B-Glucan contents in calli of *Oryza sativa* L. var Hashemi under different nutritional treatments

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Abstract

Studies show that *Oryza sativa* L. contains a structure named β-glucan with anti cancer properties. In the present study, a tissue culture for rice was carried out and β-glucan content was measured under various sugar and vitamin levels in basic MS medium. Seeds of Hashemi cultivar were cultured on MS medium. Two plant hormones, namely, 2,4-D (0, 1, 1.5, 2 and 3 mg/l) and kinetin (0, 0.5, 1, and 1.5 mg/l) were used to form callus after one week. To extract β-glucan, calluses were incubated by three different sugar treatments with concentrations 15, 30, and 45 g/l and by three vitamin treatments with concentrations 2/5, 5, and 10 cc. After 30 days, those calluses treated with sucrose 45 g/l had a deeper yellow color than other calluses. In fact, higher concentrations of sugar led to deeper color of calluses which were more rigid and dry and, at the same time, more fragile and less bright in color. The calluses were then dried in an oven, and soluble and insoluble sugars were extracted. Results indicated that highest levels of soluble and insoluble sugars (including β-glucan) were achieved in medium containing 3% sucrose. Increase in vitamins did not generally affect soluble and insoluble sugars levels, and just in the medium containing 5cc vitamin, insoluble sugars increased.

Keywords: Rice (Hashemi cultivar); callus; β-glucan; sucrose; vitamin; soluble sugar; insoluble sugar (β-glucan)

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Introduction

Rice is one of essential nutrients for 30-40% of world population. More than 90% of this product is produced in Asia. Being an important carbohydrate source for human use, rice is also used as forage. An important component of rice is β-glucan, a compound with anti cancer properties. β-glucan is a linear starch free polysaccharide containing β-glocopyranosyl units. These units are linked together by (1-3) or (1-4) bounds to form (1-3) (1-4) βdiβglucan linkage. (1-4) sequence is associated with D-glocopyranosyl units which are separated by singular bond β(1-3) units. Distribution of (1-3) and (1-4) singular bonds is not random and in fact they follow a regular pattern. (1-4) bond sequence generally is composed of 3 or 4 glucose long units separated by (1-3) singular bonds (Wood et al., 1994). Solubility of β-glucan with (1-3) bonds is more than cellulose with (1-4) bonds. β-Glucans are carbohydrates consisting of linked glucose molecules, which are major structural

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components of the cell walls of yeast, fungi and some bacteria (Volman et al., 2008).

There are several reports about various components and extracts of rice with pharmacological and biological properties. For example, a specific lipoprotein is isolated from rice which can induce apoptosis in ovarian cancer cells (Fan et al., 2000). β-(1,3)-D-glucan is the most important compound indentified in rice which has regulatory immunological properties. Different properties including anticancer and antitumor effects are attributed to glucans in cereal and rice (Volman et al. 2008). As an antitumor stimulator, β-(1,3)-D-glucan is absorbed through oral cavity and this reduces its intake time facilitating its therapeutic effects on MNCs around marrow. Several studies about biological properties of β-(1,3)-D-glucan have been carried out worldwide and there are some reports about isolation of this compound in oat (Kerckhoffs et al., 2003) and barely (Morgan and Ofman, 1998). However, few such studies have been reported about isolation of this compound from Iranian agricultural cultivars. In this study, optimal levels of sugar and vitamin are researched for increased β-glucan content in rice.

Materials and Methods

After planting rice seeds, different parts of seedlings were cultured in MS medium for callus production. These parts were then sub-cultured after one month. From among the calluses treated with 1mg.L^{-1} Kin and 2 mg.L^{-1} 2, 4-D, the larger ones were selected for the experiment and transferred to the media with modified sugar and vitamins levels. Three media containing 15, 30, and 45 g sucrose and three media containing 2.5, 5 and 10 cc vitamin were used in the study. The calluses were treated with 2 mg.L^{-1} Kin and 2mg.L^{-1} 2, 4-D along coconut sap containing three different concentrations of sucrose and vitamin in three replications. Three sugar concentrations included:

1. 2 mg.L-1 Kin + 2 mg.L-1 2, 4-D + 45 g.L-1 sucrose + 5 cc coconut sap + 5 cc vitamin

2. 2 mg.L-1 Kin + 2 mg.L^{-1} 2, 4-D + 30 g.L^{-1} sucrose + 5 cc coconut sap + 5 cc vitamin

3. 2 mg.L-1 Kin + 2 mg.L^{-1} 2,4-D + 15 g.L^{-1} sucrose + 5 cc coconut sap + 5 cc vitamin.

Three vitamin concentrations included:

1. 2 mg.L^{-1} Kin + 2 mg.L^{-1} 24-D + 30 g.L^{-1}sucrose + 5cc coconut sap + 10 cc vitamin,

2. 2 mg.L^{-1} Kin + 2 mg.L^{-1} 2, 4-D + 30g.L^{-1}sucrose + 5cc coconut sap + 5 cc vitamin,

3. 2 mg.L^{-1} Kin + 2 mg.L^{-1} 2, 4-D + 30 g.L^{-1} sucrose + 5cc coconut sap + 2.5 cc vitamin.

After 30 days treatment, calluses were harvested, dried in oven at 60° C and then used for extraction of soluble and insoluble sugars.

B_Glucan Extraction

Every sample was weighted and with mortar and pestle crushed and then added to an erlenmeyer flask. Water was added, being the mixture suspended at high stirring rate. Temperature was kept constant in a water bath (Lauda Ecoline Staredition E100, Lauda, Germany). Stirring was obtained with a multistirrer plate (Fisher Scientific, USA), as shown in Fig I. Once the extraction process was finished, solid: liquid separation was done by

Fig. I. Experimental set-up used for the extraction of β-glucans from barley centrifugation (5500rpm, 10 minutes). Solid material was discarded and liquid (supernatant) was stored at 4 °C until the moment of being analysed. All the experiments were conducted in duplicate. β-glucan determination was made at ITACYL, using the Megazyme Assay Kit for mixed linkage β-glucan analysis (Megazyme, Ireland). β-glucan concentration is expressed as % (weight) of the total sample (Brennan and Cleary,2005).

Results

Calluses treated with 45 g L^{-1} sucrose were observed to be deeper yellow than the other calluses indicating that increase in sugar level yields calluses with deeper color (Fig. I). In fact, calluses under 30 g.L^{-1} sucrose treatment were lighter in color than those under 45 g L^{-1} sucrose treatment (Fig. II). Calluses obtained from treatment containing 15 g L^{-1} sucrose were pale yellow (Fig.III). All calluses were dehydrated, hard and fragile so that as sugar level increased calluses were more rigid and fragile. This indicated that increasing sugar concentration resulted in less bright calluses which were dehydrated. The calluses were also large in size and volume.

Mean Glucan level in calluses obtained under different concentrations of vitamin are shown in Table 1. Increasing vitamin contents in the medium, Glucan contents in calluses also increased. On the other hand, the highest amount of Glucan was observed in the medium supplemented with 15 g sucrose.

Mean soluble sugars level in calluses obtained under different concentrations of vitamin are shown in Table 1. These results indicate that increasing vitamin in the culture medium has no positive effect on soluble sugar contents of calluses. The highest amount of soluble sugars was obtained at calluses cultured at low level of sucrose.

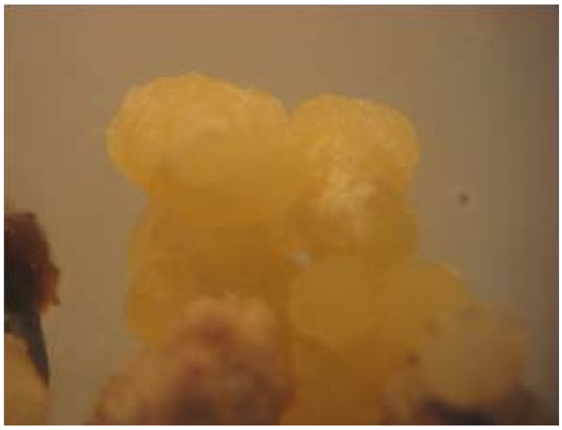

Fig. II. Callus grown in the medium containing 45 g L^{-1} sucrose

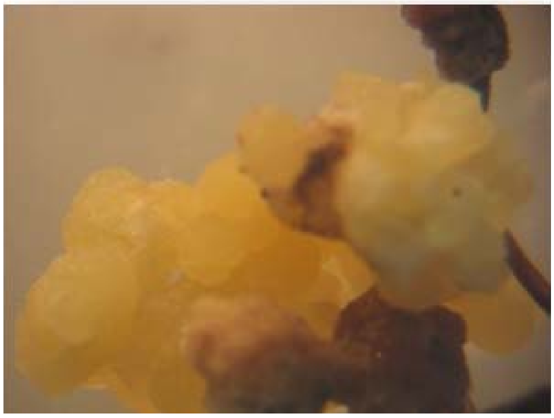

Fig. III. Callus grown in the medium containing 30g L^{-1} sucrose

Fig. IV. Callus grown in the medium containing 15g L^{-1} sucrose

Discussion

Dietary fibers (DF) are essential part of human diet. Many foods with plant origin which are not digestible in upper gastric region including cereal β-glucan, arabinogesilans and cellulose contain dietary fibers. β-glucans are starch-free polysaccharides composed of glucose polymers by linking β(1-3)bonds (nearly 30%) and glocosid β(1-4) bonds. Their main position is in wall cells of endosperm and aleuronic layer. Generally, β-glucan is an important compound of

Table 1
Glucan and soluble sugars in calluses obtained from different treatments (sugar level as mg in each 100 g callus tissue)

	Treatments	mean± SE
Glucan (mg/100g)	30g sucrose	4.42±5.10
	45g sucrose	3.22±9.11
	15g sucrose	8.14±4.26
	0.5vit	2.30 ±5.54
	1 vit	3.17±33.53
	2 vit	6.10 ±3.17
Soluble Sugars (mg/100g)	30g sucrose	3.56±9.10
	45g sucrose	3.44±6.98
	15g sucrose	6.16±3.39
	0.5vit	6.48±2.10
	1 vit	6.44±3.27
	2 vit	6.06±2.32

soluble dietary fibers that affects nutritional value and properties of foods.

Treatment of calluses in media containing sugars and vitamin showed that increase in sugar concentration in medium changed their color into deep yellow and made them hard and fragile. Optimal concentration of sucrose in calluses with the highest level of soluble and insoluble sugars (containing β-glucan) was obtained at 3% sucrose and this is consistent with results of Zenk et al. (1975) on barely and Farzami Sepehr and Ghorbanli (2005) on rhubarb.

Increase in vitamin concentration in culture medium did not increase Glucans and has no effects on soluble sugars in calluses. This is consistent with results of Waterhouse et al. (1998) on barely and Farzami Sepehr and Ghorbanli (2005) on rhubarb. Increasing of sucrose in media has negative effect on soluble sugars and Glucan contents of treated calluses. This finding is similar to Laroche and Michaud (2007) and Tariq et al (2008).

Fig. V. Callus grown in the medium supplemented with 2 vitamins

Fig. VI. Callus grown in the medium supplemented with 1 vitamin

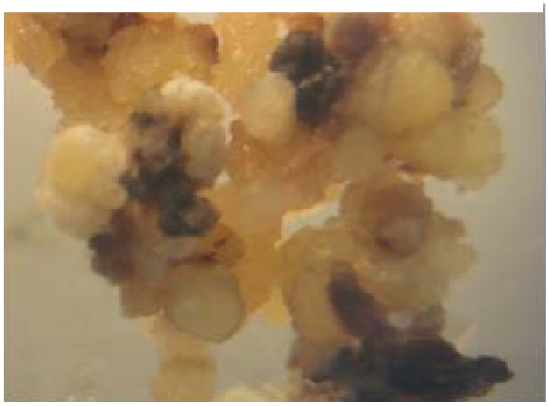

Fig. VII. Callus grown in the medium supplemented with 0.5 vitamin

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