PRELIMINARY SELECTION AND *IN VITRO* PROPAGATION OF AMORPHOPHALLUS SPECIES WITH HIGH CONTENT OF GLUCOMANNAN DISTRIBUTED IN VIETNAM

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ABSTRACT

The study was conducted to select the konjac species distributed in Vietnam having high content of glucomannan and carried out the selective *in vitro* propagation. According to analyzed samples from 5 species of Vietnam konjac, *Amorphophallus corrugatus* had a high content of glucomannan (43,5%). Corms of konjac were used for *in vitro* propagation with MS medium supplemented with basic plant growth regulators. MS medium supplemented with 3mg/L BA and 0.1 mg/L IBA showed the best of *in vitro* regeneration and growth of shoot after 8 weeks of culture (8.33 shoots per explant).

Keywords: *Amorphophallus corrugatus* konjac, glucomannan, plant growth regulators, in vitro propagation, extract.

INTRODUTION

Konjac belongs to the genus *Amorphophallus* containing about 200 species around the world. This genus has about 25 species distributed from North to South of Vietnam, even in coastal to 2000m highland areas. Konjac contains glucomannan, a polysaccharide having mannose and glucose linked by a β-(1-4)-glycosidic bond in a molar ratio of 1.6:1. Glucomannan can actively reduce lipid content (Arvill and Bodin, 1995; Sood et al., 2008), decrease blood pressure (Arvill and Bodin, 1995), and blood sugar (Sood et al., 2007). Besides, glucomannan is used in food technology, including functional food by owning the ability to stimulate the stomach and intestine peristalsis, and consequently used in aperient recipes and cosmetic industry, for producing gel in food processing and so on.

According to few reports, some konjac species have high content of glucomannan distributed in some northern provinces of Vietnam, as *Amorphophallus konjac* K. Koch, *Amorphophallus krausei* Engl., and *Amorphophallus corrugatus* (Liu, 2004). Reproduction of konjac is asexual reproduction by its corms (Edison, 2010; Follett and Douglas, 2002), but the regenerating effect is extremely low, and corms may contain some contamination factors and as a result, it can lead to many risks in cultivation of konjacs (Zhao, 2012). Corms of konjacs are the main material for glucomannan extraction. The aim of this study is to actively select the konjac species having high content of glucomannan and use *in vitro* culture techniques to propagate the target konjac species for large-scale cultivation.

MATERIALS AND METHODS

Materials and chemicals

Amorphophallus species were provided by Institute of Ecology and Biological Resources including 5 species: *Amorphophallus corrugatus*, *Amorphophallus konjac*, *Amorphophallus*

opertus, Amorphophallus paeoniifolius and *Amorphophallus coaetaneus.* These samples were collected from the investigations in 3 provinces: Ha Giang, Cao Bang, and Binh Thuan.
D-glucose, axit sunfuric, NaOH, 3,5-dinitrosalicylic acid (3,5-DNS) were purchased from Sigma-Aldrich Co.

Determine the glucomannan content in 5 samples of amorphophallus corms

Glucomannan were extracted (Sugiyama et al., 1972) and determined the glucomannan content by measuring the D-glucose concentration after hydrolysis experiment (Chua et al., 2012). After the steps of glucomannan extraction, 5ml of konjac glucomannan sample solution obtained was added to a 25ml volumetric flask and then is the adding of 2.5ml sulfuric acid (3 M). The mixture was stirred well, hydrolyzed in 1.5 hrs, followed by the addition of 2.5ml NaOH (6 M) and then mixed well. The solution was made up to 25ml with distilled water.

3,5 – DNS colorimetric assay was used to determine the glucomannan concentration in extracted solutions. 2 ml of konjac glucomannan sample solution and 2 ml of konjac glucomannan hydrolysate was added to distinct 25 ml volumetric flasks, followed by the addition of 3,5 – DNS (1.5 ml) to each flask. Each mixture was heated for 5 mins in a boiling water bath, cooled the mixture to ambient temperature and then diluted to 25ml with DI water. The colorimetric assay was carried out by measuring the absorbance at 550 nm wavelength.
The glucomannan content was determined by the equation (Chua et al., 2012):

$$G\% = \frac{5000f\,(5T-T_0)}{m}$$

Where
G: glucomannan content/dry weight of sample
f: correction factor
T: glucose content in konjac glucomannan hydrolysate (mg)
T_0: glucose content in konjac glucomannan sample solution (mg)
m: mass of analysed konjac sample

***In vitro* propagation of the target amorphophallus species**
Sterilize the explants

Corms of the target konjac species (after screening the glucomannan content) was washed by a diluted soap solution and then rinsed by DI water. The sample was continually sterilized by hypochlorite Na solution 50% (v/v) for 25 mins, followed by sterile deionized water washing and then immersed in cefotaxim (500 µg/L) for 24 hrs. The sample was then rewashed with sterile deionized water and cut into small pieces (1.5 cm × 1 cm × 0.3 cm). The sterile explant was cultured on the basic MS medium (Murashige and Skoog, 1962). The explant was observed after 2 weeks of culture.

Regenerating shoot from the sterile explant

The sterile konjac pieces was cultured on MS medium supplemented with BA (1, 2, 3, 4 and 5 mg/L) and IBA (0.1, 0.2 and 0.3 mg/L) to evaluate the efficiency of shoot regeneration. The experiment was randomly carried out with 2 interactive factors. The percentage of explants regenerating shoots and the average number of shoots was observed after 8 weeks of culture

The cultures were incubated under light intensity of 3000 Lux with 10 hrs light and 14 hrs dark cycle. The temperature was maintained at 25 – 28ºC. The culture medium was MS medium (Murashige and Skoog, 1962) adjusted to pH 5.8 and the agar concentration is 8 g/L contained 15% sucrose.

Data analysis

Each experiment was replicated 3 times. Data was analyzed by Ducan test ($p < 0.01$) in SAS software (ver. 6.12) and Excel.

RESULTS AND DISCUSSIONS

Determine the glucomannan content

The konjac samples were collected at various regions (Table 1), extracted and determined the glucomannan content. Glucomannan contains 2 constituent sugar molecules including mannose and glucose linked by a β-(1-4)-glycosidic bond in a molar ratio of 1.6:1. Hydrolysis of glucomannan will create constituent sugar molecules reacting with 3,5 – DNS and consequently produce an amino complex (3-amino-5-nitrosalicylic acid) with a red-brown color under alkaline conditions. The content of glucomannan were determined by the reduction of sugar contents when reacted with 3,5-dinitrosalicylic acid (3,5-DNS) (based on the D-glucose standard calibration curve) (Chua et al., 2012). The result showed the difference of glucomannan content among the analyzed samples (Table 1). This can be suggested that different konjac species contain different levels of glucomannan content. Besides, glucomannan content can be affected by various factors such as nutrient conditions (Chua et al., 2012; Lee et al., 1992), ages of growth (Chua et al., 2012; Kurihara, 1979), harvesting seasons (Kurihara, 1979), size of konjac (Long, 1998) and so on. According to the result in this study, *Amorphophallus corrugatus* species had the highest content of glucomannan (43.5%). This species was selected for *in vitro* propagation.

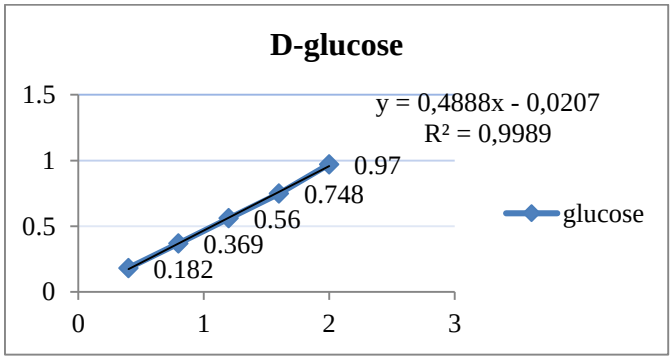
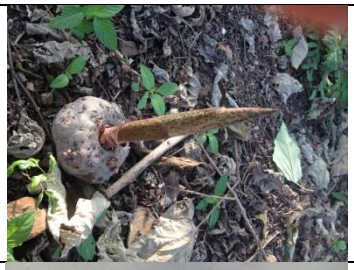


Fig 1: D-glucose standard calibration curve

Table 1: Information and glucomannan content of analyzed samples

No.	Information of Samples	Photos of samples	Glucomannan content (%)
1	Species *: Amorphophallus konjac* *Distribution: Ha Giang, Cao Bang* *Weight:182,5 g* *Diameter: 12 cm*		37.83
2	*Species : Amorphophallus coaetaneus* Distribution: Ha Giang *Weight: 201 g* *Diameter: 13 cm*		30.90
3	*Species: Amorphophallus corrugatus* Distribution: Ha Giang *Weight: 234 g* *Diameter: 14,5 cm*		43.50
4	*Species : Amorphophallus opertus* Distribution: *Binh Thuan* *Weight: 207 g* *Diameter: 15,5 cm*		29.53
5	*Species: Amorphophallus paeoniifolius* Distribution: Hà Giang, Cao Bằng *Weight: 89,6 g* *Diameter: 4-4,5 cm*		31.35

***In vitro* culture of the selected konjac species**

After sterilizing, the konjac corm was splitted into pieces (Fig 2) and was cultured on MS medium. 85% of sterilized explants were obtained after 2 weeks of culture.

Fig 2: Sterilized explant of *Amorphophallus corrugatus* species cultured on MS medium

Sterilized explants of *Amorphophallus corrugatus* species were cultured on basic MS medium supplemented with plant growth regulators to study the ability of *in vitro* regeneration and growth of shoot from explants. The data was collected after 8 weeks of culture (Table 2; Fig 3).

Table 2: Effects of plant growth regulators to *in vitro* shoot regeneration from explants of *Amorphophallus corrugatus*

Test	BA (mg/L)	IBA (mg/L)	Average number of shoot (shoot/explant)
C_1	1	0,1	$1,00^{f}$
C_2	1	0,2	$2,67^{ef}$
C_3	1	0,3	$3,33^{de}$
C_4	2	0,1	$3,67^{de}$
C_5	2	0,2	$3,33^{de}$
C_6	2	0,3	$4,00^{de}$
C_7	3	0,1	$8,33^{a}$
C_8	3	0,2	$6,00^{bc}$
C_9	3	0,3	$4,67^{dc}$
C_{10}	4	0,1	$6,67^{ab}$
C_{11}	4	0,2	$4,33^{dce}$
C_{12}	4	0,3	$3,00^{de}$
p			<0,01
CV			17,54

Fig 3: Shoots of *Amorphophallus corrugatus* regenerated from explants on C_7 medium after 8 weeks of culture

There are many reports show that auxin (IBA) itself cannot stimulate organ regeneration, it should be combined with BA for that ability (Nakano et al., 1994). In this study, MS medium supplemented with BA (3mg/L) and IBA (0.1 mg/L) in C_7 test had the highest efficiency of shoot regeneration (8.33 shoots/explant). The combination effect between BA and auxin on shoot regeneration had been recorded (Hu et al., 2008). In the experiment on *Amorphophallus albus* konjac, the combination of BA (4.44 µM) and NAA (1.07 µM) or Kinetin (2.32 µM) and NAA (0.54 µM) showed the highest efficiency of shoot regeneration as 7.8 shoots/explant and 7.5 shoots/explant, respectively. The regenerated shoots were subcultured on C_7 medium to increase the number and the size.

Fig 4: Shoots of *Amorphophallus corrugatus* on C_7 medium after 4 weeks of culture

CONCLUSIONS

Glucomannan was extracted from various *Amorphophallus* species could have a potential target because of its high value in food technology, medicine and pharmacy. In this study, we have already demonstrated that *Amorphophallus corrugatus* konjac has a high content of glucomannan (43.5 %) from analyzed samples of 5 species. The shoot regeneration from pieces of konjac corms was also evaluated: 8.33 shoots/piece cultured on MS medium supplemented with IBA (0.1 mg/L) and BA (3mg/L). This result can be applied for large-scale propagation of the selected konjac species to provide for food industry, medicine and pharmacy.

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