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Full Length Article

Evaluation and Optimizations of Media on the Tissue Culture System of Upland Rice

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ABSTRACT

Mature seeds of Malaysian upland rice, cv. Selasi were selected as starting material for callus production and regeneration response in serial experiments. Four media, three carbon sources at three concentrations and four gelrite concentrations were tested. Moreover, different concentrations of proline, casein hydrolysate and sorbitol were also examined. The results obtained from our study suggested that the optimal callus induction medium was MSB5 supplemented with 2 mg L^{-1} 2, 4-D (previous work, Shahsavari *et al.,* 2010), 40 g L^{-1} maltose, 600 mg L^{-1} casein hydrolysate and 0.4% gelrite. While maximum regeneration response was detected on MSB5 medium fortified with 0.5 mg L^{-1} NAA+2.0 mg L^{-1} Kin+2.0 mg L^{-1} BAP, 30 g L^{-1} maltose or 30 g L^{-1} sucrose plus 10 g L^{-1} sorbitol, 600 mg L^{-1}casein hydrolysate, which was solidified with 0.4% gelrite. © 2010 Friends Science Publishers

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

INTRODUCTION

Rice genetic transformation technologies appear to hold great promise for increasing rice productivity, especially in areas, where conventional breeding lacks solution and farmers have little means to counter damage caused by stress (Afolabi *et al.,* 2008). But improvement of rice production, using marker-free transformation biotechnology, has been significantly impaired due to lack of a suitable regeneration protocol for the locally preferred rice cultivars (Afolabi *et al.,* 2008).

Upland rice contributes 12% of global rice production and is interestingly, the lowest-performing ecosystem (Bernier *et al.,* 2008) Furthermore, upland rice farmers in Asia and Africa are the poorest of the poor compared to other framers around the globe and their land sizes are very small, often less than 0.5 ha (Arraudeau, 1995). Drought stress is a serious problem for crop production around the world (Misra *et al.,* 2002). It severely reduces upland rice yield (Bernier *et al.,* 2008).

In order to use upland rice in the gene transformation process, some optimizations for locally preferred rice genotypes were reported (Shahsavari *et al.,* 2010). Four upland rice cultivars, namely Kusan, Lamsan, Selasi and Siam were screened on MS medium fortified with different levels of plant growth regulators. The results showed that the cultivars response to the tissue culture system was very different. Lamsan and Selasi indicated a better response to callus induction and regeneration compared to Kusan and Siam. Our knowledge of the tissue culture system of Malaysian upland rice is limited. Therefore, the aim of this study was to specifically evaluate the impact of different types and compositions of media on callus induction and the regeneration response of upland rice, cv. Selasi.

MATERIALS AND METHODS

Mature seeds as initial explants from cv. Selasi were used for starting callus induction, because this showed the best response to tissue culture in our lab. After sterilization based on Shahsavari *et al.* (2010), seeds were placed on callus induction media and kept in the dark at 26°C for 28 days.

To understand the effects of media types on callus induction and regeneration, the four basal media used were MS (Murashige & Skoog, 1962), N6 (Chu *et al.,* 1975), MSB5 (MS macro elements, B5 micro elements (Gamborg *et al.,* 1968), B5 vitamins and N6B5 (N6 macro elements, B5 micro elements, B5 vitamins). All media were incorporated with 2 mg L^{-1} 2,4-D and 5 mg L^{-1} NAA+2.0 mg L^{-1} Kin+2.0 mg L^{-1} BAP for callus induction and regeneration, respectively. The media supplemented with 30 g L^{-1} of maltose and were solidified by 0.3% gelrite. On the basis of the results obtained from the different media experiment, the MSB5 medium was selected for other optimizations. At levels of 10, 20, 30, 40 g L^{-1}, glucose,

sucrose and maltose were used as carbohydrate sources and the medium was also tested with 0.2, 0.25, 0.3, 0.4% gelrite as a gelling agent in the callus induction and regeneration studies.

In order to examine the impact of sorbitol, proline and casein hydrolysate on somatic embryogenesis and regeneration 0, 500 mg L^{-1} proline, 0, 300, 600 mg L^{-1} casein hydrolysate and 0, 10, 15, 20 g L^{-1} sorbitol were tested on the MSB5 medium forfeited with 30 g L^{-1} sucrose and 0.3% gelrite.

Analysis of variance (ANOVA) for a completely randomized design with four replications and media as treatments was applied to find out differences between the treatments. "Callus induction frequency" and "Regeneration frequency" parameters were analyzed by Least Significant Difference (LSD %5) with using SAS 9 software (Compton, 1994).

RESULTS

In this study, calli from the tested media obtained after 18-25 days after inoculation. Moreover, two types of callus; embryogenic and non-embryogenic were detected. Calli that are nodular and compact, white to lemon in color are defined as embryogenic. By contrast, the non-embryogenic calli were completely yellow or bright brown in color and were much greater in size than the embryogenic calli. Green spots from the calli appeared on the regeneration media after 8-10 days. Then, 15-25 days after the advent of the green spots, shoot and root regeneration was produced at the same time (Fig. 1).

The ANOVA results indicated that callus induction frequency was nearly identical in the four tested media (82.1–91.1%) (Fig. 2). However, the regeneration frequency was significantly different among all media. With respect to media types, the highest (62.5%) and lowest (20%) regeneration frequency took place on MSB5 and N6B5, respectively. For this reason, MSB5 was applied for other optimizations.

It was observed that both callus induction and regeneration frequencies were statistically significant when used deferent levels of carbon source (Fig. 3). The maximum and minimum calli were obtained in the presence of 40 g L^{-1} maltose and 30 g L^{-1} glucose, respectively (95.8, 87.5%). Interestingly, cv. Selasi gave the best regeneration response using 30 g L^{-1} of maltose at an average of 79.2 and was followed by 30 g L^{-1} sucrose at 64.6%. As can be seen, a low concentration of carbon source (20 g L^{-1}) led to a decline in both callus induction and regeneration response in all treatments.

Although the difference in the callus induction frequency in gelrite concentrations was not significant, according to morphological observation, the most calli achieved from 0.4% were embryogenic compared to others (Fig. 4). The results showed that 0.4% was the optimum in regeneration media and Selasi reached the highest

Fig. 1: Different stages of tissue culture of cv.Selasi. (a) callus induction, (b) callus subculture, (c) regeneration, (d) whole plantlets

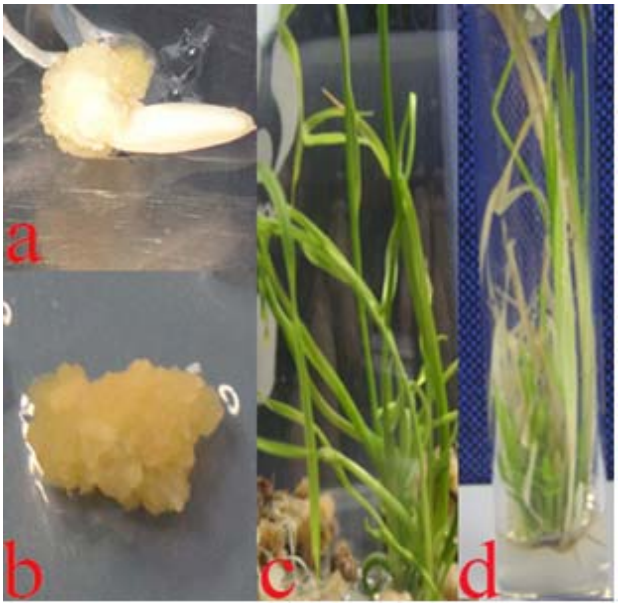
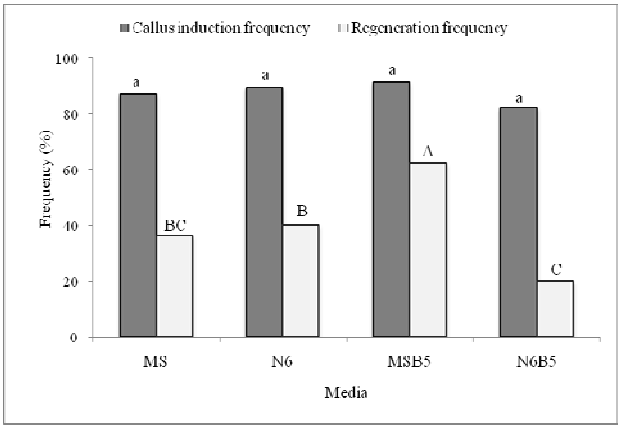


Fig. 2: Effect of media types on callus induction and regeneration frequencies of cv.Selasi after 28 days of culture

Means with the different letter were statistically significant at 0.05 probability level using LSD. Small alphabets refer to callus induction frequency differences and capital alphabets refer to regeneration frequency differences among treatments. The symbols and the meanings above also used for figures 3-7



regeneration frequency at this level. What is more, using low consternations of gelrite caused a low regeneration response.

The effect of incorporating two concentrations of proline was not significant in callus induction and regeneration experiments. However, the response to the tissue culture system at 500 mg L^{-1} was better than at 0 level (Fig. 5).

The callus induction and regeneration frequencies were different upon the addition of 300 and 600 mg L^{-1} casein hydrolysate compared to 0 mg L^{-1} (Fig. 6). No

Fig. 3: Effect of carbon source and its levels on callus induction and regeneration frequencies of cv. Selasi after 28 days of culture

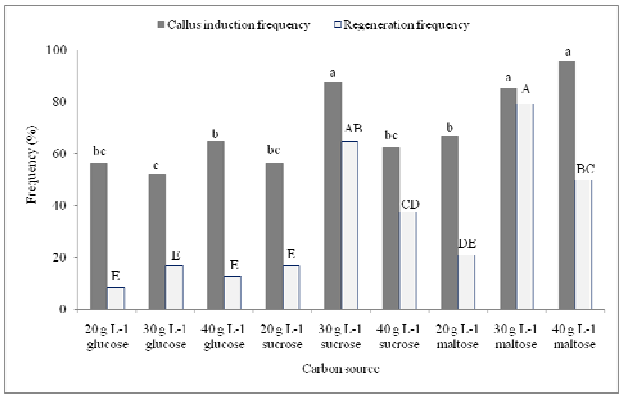


Fig. 4: Effect of different concentrations of gelrite on callus induction and regeneration frequencies of cv. Selasi after 28 days of culture

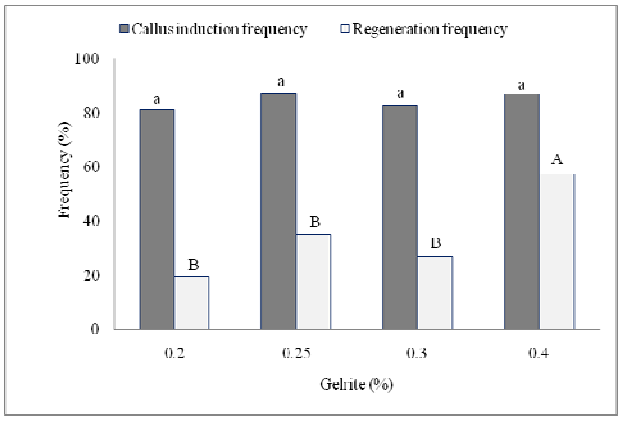
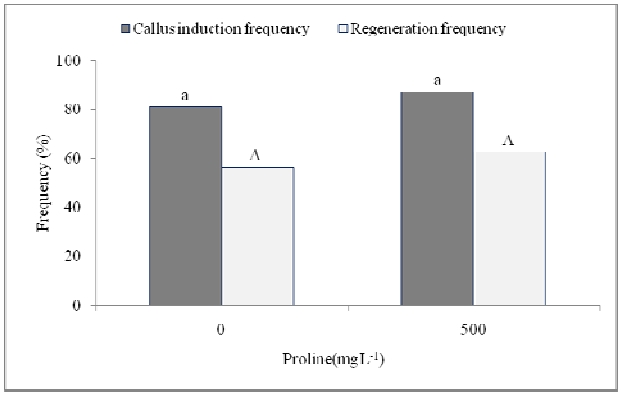


Fig. 5: Effect of different concentrations of proline on callus induction and regeneration frequencies of cv. Selasi after 28 days of culture



remarkable difference was observed when 300 mg L^{-1} casein hydrolysate was replaced with 600 mg L^{-1} in the production of calli and the regeneration response. The results obtained from the regeneration experiment showed that using 600 mg L^{-1} casein hydrolysate gave the highest response of 67.2%, while the lowest frequency of 31.1% was observed at zero level.

Fig. 6: Effect of different concentrations of casein hydrolysate on callus induction and regeneration frequencies of cv. Selasi after 28 days of culture

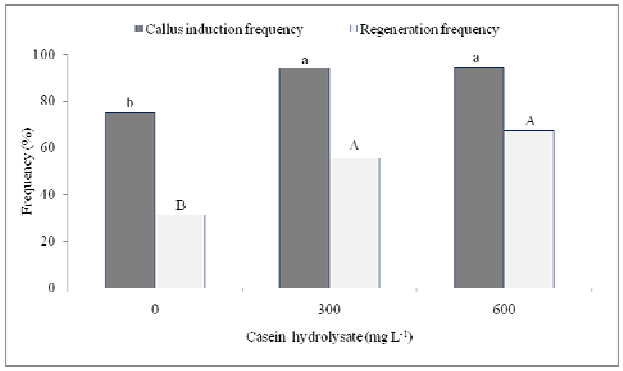
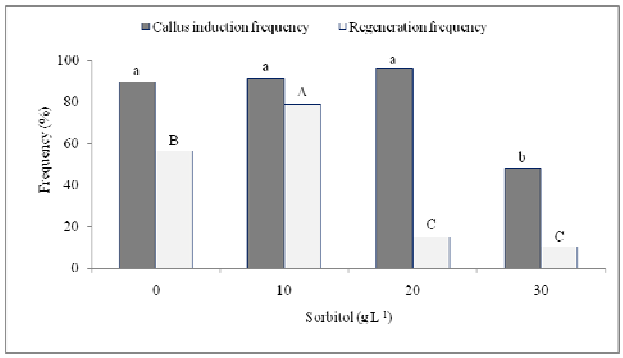


Fig. 7: Effect of different concentrations of sorbitol on callus induction and regeneration frequencies of cv. Selasi after 28 days of culture



According to Fig. 7, the positive effect of sorbitol on calli production was not significantly different. In addition, regeneration response reached its highest frequency (78.7%) at 10 g L^{-1} sorbitol and lowest (10%) at 30 g L^{-1}. Notably, media fortified with more than 10 g L^{-1} sorbitol decreed dramatically the callus induction and the regeneration ability.

DISCUSSION

In this study, the tissue culture optimizations of cv. Selasi were attempted. The findings obtained from this study have emphasized that the composition of media used for calli production and regeneration had a significant effect. Of the four media, the MSB5 medium produced more embryogenic calli than the other media and maximum regeneration was also achieved with this medium.

According to the literature, different media, such as NB, MS, N6, MSB5 have been used by researchers and although their compositions are distinctive, the ratio of nitrate nitrogen to ammoniac nitrogen (NO_3^-/NH_4^+) is the crucial parameter (Lee *et al*., 2002; Ge *et al.,* 2006; Zaidi *et al.,* 2006; Afolabi *et al.*, 2008; Geng *et al.*, 2008). This ratio probably contributes to producing somatic embryogenesis in monocots (Ge *et al.,* 2006). Furthermore, it is known that rice, which responds to a tissue culture system seems genotype unique and so it is essential to optimize these media separately for each genotype before carrying out any transformation experiments (Zaidi *et al.,* 2006; Afolabi *et al.*, 2008).

The response of cv. Selasi to tissue culture was affected by the type and concentrations of carbon source. Our results showed that maltose was more effective than sucrose or glucose with respect to both calli production and regeneration response. This finding is similar to previous works (Kumria *et al.,* 2001; Zaidi *et al.,* 2006). Lentini *et al.* (1995) reported that using maltose might control the osmotic potential of the cellular environment of callus and led to the production of embryogenic calli. Furthermore, sucrose helps excised tissues to make more ethylene under *in vitro* conditions and resulted in the callus browning. It is obvious that the replacement of sucrose with maltose diminishes ethylene production. In regard to the carbon source, a sufficient quantity of the carbon source is vital to start callus induction and promote regeneration ability.

In addition to sucrose, gelling agents also influence the response to tissue culture. The highest frequencies of callus induction and regeneration were obtained when 0.4% gelrite was used. The contribution of these agents adjusts the humidity of *in vitro* culture conditions (Zaidi *et al.,* 2006).

Proline, as an osmotic regulatory, can have a positive impact on calli and regeneration ability. Our findings showed only a slight rise in the tissue culture response, when 500 mg L^{-1} was used. However, 500-600 mg L^{-1} proline has been recommended for rice tissue culture experiments (Ge *et al.,* 2006; Afolabi *et al.*, 2008).

In the present report another factor, which stimulated callus induction and regeneration frequencies was casein hydrolysate and this provides a source of amino acids. These findings are in conformity with those of Zaidi *et al.* (2006) and Afolabi *et al.* (2008) in which the positive effect of casein hydrolysate had been shown. On the other hand, Lee *et al.* (2002) indicated that the media, which were supported with several levels of casein hydrolysate were unable to affect significantly induction of the embryogenic calli.

The impact of incorporating appropriate sorbitol levels on the tissue culture of cv. Selasi was also examined. Adding 10 g L^{-1} sorbitol to media with 30 g L^{-1} sucrose created a positive impact only on regeneration frequency. Our findings are in harmony with that of Geng *et al.* (2008). In fact they reported that addition of appropriate amounts of sorbitol in the culture media increased regeneration rate drastically. It seems using sorbitol in tissue culture acts as primary carbon source to enhance regeneration frequency of embryogenic calli (Geng *et al.,* 2008).

In conclusion, successful optimizations on the tissue culture system of upland rice, specifically cv. Selasi, were performed and the primary experiments have indicated that these can be useful for other Malaysian upland rice.

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