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Short Communication

Contribution of Sorbitol on Regeneration of Embryogenic Calli in Upland Rice

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ABSTRACT

Four upland rice cultivars were evaluated to determine the impact of 0, 10, 20 and 30 g L^{-1} sorbitol for callus induction and regeneration response on the MSB5 medium. These findings indicated that the incorporation of an appropriate amount of sorbitol significantly enhanced callus induction in only two of the cultivars, Kusan and Siam (10 g L^{-1}). The positive effect of sorbitol was also detected in the regeneration experiment with all cultivars. The optimal level of sorbitol was 20 g L^{-1} for all of the cultivars except Selasi, for which 10 g L^{-1} led to the highest regeneration frequency. Interestingly, the addition of 20 g L^{-1} sorbitol in the culture media increased regeneration response (%) from 10 and 15 to 40 and 45 in Kusan and Siam, respectively (the low regeneration capacity cultivars). However, 30 g L^{-1} sorbitol decreased the response in both callus indication and regeneration frequencies in all tested cultivars. © 2011 Friends Science Publishers

Key Words: Callus induction frequency; Plantlet regeneration frequency; Upland rice
Abbreviations: 2, 4-D, 2, 4-dichlorophenoxyacetic acid; NAA, α-naphthalene acetic acid; BAP, 6- benzylaminopurine; Kin, Kinetin.

INTRODUCTION

Plant gene transfer technology has become an important tool for rice breeding programs, but most attempts regarding upland rice have failed. The poor level of regeneration ability in such upland rice cultivars has limited the use of gene transformation (Geng *et al.*, 2008). However, recent advances in the rice tissue culture system may overcome this problem. Much attention to this theme has also brought about the establishment of more effective and less genotype dependent protocols (Yookongkaew *et al.,* 2007). With respect to independent genotype protocols, some reports have shown that using proper plant growth regulators, carbon source and adding adequate amounts of specific chemicals (i.e., tryptophan, proline & sorbitol) can result in drastic increases in the regeneration frequency, regardless of the genotype constraints (Chowdhry *et al.,* 1993; Ge *et al.,* 2006; Zaidi *et al.,* 2006; Geng *et al.*, 2008; Shahsavari, 2010). According to the literature, adding sorbitol into the tissue culture media may promote both callus induction and regeneration rates dramatically in apples(Lin & Chen, 1997), loquats (Ma & Wangm, 1996), maize (Swedlund & Locy, 1993) rice (Geng *et al.*, 2008) and wheat (Ryschka *et al.*, 1991). However, the response of the different cultivars of Malaysian upland rice to varied concentrations of sorbitol doses is not yet fully understood. Therefore, this experiment was conducted in order to discover the impact of sorbitol concentrations on the tissue culture of four cultivars of Malaysian upland rice.

MATERIALS AND METHODS

The mature seeds of four cultivars of upland rice known as Kusan, Lamsan, Selasi and Siam were used for callus induction. The MSB5 medium [MS macro elements (Murashige & Skoog, 1962), B5 micro elements (Gamborg *et al.,* 1968), B5 vitamins] was used for all experiments. This medium was fortified with 2 mg L^{-1} 2, 4-D for callus induction. The medium was supplemented with 30 g L^{-1} of sucrose and 0.4% gelrite as the gelling agent. In order to investigate the effect of sorbitol, various concentrations of sorbitol (0, 10, 20 & 30 g L^{-1}) were added to the above medium.

To discover the impact of sorbitol on subculture, one subculture was performed 28 days after inoculation and obtained calli were transferred to the same calli origin medium. For the regeneration experiment, after discarding the non-embryogenic scutellum-derived calli, the embryogenic calli produced on the callus induction medium were placed on the regeneration medium consisting of 0.5 mg L^{-1} NAA + 2.0 mg L^{-1} Kin + 2.0 mg L^{-1} BAP. Furthermore, the concentration of sucrose, gelrite and sorbitol in the regeneration medium were the same as that for the callus induction.

Two parameters including callus induction and regeneration frequencies were measured according to Shahsavari *et al*. (2010). Analysis of variance and least significant difference (LSD; P=0.05) for a completely randomized design with four replications were applied using SAS software (Compton, 1994).

RESULTS AND DISCUSSION

The scutellum region of all cultivars' mature seeds demonstrated callusing after 18–22 days following inoculation. Morphological observation showed that two types of callus appeared on the MSB5 medium. One type was embryogenic, nodular and had a compact appearance, and was white to lemon in color, while the other type was non-embryogenic soft, mucilaginous and disorganized in appearance, yellow or bright brown in color. These calli did not appear to have regeneration capability. Green spots from embryogenic calli appeared on the regeneration medium after 8–10 days. Then, 14–22 days later, shoot and root emerged simultaneously.

Different combinations of sorbitol incorporated into MSB5 were tested on callus induction and the regeneration media. Marked differences were found in both cultivars and sorbitol effects. Significant differences were also observed in interaction between varieties and sorbitol treatments. Of the cultivars, Selasi gave the highest callus induction frequency (80.8%) and Siam the poorest (46.9%) (Table I). The ANOVA for each cultivar revealed that Kusan and Siam formed callus better on the medium that had been supplemented with sorbitol compared to the sorbitol-free medium. However, with reference to Lamsan and Selasi, adding various levels of sorbitol led to no positive impact on the callus induction frequency (Table I). The optimal concentration of sorbitol for Kusan and Siam was 10 g L^{-1}. Interestingly, the response of these two cultivars to callus induction was genetically low and adding 10 g L^{-1} sorbitol enhanced the callus induction response dramatically from 43.7% to 64.6% and 47.9% to 74.6% in Kusan and Siam, respectively. It is clear from Table I that all cultivars performed poorly regarding medium supplemented with high levels of sorbitol (30 g L^{-1}).

The effect of sorbitol on subculture is presented in Table II. The cultivar Selasi demonstrated the best callus growth for all sorbitol concentrations. The growth of Lamsan calli was good in 0 and 10 g L^{-1} sorbitol, but decreased when the amount of sorbitol added to the medium increased. In Kusan, the sorbitol levels cannot increase callus growth but interestingly, calli obtained from Siam at 10 g L^{-1} sorbitol showed impressive growth. Callus browning or necrosis is another factor that can influence rice tissue culture especially gene transformation. Calli obtained from the cultivar Siam showed browning on the sorbitol-free medium, while the callus of others did not betray any browning. The high concentration of sorbitol led to callus browning in all tested cultivars.

Table I: Effect of different sorbitol concentrations on callus induction frequency of four upland rice cultivars after 28 days of culture

Sorbitol (g L^{-1})	Cultivar				
	Kusan	Lamsan	Selasi	Siam	Mean
0	43.7ab	91.7a	92.6a	47.9b	68.1B
10	64. 6a	89.6a	87.5a	74.6a	79.1A
20	50.0ab	85.4a	82.8a	45.8b	66.0B
30	29.2 b	56.3b	60.4b	20.8c	41.7C
Means	46.9 B	80.7A	80.8A	47.2B	
P value cultivars			<.0001		
P value sorbitol concentrations			<.0001		
P value cultivars × Sorbitol concentrations			0.0257		

Values with the same letter were not significantly different at the 0.05 probability level using LSD; capital alphabets (A, B, C, ...) refer to differences among cultivars and sorbitol levels in factorial analysis while small alphabets (a, b, c, ...) refer to differences based on separate analysis of variation for each cultivar

Table II: Effect of different sorbitol concentrations on subculture of four upland rice cultivars after 28 days of culture

Sorbitol (g L^{-1})	Cultivar			
	Kusan	Lamsan	Selasi	Siam
0	+++	++++	++++	++(B) *
10	+++	++++	++++	++++
20	+++(B)	+++	++++	+++(B)
30	+ (B)	+++(B)	+++(B)	+(B)

*Callus growth indicator in subculture; ++++ = very good growth; +++ = good growth; ++ = medium growth; + = low growth
The letter of B in the table indicated callus browning was happened

Table III: Effect of different sorbitol concentrations on regeneration frequency of four upland rice cultivars after 28 days of culture

Sorbitol (g L^{-1})	Cultivar				
	Kusan	Lamsan	Selasi	Siam	Mean
0	10.0b	52.5b	52.6b	15.0b	32.5B
10	8.00b	58.7b	75.0a	11.6b	38.3B
20	40.0a	80.0a	25.8c	45.0a	47.7A
30	0.00b	9.20c	15.1c	0.00b	6.10C
Means	14.5B	50.1A	42.1A	17.9B	
P value for cultivars			<.0001		
P value for sorbitol concentrations			<.0001		
P value for cultivars × sorbitol concentrations			<.0001		

Values with the same letter were not significantly different at the 0.05 probability level using LSD; capital alphabets (A, B, C, ...) refer to differences among cultivars and sorbitol levels in factorial analysis while small alphabets (a, b, c, ...) refer to differences based on separate analysis of variation for each cultivar

According to the findings of the regeneration study, it was observed that both cultivars and sorbitol effects were significant. In addition, significant differences were observed in the interaction between cultivars and sorbitol concentrations. With respect to cultivars, Lamsan showed the highest regeneration of 50.1% and Kusan the lowest with 14.5 (Table III). The frequency of regeneration differed upon the addition of sorbitol levels in all tested cultivars.

The optimum level of sorbitol on the regeneration medium was 20 g L^{-1} for all cultivars except Selasi. In Selasi, the maximum regeneration response was obtained at 10 g L^{-1} sorbitol (Table III). The regeneration frequency of the four cultivars was improved with the appropriate amount of sorbitol levels and varied from 0 to 80%. For Kusan and Siam especially, the frequency increased from 10 and 15 to 40 and 45 at 20 g L^{-1} sorbitol concentration, respectively. Interestingly, in all cultivars, the high level of sorbitol (30 g L^{-1}) did exhibit adverse effects on the regeneration response and resulted in reducing the regeneration frequency.

In this report, an attempt was made to investigate the effect of sorbitol on four Malaysian upland cultivars on both callus indication and regeneration ability. Our results focused on the positive effect of sorbitol on all cultivars in both calli production and regeneration response. Although the proper levels of sorbitol enhanced calli production, morphological observation also indicated that adding sorbitol led to the production of more embryogenic calli compared to the sorbitol-free medium. Furthermore, in Kusan and Siam, the beneficial effect of sorbitol on the tissue culture system was more noticeable compared to those for Lamsan and Selasi, which performed poorly in the regeneration medium as previously found by Shahsavari *et al*. (2010).

The contribution of sorbitol in tissue culture is based on two functions: firstly, one acts as a primary carbon source to enhance regeneration frequency of embryogenic calli; and secondly, one is an osmotic regulator which can have a positive impact on calli and regeneration ability (Swedlund & Locy, 1993; Geng *et al.*, 2008). Some studies mentioned that sorbitol can be used as an osmotic agent and for this reason calli cannot metabolize it (Wang *et al*., 1999). In this position, an appropriate amount of sucrose must also be applied to provide a carbon source in the medium. In contrast, others reported that sorbitol can also contribute as a carbon source or carbohydrate. Kumria *et al*. (2001) applied sorbitol for the first time in co-cultivation medium in the rice gene transformation technology. Their results indicated that sorbitol led to a dramatic increase in GAS activity. Geng *et al*. (2008) stated that due to the fact sorbitol functions in the cell as a complex mechanism, this complexity results in a high level of interaction in different culture stages. Sorbitol may act as an osmotic agent at an early stage of culture, and becomes a source of carbon later.

However, it seems the mechanism for how sorbitol works in the regeneration system of rice is still far from clear and further studies are required to determine the molecular functions of sorbitol in tissue culture.

CONCLUSION

In summary, sorbitol not only increased callus induction in two cultivars of upland rice, but promoted the plantlet regeneration response in all cultivars.

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