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RESEARCH ARTICLE

NUTRIENTEVALUATION, PHYTOCHEMICAL ANALYSIS AND *INVITRO* MICROPROPAGATION IN NAVARA (*ORYZASATIVA* L.)

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ABSTRACT

The study was conducted to assess the nutritional value, phytochemical compounds and invitro regeneration in Navara. The major nutrients in dehusked rice were Ca (10.3%), Fe (2.81%), Zn (2.78%), P (197%), VitB1 (0.77%), VitB2 (0.16%), VitB3 (1.26%), protein (8.8%), fat (2.4%) and carbohydrate (72.7%).GC-MS analysis of hexane extract of Navara revealed the presence of 113 phytochemical compounds, of which the major compounds were Cyclotrisiloxane hex methyl (52%), 5,7,9(11)-Androstatriene,3-hydroxy-17-oxo- (64%) and Olean-12-ene-3,16,21,22,28-pentol, 21-(2-methyl-2-butenoate), [3a,16a,21a(Z),22a]- (66%).To observe the callus induction and plant regeneration, different concentrations and combinations of growth regulators were added in MS medium. Better callus induction was observed on MS medium supplemented with 2,4-D 1mg/l, while MS medium supplemented with BAP1mg/l+NAA0.5mg/l induced the highest somatic embryogenesis. Better shoot multiplication was observed on MS medium containing BAP 0.5mg/l + IBA 1mg/l. Effective root regeneration was on MS + IBA 1mg/l. Present study provides a protocol to conserve and multiply this nearly extinct medicinal rice variety.

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INTRODUCTION

Navara is indigenous to Kerala and has important role in ayurvedic system of treatment. It is of two kinds based on glume colour; black glumed Navara and white glumed Navara (Joseph *et al*., 2007).Black colored Navara uses particularly in northern districts of Kerala, while the white or yellow glumed ones are popular among the traditional medical practitioners of southern districts (Leenakumary, 2004). Plant shows wide range of medicinal uses, mainly in the preparation of navarakizhi and navara oil. Rice is the basic food of more than half of the world's population and also acts as the primary source of calories (Pathak, 1982). It is the source of minerals, vitamins, proteins, fibres, carbohydrates, fats etc (Abbas *et al*., 2011). Among the rice varieties, Navaraconsiders as suitable rice useful as food for healthy men, women and children (Moos, 2004). Gas Chromatography - Mass Spectrometry (GC-MS) is a technology for secondary metabolite profiling in plant and non-plant species. Gas chromatography separates the components of the mixture and mass spectroscopy analyzes each of the components separately. Most of the analyzed volatile components are long chain unsaturated fatty acids which are structural elements of many valuable compounds as well as important sources of energy (Spokas *et al*. 2011). India is among the largest rice growing countries, accounting for about one third of the world acreage under the crop (Verma *et al*., 2011). Due to the increasing importance in nutrition and economy, Navara should be conserve and multiply in nature. Tissue culture techniques are often using in many crop improvement programmes of rice as a tool of plant breeding (Yamada, 1986). Callus proliferation is the essential step of micro propagation. Rice seeds have more potential for call genesis as compared to node or tip (Rashid *et al.* 2000, Gonzalez 2000; Navraj *et al*. 1999; Marasi 1996; Valdez *et al*. 1997). Callus culture on nutrient medium with suitable growth regulators shouldinduce the multiple shoots. Present study was aimed to analyze the nutrients and phytochemical compounds in Navara. Study also focuses on the standardization of suitable concentration and combination of growth regulators for callus induction, somatic embryogenesis and shoots multiplication.

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MATERIALS AND METHODS

The experiment was conducted in the tissue culture laboratory of the Department of Botany, KAHM Unity Women's College, Manjeri, Malappuram, Kerala, India. Medicinal rice variety - Navara was used for nutrient evaluation, phytochemical analysis, callus induction and plant regeneration. Seeds were

collected from Chandragiri Modern Rice Mill, Thirurangadi, Malappuram, Kerala, India.

GC-MS analysis

The hexane extract of powdered dehusked seeds was used to analyze the phytochemical compounds. GC-MS analysis was carried out using the instrument method - C:\Xcalibur\ RCEL_KKD\DRUGS\METHOD\NAT.PRODUCTS. meth from the Department of Applied Chemistry, Cochin University of Science and Technology (CUSAT), Kochi, Kerala, India.

Nutrient analysis

Nutrients in the powdered dehusked seeds were analyzed from Central Food Technological Research Institute (CFTRI), Mysore, Karnataka, India.

Explant sterilization

Seeds were selected as explant for the present study. The husks were removed manually. Dehusked seeds were thoroughly washed with distilled water for 4-5 times followed by surface sterilization with 1% bavistine for 30 minutes and 1% Tween 20 for 15 minutes. Then, the explants washed with distilled water for 3 times. In the laminar air flow cabinet, the explants were treated with 0.1% $HgCl_2$ for 3 minutes. Finally, the explants were washed with sterile distilled water for several times to remove all the sterilizing agents.

Callus induction and plant regeneration

The basal medium MS was used for callus induction (Murashige and Skoog, 1962). The proposed medium was supplemented with 2,4-D (0, 0.5 and 1mg/l).The pH of the media was adjusted to 5.8 ± 2. Surface sterilized seeds were inoculated within the laminar air flow chamber and maintained in an environmentally controlled room at 22 ± 2°C with a photoperiod of 8 h daylight and light intensity of $1500\mu Em^{-2}S^{-1}$.After 21 days, callus induction frequency was recorded. Callus was sub-cultured to the MS medium supplemented with 2,4-D1mg/l and maintained for one month. For plant regeneration, the embryogenic callus was excised and sub-cultured on MS medium supplemented with BAP0.5mg/ l+IBA1mg/l,BAP1mg/l+IBA0.5mg/l, BAP1mg/ l+NAA0.5mg/ l and BAP0.5mg/l+NAA1mg/l. The pH of media was adjusted to 5.8 ± 2 and autoclaved. Cultures were maintained in an environmentally controlled room at 22 ± 2°C with a photoperiod of 8 h daylight and light intensity of $1500\mu Em^{-2}S^{-1}$. After one month, shoot multiplication and somatic embryogenesis frequencies were recorded. For root regeneration, the shoots were sub-cultured on MS medium supplemented with different concentrations of IBA (0, 0.5, 1.0, 1.5 and 2mg/l).

RESULTS AND DISCUSSION

Rice is a dietary staple food and one of the most importcereal crops, especially for people in Asia (Rohman *et al.,* 2014). It includes the nutrients likeprotein, fat, crude fibre, carbohydrate, ash, minerals (Ca, P, Fe, Na, K and Vitamins viz. Thiamine, Riboflavin, Niacin, Tocopherol) etc. which plays an important role in disease prevention (Gayen *et al*., 2013). Medicinal rice variety - Navara uses to prepare healthy food due to the presence of enormous nutrients and medicinal compositions. In the present study, macro nutrients (calcium and phosphorous), micro nutrients (Fe and Zn) and vitamins (Vitamin B1, Vitamin B2 and Vitamin B3) were analyzed in Navara (Table 1). Among the mineral nutrients, Phosphorus showed the highest quantity. It was also rich with carbohydrate, protein, fat, insoluble dietary fibres, soluble dietary fibres and crude fibres. The caloric value was 348 K.cals/100g. Deepa *et al*. were reported the higher nutritional value of Navara rice compared to IR64 and Jyothi in 2007.

Table 1. Analyzed nutritional contentsin dehusked Navara rice

Sl. No.	Parameters	Quantity (mg/100g)
1	Calcium	10.3
2	Iron	2.80
3	Zinc	2.80
4	Phosphorous	197
5	Vitamin B1	0.77
6	Vitamin B2	0.16
7	Vitamin B3	1.30
8	Protein	8.80
9	Carbohydrate	72.7
10	Fat	2.40
11	Total ash	1.20
12	Moisture	13.7
13	Crude fibre	1.10
14	Insoluble dietary fibre	12.2
15	Soluble dietary fibre	0.30

For the analysis of plant volatile compounds using GC-MS analysis, different solvents including ethanol, hexane, methanol, ethyl acetate etc. were used (Mamza *et al*., 2012). In the study, GC-MS analysis of hexane extract of dehusked ricerevealed the presence of 113 phytochemical compounds, of which cyclotrisiloxane hexamethyl showed the lowest RT value (4.06) with 52% probability (Table 2). The compounds, 5,7,9(11)-Androstatriene,3-hydroxy-17-oxo- and Olean-12-ene-3,16,21,22,28-pentol,21-(2-methyl-2-butenoate), [3a,16a, 21a(Z),22a]- showed the probability of 64 and 66% while the RT values were 168 and 184 (Table 2).The range of retention time varies from 4.06 to 215.56. Similarly, the metabolomics of rice using GC-MS analysis was analyzed in different research institutes (Ogawa *et al*., 2017). Krishnaveni (2015) was reported the major phyto-constituents in brown rice with retention time ranging from 4.79 to 35.15, that including 6-(1-Hydroxymethylvinyl)-4, 8a-Dimethyl-3, 5, 6, 7, 8, 8a-hexahydro-1H-naphthalen-2- one, 26-Nor-5-cholesten-3β-ol-25-one, Propane, 1,1-diethoxy- and Stigmastan-3,5-diene. The demand for rice is continuously growing with the increasing population, thus suitable biotechnological methods are essential to enhance the productivity. Efficient plant regeneration through *in vitro* micropropagation is very essential for the successful utilization of biotechnology in rice crop improvement. In rice, *in vitro* plant regeneration from scutellum has been reported by Wani *et al*. (2011).

The use of mature seed embryos has distinct advantage over other explants as staring material for *in vitro* regeneration (Jubair *et al*. 2008). In the present study, callus induction was started from Navara seed embryo. Within 21 days, callus was regenerated from embryonic tissue on MS medium supplemented with 2,4-D 1mg/l. Callus was creamy white in colour, friable and embryogenic, which was fragmented and sub-cultured to the same medium for further experiments (Figure 1).However, supplementation of 2-2.5 mg/l 2,4-D on MS medium induced better callus tissue in Super Basmati, Basmati-370, Basmati-371 and Fakhre Malakand (Tariq *et al.,* 2008; Revati and Pillai, 2011).

Table 2. Compounds analyzed in hexane extract of dehusked Navara rice with probability of above 30%

Sl. No.	Compounds	Molecular weight	Probability (%)	RT value
1	Cyclotrisiloxane, hexamethyl	222	52.38	4.06
2	Benz[e]azulene-3,8-dione, 3a,4,6a7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-5-(hydroxymethyl)-7-(1-hydroxy-1-methylethyl)-2,10-dimethyl-,[3aR-(3aa,6aa,7a,10a,10aa,10ba)]-	364	47.18	4.93
3	Cyclobarbital	236	47.93	7.65
4	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethenyl)-, trans-	152	39.61	19.21
5	2-Bromotetradecanoic acid	306	46.91	73.70
6	Cyclopropanebutanoic acid,2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl] methyl]cyclopropyl]methyl]-,methyl ester	374	33.24	78.19
7	aR-Turmerone	216	34.54	79.54
8	Tridecanoic acid, 12-methyl-, methyl ester	242	50.35	86.85
9	n-Hexadecanoic acid	256	34.35	112.49
10	Phorbol	364	47.94	123.85
11	Hyodeoxycholic acid	392	35.42	156.90
12	5,7,9(11)-Androstatriene,3-hydroxy-17-oxo-	284	64.89	168.83
13	Olean-12-ene-3,16,21,22,28-pentol,21-(2-methyl-2 butenoate),[3a,16a,21a(Z),22a]-	572	66.06	184.23

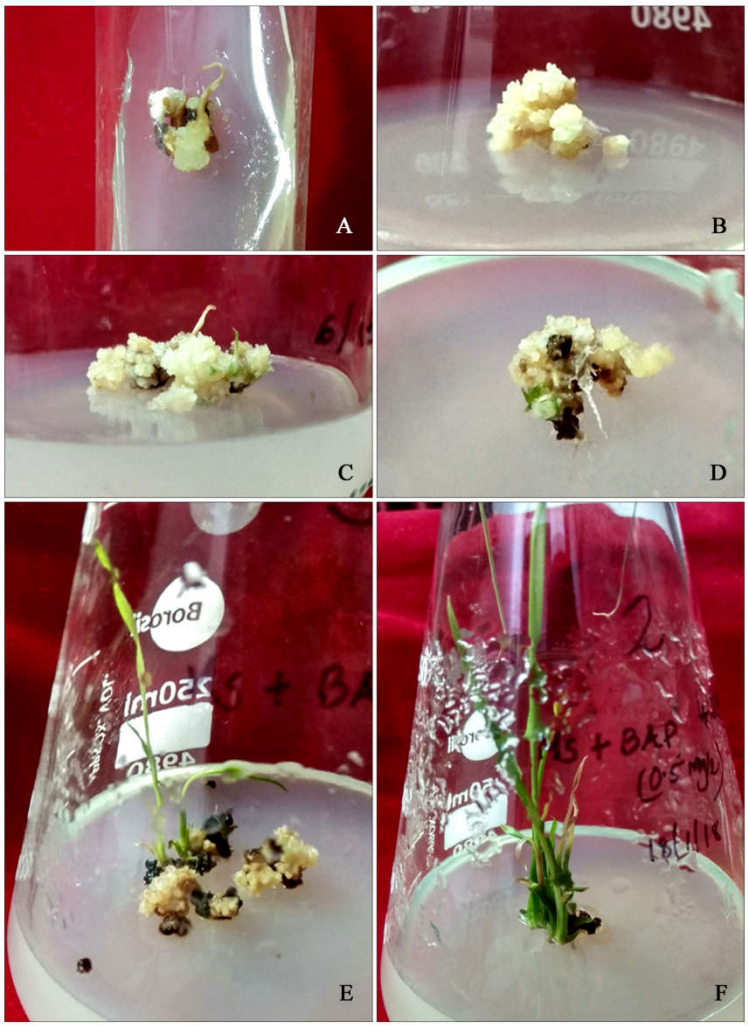

Figure 1. A. Callus induction from seeds on MS+2,4-D 1mg/l (3rd week), B. Sub-culture of callus on MS+2,4-D1mg/l (5th week), C. Sub-culture of callus on MS+BAP1mg/l+NAA0.5mg/l(5th week), D. Sub-culture of callus on MS+BAP1mg/l+IBA0.5mg/l (5th week), E. Sub-culture of callus on MS+BAP1mg/l+NAA0.5mg/l (7th week), F. Sub-culture of callus on MS+BAP0.5mg/l + IBA1mg/l (5th week)

In contrast, the optimal callus induction frequency at 90% was obtained in rice cultured on MS media containing 2,4-D 3 mg L^{-1} and NAA 2 mg L^{-1} (Din *et al.,* 2016). Growth regulators in different combinations and concentrations induce plant regeneration from calli. In the study, BAP, NAA, IBA and 2,4-D were showed different responses in calli induction and plant regeneration. Highest embryonic responses were reported by Verma*et al*. (2011) in some rice varieties (Govind and Pusa Basmathi) by supplying the MS medium provided with 2,4-D (0.4µM) and kinetin (0.4-2.0 µM). Our results also showed better callus proliferation and somatic embryogenic response on MS medium supplemented with BAP1mg/ l+NAA0.5mg/l. Shoot regeneration was highest in calli grown on MS medium supplemented with BAP0.5mg/ l+IBA1mg/l (18 ± 2) (Figure 1).Moreover, Basmati-370 and Basmati-371 were showed better regeneration on N6 media containing NAA 1 mg L(-1) and BAP 2.5 mg L(-1) (Tariq *et al.*, 2008). In the study of Ullah *et al.* (2007), callus induction from Basmati-370

and 385 on MS medium supplemented with 2,4-D (2-2.5mg/l) and BAP (0.1-0.5mg/l) were reported. In the present experiment, MS medium provided with BAP0.5mg/ l+NAA1.0mg/ l never induced callus and plant regeneration, while 2-4 roots of 0.3-0.5 cm were produced. On hormone free MS medium and MS medium supplemented with BAP1mg/l+IBA0.5mg/l, the callus showed better proliferation, of which the calli were creamy-white in colour, friable and non-embryogenic. Supplementation of auxins in MS medium induces the root regeneration in *invitro* cultures. In the study, the multiple shoots cultured on MS+IBA1mg/l showed better root length(4.5 ± 1.08 cm) compared to shoots grown on MS medium without growth hormones (3.0 ± 0.7 cm).The number of roots was higher in multiple shoots supplemented with hormone free MS medium (12 ± 1.2) (Figure 2). The plants were hardened in plastic pot contained sterilized soil and maintained under green house.

Figure 2. Root regeneration in multiple shoots cultured on MS medium supplemented with IBA1mg/l (A) and on hormone free MS medium (B) within 14 days

Conclusion

Navara is a nutritionally and medicinally important rice variety. It consisted of minerals, vitamins, proteins, carbohydrates, fats, dietary fibres etc. The variety shows the presence of 113 volatile phytochemical compounds with many medicinal values. In *invitro* culture, better callus proliferation and somatic embryogenesis were on MS medium supplemented with 2, 4-D1mg/l and BAP1mg/l + NAA0.5mg/l respectively. While the highest shoot multiplication frequency was observed on MS + BAP0.5mg/l + IBA1mg/l. It is a successful protocol of high frequency regeneration from mature embryo through somatic embryogenesis in Navara which would be useful for genetic transformation using any selected gene transfer method in future.

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