EFFECT OF DIFFERENT SUGARS ON SHOOT INDUCTION IN CV. BASMATI

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ABSTRACT

Effect of various concentrations of different sugars was investigated for induction of root and shoot from basmati rice. Explant, seeds (grains) were transferred in ½ MS basal media fortified with different concentrations of carbon source like sucrose, maltose and dextrose. *In vitro* regenerated rice plantlets were healthy and attained a length of 16.17cm at 0.25 % concentration within a week. Only low concentration (0.25%) of dextrose exhibited the maximum shoot and root growth, out of three sugars. Maltose showed the moderate growth response of shoot and root length in all the concentrations.

INTRODUCTION

The composition of culture medium is a major determinant of *In vitro* growth of plants. The mineral salts sugar as carbon source and water are the main components for most plant tissue culture media (Gamborg and Phillips, 1995). Sugar is an important component in medium and its addition is essential for *In vitro* growth and development of plants because photosynthesis is insufficient, due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis (Pierik, 1997). The sugar concentration chosen is dependant on the type and age of growth material; very young embryos require a relatively high sugar concentration. Generally the growth and development increases with sugar concentration, until an optimum is reached and then decreases at high concentrations. The most commonly used source of carbon is sucrose at a concentration of 2- 5%. Glucose and fructose are also known to support good growth of some tissues (Bhojwani, Razdan, 2004).

Key words: Sugar, Sucrose, Dextrose, Maltose, Rice, Tissue culture, Rice

MATERIALS & METHODS

Plant Material and explants: Matured certified seeds of rice were obtained from National Agriculture Research Council, Khumaltar, Kathmandu (Nepal). The seeds were washed under running tap water for 30 minutes and with Teepol for 5 minutes. After rinsing with sterile DDW, they were surface sterilized in 0.1% (w/v) $HgCl_2$ for 10 minutes and again rinsed thoroughly with sterile DDW. The rice grains were then dissected for excision embryo. Excised embryo was then transferred on 1/2MS and full MS (Murashige, Skoog, 1962) basal medium containing 0.5- 10% w/v different sugars concentration and solidifies with 0.8% agar. The employed sugars were sucrose, maltose and dextrose.

Medium and cultural conditions: Half and full MS basal media containing various concentrations of sugars (0.5% to 10%) were used for regeneration of shoots from the embryo of rice. The pH of medium was adjusted to 5.8 prior to the addition of agar 0.8% (w/v) agar and autoclaved at 15 lbs and 121^0C for 20 minutes. All the cultures were maintained at 25 ± 2^0C with 16 hrs photoperiod under 3000 lux fluorescent light intensity.

Hardening and acclimatization: Well rooted plantlets were carefully removed from culture vessels, washed under running tap water to remove the remnants of agar. After proper removal of agar dip the plantlet in the IAA solution (0.1%) for 1 minute and transferred to tray containing sand. For 1 week, the potted plantlets were kept under transparent polythene membrane to ensure high humidity, and then they were kept in open in diffuse light for hardening. After 7 days, the surviving plants were transferred to pots containing garden soil and maintained in green house for acclimatization.

Statistical Analysis: Each experiment was repeated three times and each treatment had 10 replicates. The number of explants exhibiting regeneration was identified and the size of the shoot and root were determined. The data on size of shoot and root per explants were analyzed using descriptive statistical method to arrive at mean of shoot length; for variability in shoot lengths between the treatments was computed by calculating the standard deviation.

RESULTS AND DISCUSSION

A perusal of the data in Table 1-3 reveals that effect of different concentration of sugars on shoot regeneration in rice grain. Among the different sugars, dextrose was found to be superior for plant regeneration when explanted was seed. Observation on shoot response of rice recorded that the root & shoot length decline with the increase in the concentration of dextrose. Root and shoot length at 0.25% concentration was found to be maximum i.e., 16.17 cm and 5.25 cm, respectively (Table: 2). Observations with regard to the effect of various concentration of maltose on the root & shoot length were revealed that high concentration of maltose retard the shoot & root length. Maximum root (2.84 cm) and shoot (10.1 cm) were observed at 0.25% and 2.84, respectively (Table: 1). In case of sucrose, 0.25% concentration shows the maximum shoot and root length. Root and shoot growth were promoted by the lowest concentration (0.25%).

Present findings show variations against earlier observations recorded by tissue culturists (Bhojwani & Razdan, 2004). In the present work, variation in shoot response was observed in different sugars and author found that lower concentration of dextrose enhance the root and shoot growth in comparison to sucrose and maltose. Thus, the protocol established in the present study in which sucrose has been replaced by dextrose is more efficient and can be used for further tissue culture experiments of rice grain.

Table 1: Effect of Starch on shoot regeneration from embryo of Rice in ½ MS media after a week of culture

Dextrose (%)	Regeneration per explant (%)	No. of shoots (cm)	No. of roots (cm)
0.25	100	10.1 ± 1.8	2.1 ± 0.5
0.5	100	8.3 ± 3.0	2.0 ± 0.2
0.75	100	9.3 ± 6.4	2.5 ± 0.7
1	100	5.3 ± 2.8	2.8 ± 0.9
2.5	100	8.2 ± 0.6	2.7 ± 0.4
5	100	9.5 ± 2.2	3.5 ± 0.5
10	-	3.1 ± 2.8	2.6 ± 0.2

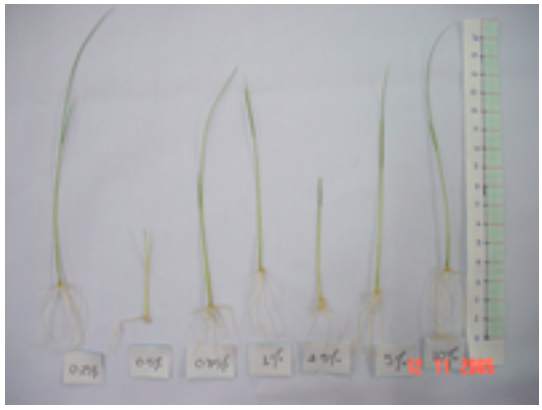
Table 2: Effect of Dextrose on shoot regeneration from embryo of Rice in ½ MS media after a week of culture

Dextrose (%)	Regeneration per explant (%)	No. of shoots (cm)	No. of roots (cm)
0.25	100	16.2 ± 3.1	5.3± 1.7
0.5	100	14.8 ± 1.1	4.2 ± 1.9
0.75	100	10.0 ± 4.8	2.2 ± 0.1
1	100	13.1 ± 7.3	3.9 ± 1.1
2.5	100	10.5 ± 4.3	4.7 ± 0.3
5	100	5.3 ± 4.6	3.3 ± 0.6
10	-	-	-

Table 3: Effect of Maltose on shoot regeneration from embryo of Rice in ½ MS media after a week of culture

Dextrose (%)	Regeneration per explant (%)	No. of shoots (cm)	No. of roots (cm)
0.25	100	15.0 ± 0.8	3.9 ± 0.1
0.5	100	12.5 ± 3.0	3.5 ± 0.2
0.75	100	11.5 ± 4.8	2.9 ± 0.2
1	100	14.6 ± 1.9	3.59 ± 0.1
2.5	100	8.3 ± 2.6	3.58 ± 0.9
5	100	12.9 ± 2.4	3.75 ± 0.6
10	100	8.35 ± 0.2	2.1 ± 1.0

Figure 1. Effect of different concentrations of sucrose on shoot regeneration of rice cv. 'Basmati'



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REFERENCES

1. Bhojwani, S. S, Rajdan, M. K., 1996. Plant tissue culture: theory and Practice, a revised Edition, Elsevier Publication, Netherland, 47- 48.

2. Gamborg, O. L., Phillips, G. C., 1995. Plant cell, tissue and organ culture. Spring-Verlag Berlin Heidelberg, 21- 22.

3. Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15: 431- 497.

4. Pierik, R. L. M. (1987). *In vitro* culture of higher plants. Martinus Nijhoff, Dordrecht, 24- 26.