Effect of some of the natural organic sources on rice tissue culture

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Objective
The main goal of this investigation was to evaluate the effect of glutamine, tryptophan, and casein hydrolysate on callus induction and shoot regeneration in two Egyptian rice cultivars (Sakha104 and Giza178).
Materials and methods
Different concentrations of tryptophan, glutamine, and casein hydrolysate were investigated separately for the maximum production of callus and shoot regeneration.
Results and conclusion
Although tryptophan demonstrated a stimulatory effect on callus induction of Sakha104 cultivar, it showed no positive effect on callus initiation of Giza178 genotype and shoot regeneration of both cultivars. Inclusion of glutamine did not enhance either callus induction or shoot regeneration in both cultivars. Supplementation of appropriate amounts of casein hydrolysate resulted in positive response in both callus induction and shoot regeneration. In addition, these responses varied significantly between the two tested cultivars. Irrespective of type and concentration of the natural organic source, Sakha104 proved to have better regeneration capacity than that of Giza178.

Keywords:
callus induction, nutritional supplements, *Oryza sativa* L, shoot regeneration

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Introduction

Rice (*Oryza sativa* L.) as a stable diet for greater than half of the world's population is one of the most important food crops worldwide. To cope with the ever-growing world population, total rice production has to be increased 50% by 2025 [1]. This goal cannot be achieved by raising the area under rice cultivation because of the unavailability of suitable lands and limited water resources. On the contrary, farm areas are being converted to residential areas especially in the developing countries including Egypt. The most viable solution is to improve rice productivity by developing new genotypes tolerant to biotic and abiotic stresses with high yielding capacity.

Modern biotechnological tools including genetic transformation enable breeders to combat such problems. In fact, considerable efforts were made for the genetic improvement of rice throughout the last two decades [2,3]. However, significant genotype-specific morphogenetic response is a major limitation in rice tissue culture. Therefore, optimizing of an efficient regeneration protocol for specific cultivar(s) is an essential step before applying transformation methods. Besides the genotype dependency, the composition of the nutrient media (basal salts, organic components, and growth regulators) is a major factor influencing the regeneration frequency of rice [4,5].

Organic nitrogen sources such as tryptophan, glutamine, and casein hydrolysate have been reported to promote callus induction and somatic embryogenesis in rice [6,7]. However, to date, there have been no reports regarding the effect of these nutritional supplements on the tissue culture of Egyptian rice. In a previous study, we investigated many factors affecting rice regeneration and developed an efficient protocol for two Egyptian rice cultivars, namely, Sakha104 and Giza178 [8]. Yet, there is room to research other factors that might maximize the frequency of regeneration. The main aim of this study was to evaluate the effect of tryptophan, glutamine, and casein hydrolysate on callus induction and shoot regeneration of the two Egyptian rice cultivars. The findings from this

investigation might be useful for improving the efficiency of transgenic Egyptian rice production.

Materials and methods

Plant material and surface sterilization of seeds

Seeds of Egyptian rice cultivars Sakha104 and Giza178 were kindly provided by the Rice Research Program, Field Crop Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. They were first sterilized with 70% ethanol for 2 min and then with 50% commercial Clorox (5% NaOCl) for 30 min. The seeds were further washed three times with sterilized distilled water.

Callus induction

Aseptic mature seeds were cultured on N6 medium [9] supplemented with different concentrations of either glutamine (10, 20, 30, and 40 mg/l), tryptophan (10, 20, 30, and 40 mg/l), or casein hydrolysate (100, 200, 300, and 400 mg/l). All media were adjusted to pH 5.8 and fortified with 0.7% agar, 3% sucrose, and 2 mg/l 2,4-dichlorophenoxy acetic acid. The cultures were incubated at 27±1°C under dark condition for 3–4 weeks. The responded explants that succeeded to induce callus were scored and also the explants that did not show any response or failed to keep alive were scored.

Shoot regeneration

Callus was cultured on MS medium [10] supplemented with different concentrations of either glutamine (10, 20, 30, 40, and 50 mg/l), tryptophan (10, 20, 30, 40, and 50 mg/l) or casein hydrolysate (100, 200, 300, 400, and 500 mg/l). All media were adjusted to pH 5.8 and fortified with 0.7% agar, 3% sucrose, 2% sorbitol, 2 mg/l kinetin, and 0.2 mg/l naphthalene acetic acid. The cultures were incubated at 27±1°C under 16/8 h (light/dark) photoperiod for 4 weeks. Shoot regeneration frequency was determined by establishing the ratio between the number of responded callus that managed to form shoots versus the total number of used callus.

Statistical analysis

Experiments were set up in a randomized completely blocks designs, 20 explants were used per treatment, and each experiment was repeated three times. Means and SEs were obtained from analysis for each treatment. Data were presented as mean±SE.

Results

Callus induction

To improve callus initiation frequency, addition of three supplements (tryptophan, glutamine, and casein hydrolysate) was examined. Data presented in Tables 1–3 showed a broad spectrum of callus induction efficiencies. In general, irrespective of type and concentration of the natural organic source, the callus induction capacity of Sakha104 cultivar was significantly higher than that of Giza178 cultivar.

With respect to tryptophan, although it showed no effect on Giza178 cultivar, it demonstrated a promotive effect on Sakha104 cultivar (Table 1). The highest callus induction percentage for Sakha104 cultivar (80%) was recorded on medium supplemented with 20 mg/l tryptophan. The results also revealed that the inoculation of various levels of tryptophan into the medium could not lead to significant response in Giza178 cultivar.

Unlike tryptophan, inclusion of glutamine did not enhance callus induction frequency in both rice cultivars (Table 2). Our results showed that in most cases, the effect of glutamine was not statistically significant. Moreover, high concentration of glutamine (40 mg/l) led to negative effect on callus induction frequency in Giza178 cultivar. The highest callus induction frequency was observed on medium fortified with either 10 mg/l for Sakha104 cultivar or 20 mg/l for Giza178 cultivar. However, no significant differences between both of them and their control treatments were recorded.

Regarding the addition of casein hydrolysate, it greatly promoted the frequency of callus induction in both cultivars (Table 3). In Sakha104 cultivar, the highest callus induction frequency (87%) was recorded in medium supplemented with 300 mg/l casein hydrolysate whereas the lowest frequency (74%) was

Table 1 Effect of tryptophan on callus induction

Tryptophan (mg/l)	Callus induction (mean±SD)	
	Sakha104	Giza178
0	74±1.7	66±2.1
10	76±1.3	67±1.8
20	80±1.2	65±1.7
30	78±1.5	66±1.5
40	74±1.8	64±2.3

Table 2 Effect of glutamine on callus induction

Glutamine (mg/l)	Callus induction (mean±SD)	
	Sakha104	Giza178
0	74±1.7	66±2.1
10	75±2.0	66±2.2
20	73±1.7	67±2.1
30	72±1.5	64±1.7
40	72±1.8	58±2

Table 3 Effect of casein hydrolysate on callus induction

Casein hydrolysate (mg/l)	Callus induction (mean±SD)	
	Sakha104	Giza178
0	74±1.7	66±2.1
100	81±1	72±1.3
200	86±1.2	78±1.2
300	87±1.5	83±1.9
400	85±1.7	82±1.3

observed in control medium (no casein hydrolysate). All casein hydrolysate treatments showed positive response with various degrees compared with control. The same trend was observed also in Giza178 cultivar. The highest (83%) and lowest (66%) callus induction rates belonged to 300 mg/l casein hydrolysate treatment and control treatment, respectively.

Shoot regeneration

For efficient induction of shoots from callus, the aforementioned three nutritional supplements were tested. Differences in shoot regeneration frequency were observed based on organic nitrogen source type and concentration and nature of genotype (Tables 4–6). Similar to callus induction result, Sakha104 genotype was observed to have significantly higher response for shoot regeneration than Giza178 cultivar. Taken together, our results indicated the superiority of Sakha104 cultivar over Giza178 cultivar in terms of regeneration capacity.

Considering tryptophan and glutamine supplementations, we were unable to detect marked positive difference in the shoot regeneration frequency when the different concentrations of either tryptophan or glutamine were incorporated for each cultivar (Tables 4 and 5). In Sakha104 cultivar, although there was no significant difference between control treatment and the addition of 10, 20, or 30 mg/l of either tryptophan or glutamine, there was significant negative effect when 40 mg/l of either of them was used. Likewise, high levels of tryptophan (40 mg/l) or glutamine (30 or 40 mg/l) decreased significantly the shoot regeneration frequency of Giza178 cultivar, whereas no significant differences were recorded between control treatment and low levels of either of tryptophan or glutamine.

For casein hydrolysate supplementation, it was observed that optimum concentration of it tremendously increased shoot regeneration frequency in both cultivars (Table 6). In this respect, medium supplemented with 100 mg/l was found to exert the maximum response with 85% shoot induction frequency followed by medium fortified with

Table 4 Effect of tryptophan on shoot regeneration

Tryptophan (mg/l)	Shoot regeneration (mean±SD)	
	Sakha104	Giza178
0	79±1.7	70±2.3
10	80±2.1	72±2.4
20	78±1.5	70±2
30	79±1.9	71±1.7
40	72±1.7	63±2

Table 5 Effect of glutamine on shoot regeneration

Glutamine (mg/l)	Shoot regeneration (mean±SD)	
	Sakha104	Giza178
0	79±1.7	70±2.3
10	78±1.5	71±2.6
20	80±1.8	70±2.0
30	77±1.7	64±1.5
40	71±1.3	62±2.1

Table 6 Effect of casein hydrolysate on shoot regeneration

Casein hydrolysate (mg/l)	Shoot regeneration (mean±SD)	
	Sakha104	Giza178
0	79±1.7	70±2.3
100	85±1.7	71±1.9
200	82±2.3	76±1.3
300	79±1.9	70±1.7
400	73±1.3	64±1.5

200 mg/l (82%) whereas medium containing 400 mg/l exhibited the lowest frequency of shoot regeneration (73%) in Sakha104 genotype. Shoot regeneration efficiencies ranged from 64 to 76% in Giza178 cultivar. Among the different concentrations of casein hydrolysate used, 200 mg/l was the best for shoot regeneration in Giza178 cultivar.

Discussion

The present investigation studied the effect of tryptophan, glutamine, and casein hydrolysate on callus induction and subsequent shoot regeneration of two Egyptian rice cultivars, that is, Sakha104 and Giza178. Although our results clearly showed that there was a significant response in embryogenic callus production of Sakha104 cultivar by adding tryptophan to the medium, supplementation of tryptophan did not significantly yield higher callus induction frequency of Giza178. The variant response of the two rice cultivars could be attributed to the differences in their genotypes. Different genotypes had different sensitivity to the nutritional additives. A complex genetic interaction is engaged in rice tissue culture, and one suggestion is to supplement natural organic additives to cope with the genotypic barriers [11]. Numerous previous investigations

showed the positive effect of tryptophan on the tissue culture system of rice. For example, Zaidi *et al.* [12] demonstrated that the supplementation of amino acid tryptophan promoted callus induction and regeneration of numerous genotypes of rice. It is well known that tryptophan is an essential amino acid that acts as a precursor of IAA, an important auxin for somatic embryogenesis in cereals.

In this work, usefulness of glutamine was observed for both callus induction and shoot regeneration of the two tested rice cultivars. Conflict studies were reported regarding the effect of glutamine on rice tissue culture. Although some researchers indicated its privilege, and recommended its addition in rice tissue culture [7,13], other investigators could not enhance their regeneration frequency when they used it [6,14]. It is worthy to say that though the amino acid glutamine is recommended by some scientists, they generally have not proven or discussed its contribution in details.

Casein hydrolysate is a fruitful source of vitamins, various microelements, and most importantly a mixture of up to 18 amino acids. Many researchers have demonstrated that casein hydrolysate is more effective for plant tissue cultures than the supplementation of the major amino acids, [15,16] owing to the fact that it provides the plant cultures with more accessibility to a nitrogen source. In this work, optimum concentration of casein hydrolysate resulted in positive response in both callus induction and shoot regeneration. In addition, these responses varied significantly between the two tested cultivars. Our results are in agreement with numerous studies that reported the beneficial effect of casein hydrolysate on the frequency of callusing and regeneration in rice [12,17–19]. Although many studies agreed with the promotive effect of casein hydrolysate on rice tissue culture, they disagreed about the appropriate amount of it. In most cases, the optimum amount of casein hydrolysate ranged from 100 to 600 mg/l. This difference in the appropriate amount might be explained by the use of different genotypes, culture conditions, nutrient compositions, and regeneration procedures.

Exogenous supply of amino acids in culture medium has been reported to promote the production of embryogenic callus and regeneration in a number of plant species including rice. The most commonly used amino acids are glycine, tryptophan, glutamine, proline, and casein hydrolysate as an exclusive source of amino acids [12,20,21]. Amino acids represent an available source of nitrogen, which can be taken up faster than inorganic sources of nitrogen, thereby stimulating faster cell growth and development [7,22]. Varied responses of rice cultures in our experiments confirm the requirement of specific amino acids for specific events in particular genotypes during in-vitro morphogenesis. Because of the complexity of amino acid metabolism, future research should investigate the properties of amino acids combination that play a major role in tissue culture of Egyptian rice cultivars. Up to our knowledge, there have been no reports regarding the effect of these amino acids on the tissue culture of Egyptian rice.

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Conflicts of interest

There are no conflicts of interest.

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