Impact of tryptophan and glutamine on the tissue culture of upland rice

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ABSTRACT

In order to evaluate the effect of tryptophan and glutamine on the tissue culture of upland rice cultivars, serial experiments were conducted using four cultivars: Kusan, Lamsan, Selasi and Siam. Mature seeds from these cultivars were subjected to 4 levels of tryptophan and glutamine on the MSB5 (MS macro elements, B5 micro elements and B5 vitamins) medium. Callus induction results showed a positive effect of tryptophan on all cultivars except Selasi. The optimal tryptophan concentration for callus induction in cultivars Kusan and Siam was 100 μmol, while in Lamsan the optimum was 200 μmol. With the exception of the Lamsan cultivar, incorporation of glutamine generally did not result in the enhancement of callus induction response that incorporation of tryptophan did. Plantlet regeneration frequency was significantly increased when an appropriate level of tryptophan was added to culture media, the optimum being 100 μmol or Kusan, Selasi and Siam, compared to an optimum of 200 μmol for Lamsan. Glutamine did not affect regeneration frequency in any of the cultivars under the conditions tested. In summary, the results showed that tryptophan is a useful additive for upland rice tissue culture.

Keywords: callus induction frequency; plantlet regeneration frequency; embryogenic calli

Around the globe, rice is a very important crop as a staple food and a model plant for genomic study (Tyagi and Mohanty 2000, Bajaj and Mohanty 2005). However, rice is a very sensitive plant to water deficiency and for this reason, many attempts were made to improve rice cultivars that are able to produce comparatively high yield in both stressed and non-stressed environments (Bernier et al. 2008, Geng et al. 2008). In the mean time, upland rice was taken into consideration for more studies in recent years because it has the potential to survive in drought conditions with high yield (Bernier et al. 2008, Geng et al. 2008).

Biotechnological tools, including gene transformation, open new windows to plant breeders to use these approaches to lessen breeding program times (Dabul et al. 2009). However, the main problem about applying gene transformation in upland rice is finding an effective tissue culture system. It was shown that the regeneration rate of upland rice is relatively low and varies among cultivars (Geng et al. 2008). For these reasons, optimization for specific types and components of media for such cultivars are required before applying gene transformation methods (Ge et al. 2006, Zaidi et al. 2006).

The previous investigations showed the positive effect of tryptophan on the tissue culture system of rice (Siriwardana and Nabors 1983, Chowdhry et al. 1993). Furthermore, glutamine is an ordinary organic nitrogen source used in higher plant tissue culture media and is recommended by researchers in rice tissue culture (Ge et al. 2006, George et al. 2008). To date however, there have been no reports regarding the effect of these chemicals on the tissue cultures of Malaysian upland rice cultivars. The purpose of this study was to evaluate the effect of tryptophan and glutamine on upland rice callus induction rate and regeneration response.

MATERIAL AND METHODS

Mature seeds of four cultivars of Malaysian upland rice namely Kusan, Lamsan, Selasi and Siam were used for callus induction. After sterilisation based on Shahsavari et al. (2010), the seeds were placed on callus induction media and were kept in the dark at 26°C for four weeks. MSB5 medium [MS macro elements (Murashige and Skoog 1962), B5 micro elements (Gamborg et al. 1968), B5 vitamins] containing 2 mg/L 2,4-D and 0.5 mg/L NAA + 2.0 mg/L Kin + 2.0 mg/L BAP was used for callus induction and plantlet regeneration, respectively. The medium supplemented with 30 g/L of sucrose and solidified by 0.4% gelrite. To understand the effect of tryptophan and glutamine on callus induction and regeneration system, various tryptophan and glutamine concentrations 0, 100, 200 and 300 μmol were added to the above medium. After preparation of these amino acids, they were inoculated separately after filter-sterilization to the autoclaved medium.

Callus induction and plantlet regeneration frequencies were measured according to the method of Shahsavari et al. (2010). A completely randomized design was used with four replications. Each replication per treatment consisted of 10–12 seeds for callus induction and 4–6 embryogenic calli for plantlet regeneration. Data were subjected to analysis of variance (two way-factorial analysis of variance) with genotype as one treatment and amino acid concentrations as the other treatment using the SAS statistical program. Mean values were separated using the Least Significant Difference (*LSD*) test, where the *F*-value was significant (Compton 1994).

RESULTS

After swelling of the scutellum region, calli were obtained from the four cultivars after 3–4 weeks. The calli could be distinguished by two distinct types. Embryogenic calli were nodular and compact, and white to lemon in color. The non-embryogenic calli were completely yellow or bright brown in color and were much greater in size than the embryogenic calli. Greens spots from the calli formed on the regeneration media after 1–2 weeks. Then after 2 weeks, shoots and roots emerged from these green spots at the same time (Whole plantlet).

According to ANOVA results from callus induction experiments, it was observed that both cultivars and tryptophan effects were significant. In addition, no differences were observed in the interaction between cultivars and medium treatments. With respect to cultivars, Lamsan gave the maximum callus induction frequency (85.6%) and Siam gave the minimum (47.4%) (Table 1).

According to the separate analysis for each cultivar, the callus induction response was significantly different upon the addition of different levels of tryptophan for all cultivars except Selasi. The optimal tryptophan concentration for the callus induction in the cultivars Kusan and Siam was 100 μmol while in Lamsan, the maximum response was obtained at 200 μmol and at these concena trations, the three cultivars showed a maximum callus induction frequency. By contrast, according to the results from the glutamine experiment, the effect of glutamine was not statistically significant. However, the two-way ANOVA confirmed significant differences between cultivars and interaction between glutamine levels and the cultivars. Similar to the addition of tryptophan in the callus induction medium, among the tested cultivars, Lamsan gave the best callus induction response of 84.2% while Siam gave the poorest at 48.4%. However, separate analysis for each cultivar indicated only the positive effect of glutamine on the Lamsan callus indication frequency. In fact, in Lamsan, adding 100 μmol led to the highest response of 97.9%.

The results of plantlet regeneration experiments are presented in Table 2. As can be seen in the tryptophan study, remarkable effects were detected between cultivars, tryptophan levels and the interaction between them. The regeneration

Table 1. Effect of different levels of tryptophan and glutamine on callus induction frequency of four upland rice cultivars after 4 weeks of culture

Cultivars	Tryptophan (μmol)					Glutamine (μmol)				
	0	100	200	300	means	0	100	200	300	means
Kusan	48.7^{b}	68.7^{a}	47.9^{b}	46.6^{b}	52.9^{B}	54.2^{a}	52.1^{a}	45.8^{a}	43.3^{a}	48.9^{B}
Lamsan	73.7^{b}	95.8^{a}	96.2^{a}	76.9^{b}	85.6^{A}	70.8^{c}	97.9^{a}	85.4^{b}	82.9^{b}	84.2^{A}
Selasi	79.1^{a}	77.1^{a}	87.5^{a}	81.7^{a}	81.3^{A}	79.2^{a}	77.1^{a}	87.5^{a}	83.3^{a}	81.7^{A}
Siam	50.0^{ab}	66.6^{a}	41.7^{ab}	31.2^{b}	47.4^{B}	47.9^{a}	45.8^{a}	56.2^{a}	43.7^{a}	48.4^{B}
Means	62.9^{B}	77.0^{A}	68.3^{AB}	59.1^{C}		63.0^{A}	68.2^{A}	68.7^{A}	63.3^{A}	

Values with the same superscript were not significantly different at the 0.05 probability level using *LSD*; capital alphabets ($^{A, B, C,}$ ...) refer to differences among cultivars and tryptophan, glutamine levels in factorial analysis while small alphabets ($^{a, b, c,}$ ...) refer to differences based on separate ANOVA for each cultivar

Table 2. Effect of different levels of tryptophan and glutamine on regeneration frequency of four upland rice cultivars after 4 weeks of culture

Cultivars	Tryptophan (μmol)					Glutamine (μmol)				
	0	100	200	300	means	0	100	200	300	means
Kusan	0.00^{b}	15.0^{a}	0.00^{b}	0.00^{b}	3.70^{B}	5.00^{a}	5.00^{a}	13.3^{a}	5.00^{a}	7.00^{B}
Lamsan	57.1^{b}	50.5^{b}	75.0^{a}	19.2^{a}	50.5^{A}	53.3^{a}	48.7^{a}	55.0^{a}	38.3^{a}	48.8^{A}
Selasi	55.8^{ab}	65.0^{a}	30.0^{c}	38.3^{bc}	42.3^{A}	50.0^{a}	55.0^{a}	45.0^{a}	43.3^{a}	48.3^{A}
Siam	5.00^{b}	40.0^{a}	10.0^{b}	5.00^{b}	15.0^{B}	8.4^{a}	10.0^{a}	5.00^{a}	5.00^{a}	7.1^{B}
Means	29.5^{B}	42.6^{A}	28.7^{BC}	15.6		29.1^{A}	29.7^{A}	29.6^{A}	22.9^{A}	

Values with the same superscript were not significantly different at the 0.05 probability level using *LSD*; capital alphabets ($^{A, B, C,}$ ...) refer to differences among cultivars and tryptophan, glutamine levels in factorial analysis while small alphabets ($^{a, b, c,}$ ...) refer to differences based on separate ANOVA for each cultivar

frequency varied from 0.0 up to 75.0% among the different cultivars and tryptophan levels (Table 2). As is shown in Table 2, Lamsan gave the highest regeneration response (50.5%) and Kusan the poorest (3.70%). Based on separate ANOVA, adding tryptophan had a positive impact on the plantlet regeneration response. It is clear that all tested cultivars, with the exception of Lamsan, reached their highest regeneration frequency at 100 μmol tryptophan. Lamsan performed better in the medium with 200 μmol tryptophan. Interestingly, in the cultivar Siam (with a low regeneration frequency according to Shahsavari et al. 2010), the incorporation of 100 μmol of tryptophan increased the regeneration frequency from 5% to 40%.

The results also revealed that the inoculation of various concentrations of glutamine into the medium could not lead to significant response in regeneration system in all tested cultivars. However, both the cultivars effects and interaction between cultivars and glutamine levels were statistically significant. As Table 2 shows, we were also unable to detect marked differences when the various levels of glutamine were incorporated in separate ANOVA for each cultivar.

DISCUSSION

We have evaluated the effect of tryptophan and glutamine on the tissue culture of four upland rice cultivars. Our results showed that when tryptophan was added to the media for both callus induction and plantlet regeneration, it promoted callus induction in cultivars Kusan, Lamsan, and Siam, and promoted regeneration response in all tested cultivars. By comparison, glutamine only enhanced callus production in the cultivar Lamsan and did not enhance plantlet regeneration frequency in any cultivar.

Morphological observation revealed that tryptophan helped to increase the formation of green spots and also the frequency of embryogenic calli which led to a drastic increase in regeneration frequency in all cultivars. This finding is consistent with that of other researchers who investigated this effect on rice varieties. Koetje et al. (1989) reported that tryptophan increased the mass of embryogenic callus in immature embryo cultures of rice. Similarly, Chowdhry et al. (1993) reported that addition of tryptophan to callus induction medium could not lead to a general increase in callus induction frequency but rather only to enhanced frequency of embryogenic callus formation.

It is well known that tryptophan acts as a precursor of IAA in plants and that it may promote growth or induce morphogenesis, and we observed this in the current study.

We were not able to see any positive effect of glutamine on the tissue culture system of rice (except cultivar Lamsan regarding to callus induction). A positive effect of glutamine was shown on the tissue culture of carrot, sugi (*Cryptomeria japonica*), and on pollen embryogenesis in tobacco and potato (George et al. 2008). In rice tissue culture, although glutamine is recommended by researchers, they generally have not proven or discussed the contribution of glutamine in detail.

Addition of amino acids provides a readily available, primary source of nitrogen in tissue culture systems and uptake can occur much more rapidly than from inorganic nitrogen in the same medium (George et al. 2008). In summary, a positive response in both callus induction and regeneration was observed when appropriate amounts of tryptophan were added, and these responses differed significantly between cultivars.

Acknowledgement

The author would like to thank Dr. Bob Moore for reviewing the text.

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Received on May 15, 2010

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