IMPROVEMENT OF INDICA RICE PLANT REGENERATION FROM CALLUS THROUGH MANIPULATION OF CULTURE CONDITIONS

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Introduction

Since the early reports on plant regeneration from rice callus (1, 2), most of the attention has been paid to concentration of nutrients and phytohormones, although osmolarity of media and tissue water content are also important (3, 4). In the present study, we report simple changes which yielded relevant improvements of plant regeneration.

Materials and Methods

Callus

Callus cultures from mature seeds of the commercial Indica-type Perla variety were established and maintained for six weeks according to reported procedures (5).

Plant regeneration

Regeneration medium had 3 % sucrose and 0.7 % agar (unless otherwise stated). Callus were incubated for six weeks at 27 ± 1 °C in a glass room under natural sunlight.

When conditions were tested, this medium was used as control.

Regeneration media: three regeneration media based on MS (6) were tested: *KIBAN* (kinetin 3, BAP 0.5, NAA 1.0 mg/L); *MB* (BAP 0.5 mg/L); *MZA* (IAA 1.0, zeatin 0.05 mg/L). All further regeneration experiments were done using KIBAN medium.

High concentration sucrose: before regeneration, callus were incubated for seven or fourteen days on 6 % sucrose, and the effects on the regeneration compared with the usual 3 % concentration.

Increased agar concentration: regeneration of callus on agar (Sigma A 9915) concentrations of 1.0 and 1.3 % was compared with the recommended 0.7 % concentration.

Increased Phytagel™ concentration: regeneration on Phytagel™ (Sigma P 8196) at 0.42 or 0.64 % was compared with the recommended 0.3 % concentration.

Dehydration treatment: regeneration of callus dehydrated on sterile filter paper discs at 27 ± 1 °C for 3, 6, 12, 24, 48, or 72 h was compared with no treated callus.

Results and Discussion

Table 1 shows mean values of results obtained. Of the three described and previous regeneration media tested in our lab, KIBAN gave the best results, increasing the number of callus with regenerated plants up to 26 %. With this medium we obtained for the first time in the variety Perla an average of more than one plant per each plated callus. Taking into account this result, KIBAN medium was used in all subsequent experiments looking for increasing regeneration.

For both, Phytagel™ and agar, increasing gelling agent concentration in the regeneration medium resulted in higher values of regenerating callus and average of plants per callus, reaching the significant level of the latest (3.22 per callus) with Phytagel™ at 0.42 %. Preculture of callus on sucrose 6 % for one week almost doubled regeneration efficiency, but the average of plants per callus remains unchanged.

Dehydration of callus for 24 h, in spite of being the simplest, cheapest, and fastest treatment, increase dramatically both regeneration efficiency and average of regenerated plants per callus, resulting in values more than times higher as compared with no treated.

Starting from the preliminar results above described, we have in mind to combine more than one treatment to test whether this approach result in further increase of regeneration.

Table 1.

Manipulated factor	Best treatment	Regeneration %	Plant / callus mean
Regeneration medium	KIBAN	26	1.06
Phytagel concentration	0.42 %	30	3.22
Agar concentration	1.3 %	44	2.16
Sucrose concentration	6 % / one week	46	1.07
Dehydration	24 h	65	2.50

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