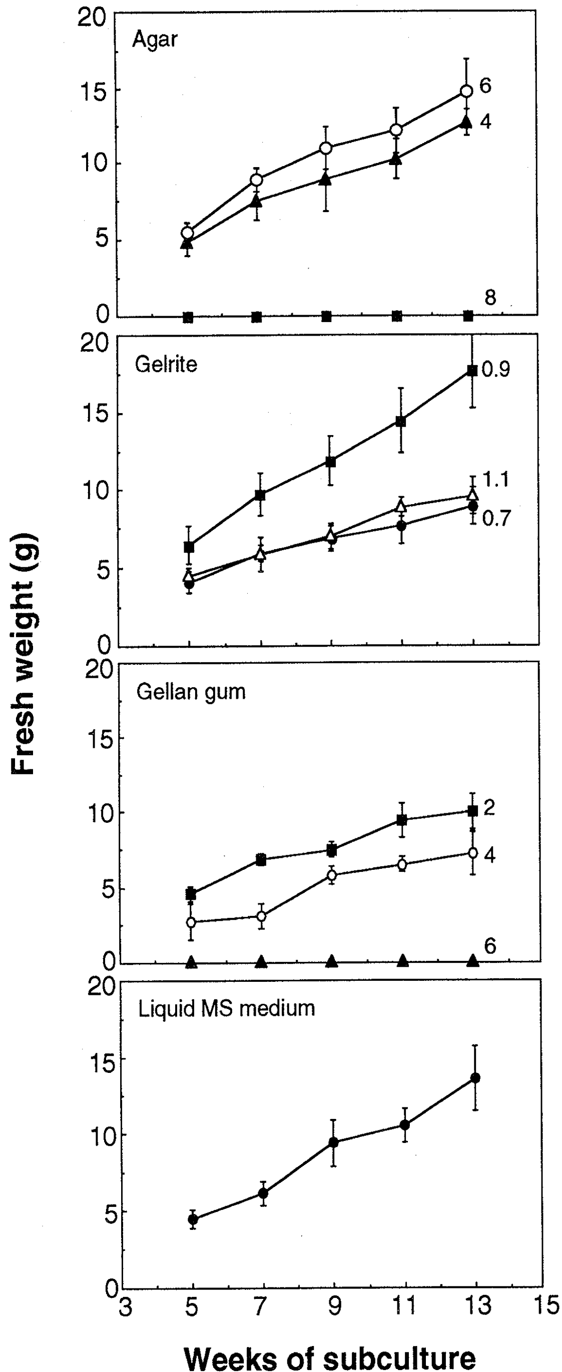

Fig. 1. Fresh weight gain of banana shoots cultured on three gelling agents at different concentrations (g L^{-1}) (numerals in the figure) for 13 weeks. Vertical bars show standard deviations.

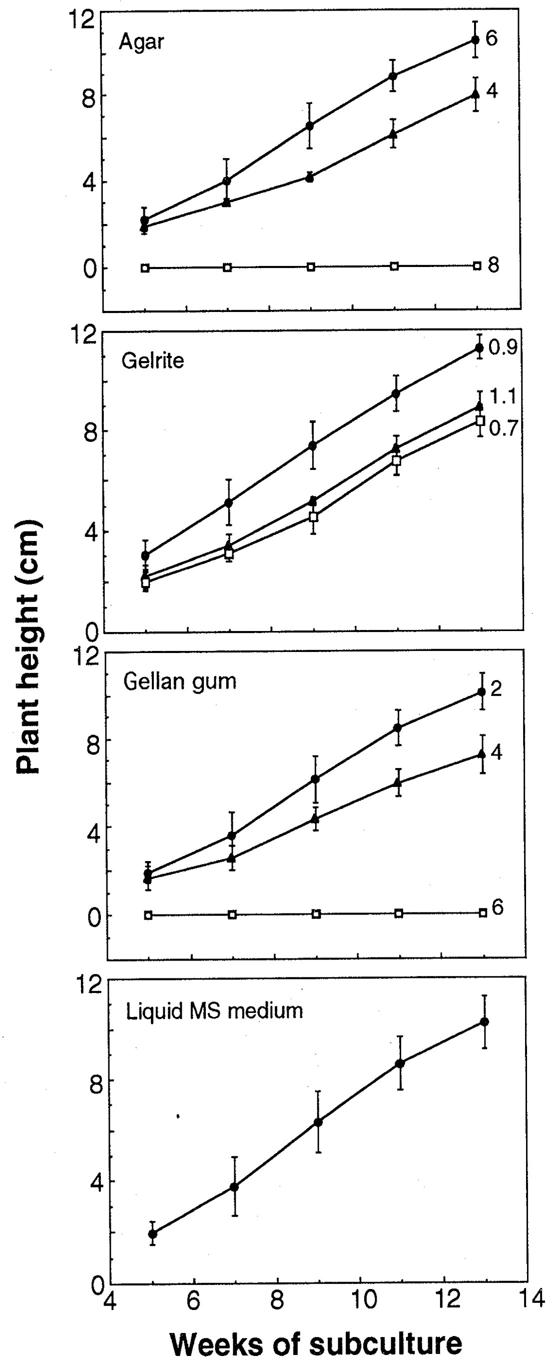

Fig. 2. Height of banana shoots cultured on three gelling agents at different concentrations (g L^{-1}) (numerals in the figure) for 13 weeks. Vertical bars show standard deviations.

the leaves. It was also noted that small differences in medium water potential caused large differences in the leaf water potential.

Discussion

In previous study on the *in vitro* micropropagation of banana various gelling agents at various concentrations have been used and no attention was paid to the type and concentration of gelling agent. The elemental concentration varied with gelling agents (Table 1). However, the concentration of the gelling agent in the medium was very low and the addition of 1.1 g of gelrite to 1 liter of medium adds only 0.07 mg of P to the medium. Therefore, the differences in the growth of the shoots on the medium with different gelling agents may not be attributed to the chemical concentrations of the gelling agent in the medium. With a few exceptions, the concentrations of various elements in the shoots were not correlated with the concentration of the gelling agent used, in agreement with the report of Singha et al. (1985). Data on the comparative elemental analysis of gellan gum and gelrite for comparison does not exist.

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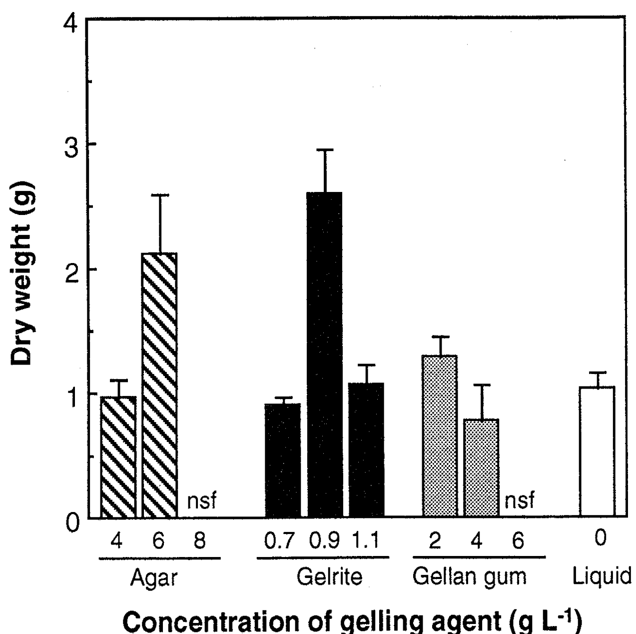

Fig. 3. Dry weight of banana plants cultured on three gelling agents at different concentrations. Data were obtained after 13 weeks of culture. Vertical bars show standard deviations. nsf, no shoot formation.

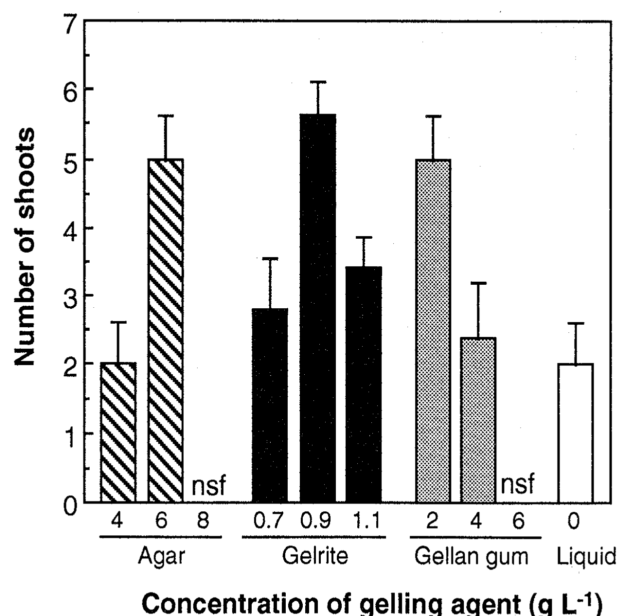

Fig. 4. Number of shoots per banana explants cultured on three gelling agents at different concentrations for 13 weeks. Vertical bars show standard deviations. nsf, no shoot formation.

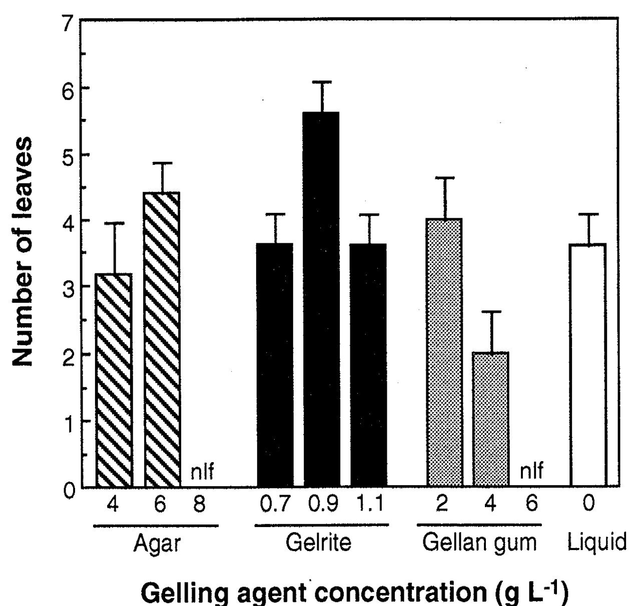

Fig. 5. Number of leaves per banana shoot cultured on three gelling agents at different concentrations for 13 weeks. Vertical bars show standard deviations. nlf, no leaf formation.

Table 1. Concentration of elements in the gelling agents used in this experiment.

Element	Gelling agents		
	Agar	Gelrite	Gellan gum
B	0.08±0.01*	1.29±0.03	1.44±0.07
Mg	39.87±1.13	49.22±0.63	30.16±0.78
Al	3.27±0.09	2.85±0.09	14.56±0.04
Si	2.06±0.06	3.00±0.03	1.65±0.02
P	35.56±0.22	65.10±0.26	6.42±0.16
S	21.13±0.23	273.93±0.10	63.53±0.19
Ca	128.90±0.09	116.34±0.09	72.17±0.25
Mn	0.09±0.01	0.45±0.04	0.11±0.00
Fe	5.40±0.48	8.68±0.45	2.87±0.07
Cu	0.24±0.04	0.17±0.02	0.06±0.00
Zn	0.37±0.04	0.37±0.01	0.15±0.02
Mo	0.02±0.01	0.03±0.01	0.01±0.00
Na	152.41±7.38	212.15±7.75	29.32±2.86
K	590.49±1.77	1,774.59±13.29	6.43±0.83
N	0.4±0.03**	0.81±0.08	0.75±0.07
C	413.54±1.44	363.54±4.83	339.40±10.45

*, Elemental concentration expressed as μg (g DW)$^{-1}$
**, C and N concentrations expressed as mg (g DW)$^{-1}$
Values are shown in mean±SD.

The favorable growth of banana shoots on 0.9 g L^{-1} gelrite, 6 g L^{-1} agar and 2 g L^{-1} gellan gum could be partly attributed to the availability of mineral nutrients, such as, K, P, Ca, Mg and Na which are needed for the growth of banana shoots (Table 2). Although Al at the concentration of 4−35 μg g^{-1} DW of gelling agent has been reported to be toxic under *in vitro* conditions (Lahav, 1996), we did not observe any clear correlation between the growth of the shoots and the Al concentrations in the shoots. Therefore, it was not possible to attribute the growth suppression at the high concentration of gelling agent to Al toxicity.

The water potential of the medium decreased with increasing concentration of gelling agent in agreement with the report of Deberg et al. (1981) and Kusumoto (1980) who reported that high agar concentrations results in decreased media water potential and increased solidity of the medium. A slight difference in medium water potential caused a large effect on the leaf water potential. For example, the difference between the water

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Table 2. Elemental concentrations of banana shoots cultured on three gelling agents at diffeent concentrations.

Element	Agar		Gelrite			Gellan gum		Liquid
	40*	6.0	0.7	0.9	1.1	2.0	4.0	
Mg	335.2±0.0**	372.9±1.3	436.9±0.7	465.4±9.6	429.6±7.5	327.8±24.6	302.4±13.8	307.2±1.9
P	375.6±1.4	641.1±0.0	625.6±21.4	834.3±0.97	685.4±5.6	1036.6±12.6	844.9±7.6	319.3±1.9
Na	194.5±0.9	244.6±0.8	309.3±6.2	552.5±7.9	279.1±0.9	327.2±13.2	199.4±7.3	157.2±0.1
K	9876.8±1.7	8624.9±1.0	9378.4±1.9	10940.0±6.8	8664.4±1.4	9246.0±1.8	8606.0±3.1	7803.3±0.6
N***	50180.0±0.0	56290.0±0.0	47280.0±0.8	58680.0±0.5	54420.0±0.4	56110.0±3.5	46310.0±1.3	30100.0±0.3
Al	35.6±2.1	39.0±2.0	12.1±1.0	13.3±0.2	18.5±0.1	42.2±2.5	43.0±0.8	12.5±6.0
Mn	102.9±2.4	176.0±0.0	2.1±0.2	18.2±0.5	25.3±1.0	17.3±0.1	15.4±0.5	93.2±7.5
Cu	0.3±0.0	17.9±1.1	0.1±0.0	0.6±0.0	16.8±0.0	0.6±0.0	17.1±0.0	1.3±0.0
Zn	0.1±0.0	14.9±0.0	15.8±0.2	16.1±5.0	17.1±5.0	17.0±1.1	20.5±0.9	0.1±0.0

*Concentration of gelling agents in g L^{-1}; **Concentration of elements in μg $(gDW)^{-1}$; ***Measured by N/C analyzer.

Table 3. Water potential of media contaning various gelling agents at various concentrations and that of banana leaves cultured on them for 13 weeks.

Gelling agent	Concentration (g L^{-1})	Water potential (MPa) Medium	Leaf
Agar	4.0	−0.496±0.004	−0.192±0.012
	6.0	−0.504±0.005	−0.292±0.032
	8.0	−0.578±0.002	nd
Gelrite	0.7	−0.411±0.005	−0.144±0.012
	0.9	−0.501±0.010	−0.198±0.007
	1.1	−0.534±0.015	−0.348±0.003
Gellan gum	2.0	−0.499±0.010	−0.237±0.016
	4.0	−0.576±0.010	−0.429±0.003
	6.0	−0.628±0.043	nd
Liquid MS medium		−0.398±0.014	−0.104±0.004

Values are shown as means±SD. nd, no data available.

potentials of medium with 0.9 and 1.1 g L^{-1} gelrite was 0.033 MPa whereas the difference between the water potentials of the leaves was 0.150 MPa. Deberg et al. (1981) and Kusumoto (1980) also reported that small changes in medium water potential results in large differences in leaf water potential. It was observed that the optimal concentration for shoot growth of gelling agent gelrite, agar and gellan gum was 0.9, 6 and 2 g L^{-1} respectively and the water potentials at each concentration was about −0.5 MPa. The growth of the shoot seemed to be favorable in media with a water potential of about −0.5 MPa. Growth was suppressed at a higher or lower concentration of gelling agent.

The variation in the growth and multiplication of shoots on various kinds of media at various concentrations may be attributed partly to the differences in the water potential of the medium which is reflected in the water potential of the leaves and affects the growth of plant. For example, Ghashghaie et al. (1991), Nonami and Boyer (1989) and Boyer (1968) all reported the reduced shoot growth at low leaf water potential and this was attributed to decreased photosynthesis. Boyer (1968) reported that leaf water potential of maize ranging from −0.46 to −1.0 MPa resulted in high photosynthetic activity and that the growth was best at −0.58MPa. Ghashighaie et al. (1991) also reported that shoot growth of rose plants decreased as leaf water potential decreased from −0.27 to −8.0 MPa. They reported a decrease in fresh weight from 700 to 400 mg and dry weight from 500 to 250 μg within the above range of leaf water potential. The optimum leaf water potential in our experiment coincides with the range of Ghashighaie et al. (1991), and we believe that at the optimal concentrations of the gelling agents, the leaves had water potentials that favored photosynthetic activity and growth accompanied with the high nitrogen concentrations of the shoots.

Unlike nursery conditions where humidity is low, plants cultured *in vitro* are subject to high humidity inside the culture vessels, resulting in low transpiration. This reduces the effect of transpiration on water and mineral nutrient absorption. Thus absorption of water and mineral nutrient under *in vitro* conditions is mainly by active mechanisms. Boyer (1988) and Kramer and Boyer (1995) reported that the high availability of water from the medium results in the high leaf water potential and the extension of the cells. This creates an active water potential difference between the medium and the leaf, called growth-induced water potential, which makes the movement of water into the plant less dependent on transpiration. Hence, the optimal medium conditions that promotes growth, resulting in the creation of growth-induced water potential is very important for the absorption of water and mineral nutrient *in vitro*.

Most of the shoots cultured in the liquid medium had abnormal leaves and the number of shoots formed was lower than those cultured on the medium solidified with optimal concentrations of gelling agents. Most of the shoots in the liquid medium had long, slender and weak stems, with thick rigid and curled leaves. Romberger and Tabor (1971) have similarly reported the formation of thicker, abnormal rigid cell walls in the plant cultured *in vitro* which was due to the submergence of the shoot apical meristem in the liquid medium which consequently affected their growth and multiplication. Novak et al.

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(1986), Hiratsuka et al. (1989), Vessey and Rivera (1980) and Hirimburegama and Gamage (1997) have cultured banana on different concentrations of agar and reported that agar concentration higher than 7 g L^{-1} or lower than 5 g L^{-1} results in low shoot multiplication and poor growth *in vitro*.

The leaf water potential of the shoots cultured on the media solidified with three kinds of gelling agents, each at the optimum concentration, somewhat differed with each other (Table 3). In addition, shoot growth was not correlated with the leaf water potential. For example, the growth of shoots cultured on 6 g L^{-1} agar was better than that on 2 g L^{-1} gellan gum but the leaf water potential of shoots cultured on 6 g L^{-1} agar was lower than that on 2 g L^{-1} gellan gum and 0.7 g L^{-1} gelrite. In liquid medium (control), both medium water potential and the leaf water potential for the control treatments were higher than in other media but shoot growth was almost the same as that on 6 g L^{-1} agar. Romberger and Tabor (1971) reported that, certain reactions between agar and sucrose proceed under the autoclaving conditions. He reported that such reactions may produce some substances that affect the growth of plants *in vitro*. It is possible that such substances affected the growth of the banana plants, and so further work is necessary in this respect.

The solidity of the medium increases with increasing concentration of gelling agent and this might affect the growth of the banana explants. Bornman and Vogelman (1984), Singha et al. (1985) and Ghashigaie et al. (1991) reported that the absorption of cytokinin and mineral nutrient from the medium was reduced at high gelling agent concentration.

Under *in vitro* conditions, sucrose is often added to the medium to supply carbon and energy. The breakdown of sucrose into fructose and glucose in the medium is very important for the growth of the explants. Invertase which diffuses out from the plant into the medium has been reported to be responsible for the breakdown of sucrose but at high concentrations of gelling agents, the diffusion of invertase into the medium is restricted (Romberger and Tabor, 1971). They reported that this restriction on the diffusion of invertase accounts for about 51% of the agar concentration effect on reduced plant growth *in vitro*.

We did not analyze the invertase concentrations in the medium but we suppose that its diffusion might have been inhibited at high concentrations of gelling agent.

All the shoots in the present experiments were cultured under the same conditions except for the types and concentrations of gelling agents. Therefore any difference in the growth of banana plants *in vitro* should be attributed to the difference in the type and concentration of gelling agent. Each of the gelling agents had an optimal concentration for plant growth, but 0.9 g L^{-1} gelrite gave better growth and multiplication than other gelling agents at the optimal concentrations.

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