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Seed Germination Behaviour and Preliminary Screening of Bioactive Components in Buckwheat (*Fagopyrum*spp.)

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ABSTRACT

Buckwheat, a traditional and underexploited crop, has high nutritious value and is tolerant to infertile soils. In this study, seed samples of *Fagopyrum esculentum* (Sample i.d.: F1, F2 and F3) and *Fagopyrum tataricum* (Sample id. :F4, F5) collected from diverse ecological zones of India were studied for their germination characteristics and nutrition mainly antioxidant potential to act as nutraceuticals. The dietary attributes of buckwheat place this crop in an excellent position for value added processing and increased utilization. Seed germination behaviour was observed with petri plate and germination paper method under controlled conditions. The results indicated high germination percentage in F2, F4 and F5 seed samples representing *F.esculentum* and *F. tataricum*. The phytochemical screening of buckwheat seeds was done with aqueous and ethanolic extracts which revealed the presence of alkaloids, glycosides, saponins, diterpenes, flavonoids, coumarins, phytosterols, lignins and terpenoids. The present study showed that bioactive component, flavonoid was obtained only in ethanolic extracts and was found abundant in samples F2, F4 and F5. In comparison, aqueous extracts had high amounts of other components such as alkaloids, coumarins and diterpenes. Therefore, introduction of buckwheat seed extracts in therapeutic formulation scan provide new avenues for consideration in advancing the utilization of this plant.

Keywords:Buckwheat, bioactive compounds, nutraceutical, functional food

INTRODUCTION

Buckwheat is an annual, short duration crop. It is a pseudocereal with its grains sans gluten have similar chemical composition to the cereals such as rice, wheat and maize (1). Grain flour is popularly known as ‘kuttu ka atta’ and sold at premium price during religious fasting among North Indians. The two cultivated buckwheat species are common buckwheat (*F. esculentum*) and tartary buckwheat (*F. tataricum*) (2). Buckwheat is produced extensively in many regions of the world. The range of distribution of buckwheat extends from the mountainous regions of India, Pakistan, Nepal, Bhutan and Myanmar. It is also grown in China, Japan, Korea, Iran and Afghanistan. In India, the buckwheat crop is grown in the Himalayan region from Jammu and Kashmir in the North to Arunachal Pradesh in the East (3). Kuttu is nutritionally equivalent to many of the cereals and

hence could be be used to improve the nutritional quality of the cereal grains. It is high in essential amino acids e.g. lysine is nearly twice the amount found in wheat and white rice. The main protein solubility fraction in buckwheat is globulin. Buckwheat proteins can show a strong supplemental effect when combined with vegetable proteins to improve the dietary amino acid balance (4).The human body can utilize 74% of the available protein in buckwheat. Although other foods are also high in available protein but majority of them contain high levels of fat.

Buckwheat grains and their hull consist of certain bioactive components with high nutritional value and biological activity, i.e. flavonoids and flavones, phenolic acids, tannins, phytosterols, diterpenes, coumarins and fagopyrins (5, 6). Their contents and compositions differ depending on the buckwheat species and growing conditions. These components represent wide range of biological activities such as hypocholesterolemic, hypoglucemic, anticancer and anti-inflammatory action (7).

Study of seed germination parameters in plants is indicative of seed performance in a particular environment. Information about the seed health and seedling vigour is obtained as the seed germinates in a specific time, location, temperature, and light conditions. Thus, an attempt was made to record seed germination parameters of buckwheat seeds obtained from different agro-ecological zones of India. In addition, phytochemical screening assay which is a simple, quick, and inexpensive procedure and an important tool in bioactive compound analyses was performed. Extensive research has been conducted in the nutritional properties of buckwheat seed proteins, flavonoids, flavones and phytosterols. However, there are few studies on morphological characterization and phytochemical analysis of buckwheat seeds obtained from different agro-ecological zones. Thus, the study was formulated to screen the presence of nutritional components in the seeds collected from different agro-ecological zones of India.

METHODOLOGY

Plant material

The experimental material consisted of buckwheat seeds collected from various locations in India (Table I). The seeds were labelled as F1, F2, F3, F4 and F5.The seed samples F1, F2 and F3 representing *Fagopyrum esculentum* and F4, F5 that of *Fagopyrum tataricum*. F3 seeds were dehulled.

Seed germination parameters

Buckwheat seeds labelled as F1, F2, F3, F4 and F5 were surface sterilized with 1% sodium hypochlorite solution. The seeds were then rinsed with distilled water thrice and then kept for germination in sterilized petri-plates lined with moist filter paper. In addition, seeds were kept for germination in germination paper for a period of 10 days and seed germination parameters were recorded. The experiment was done in replicates.

Extraction procedure for bioactive components

Briefly, 5 g of each seed sample was grounded into fine powder with mortar and pestle and dissolved in 50 ml of distilled water and ethanol(AR grade, 98% purity).The seed samples were kept on shaker for 2hrs. The aqueous and ethanolic solutions were filtered through Whatmann filter paper no.1 and the filterate obtained was used for phytochemical screening tests.

Phytochemical screening of secondary metabolites

Phytochemical analyses were carried out for all the extracts as per the methods mentioned below. The tests were performed in replicates.

Test for Flavonoids- Extracts were treated with few drops of sulphuric acid. Formation of intense yellow colour indicates the presence of flavonoids.

Test for Alkaloids (Dragendroff's test)-Extract was dissolved in dilute hydrochloric acid and filtered. Filtrates were treated with Dragendroff's reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

Test for Saponins (Foam test)- 0.5 gm of extract was shaken with 2ml of water. If foam produced persists for 10 minutes, it indicates the presence of saponins.

Test for Tannins and Phenolics(Ferric chloride test) - To the extract, few drops of dil. ferric chloride solution (5%) was added. Formation of blue blackcolour indicates the presence of tannins and phenolics.

Test for Phytosterols (Libermann Puchards test) - To the extract, 1ml of chloroform was added and the solution was filtered. The filterate was treated with 10 drops of acetic anhydride, boiled, cooled and few drops of conc. H_2SO_4 was added to it. Formation of brown ring indicates presence of phytosterols.

Test for Terpenoids (Salkowski test)- To the extract, few drops of chloroform was added and to it conc. H_2SO_4 was added. Formation of red brown colour indicates presence of terpenoids.

Test for Coumarins- To the extract, few drops of dilute 1N NaOH was added. Yellow coloration indicates presence of coumarins.

Test for Diterpenes- To the extract, few drops of copper acetate solution was added. Formation of emerald green colour indicates presence of diterpenes.

Test for Lignins- To the extract, few drops of phloroglucinol solution was added. Formation of pink colour indicates presence of lignins.

Test for oils and fats- To the extract, few drops of Sudan III was added. Formation of reddish pink colour indicates presence of oils and fats.

Test for Carbohydrates

Reducing sugars-To the extract, 0.5 ml of Fehling A and Fehling B were added. Extract was heated. Formation of orange red precipitate indicates presence of reducing sugars.

Non-reducing sugars-To the extract, few drops of sulphuric acid was added followed by a pinch of sodium carbonate and 0.5 ml of Fehling A and Fehling B. Formation of orange red precipitate indicates presence of non-reducing sugars.

Glycosides- To the extract, 2ml of glacial acetic acid was added. To it few drops of ferric chloride solution was added. Formation of brown ring indicates presence of glycosides.

RESULTS

Seed germination parameters

The seed samples used in the present study were collected from different agro-ecological zones of India representing two *Fagopyrum* species (Table I). Seeds of F1 and F2 belonging to *F. esculentum* were black to brown in color, triquetrous shape with smooth, flat surface. However, F3 represented dehulled seeds of *F. esculentum*, whitish cream in color and flat surface. The seed

samples designated as F4 and F5 represented *F. tataricum*, with color ranging from greyish black to brown and triquetrous shape with deep grooves present on the surface (Figure I)

Table-I: *Fagopyrum* seed samples used in the present study.

Sample i.d.	Species	Seed colour	Seed shape	Place of Collection
F1	*Fagopyrum esculentum*	Light/dark brown	Triquetrous	District Mahindergarh, Haryana
F2	*Fagopyrum esculentum*	Dark brown/black	Triquetrous big	Chattisgarh, West Bengal
F3	*Fagopyrum esculentum*	Whitish cream	Triquetrous small (dehulled seeds)	Nagar bazar, District Basti, Uttar Pradesh
F4	*Fagopyrum tataricum*	Greyish black	Triquetrous elongated with deep grooves	Village Thapak, Patan Valley, Lahaul
F5	*Fagopyrum tataricum*	Dark brown/black	Triquetrous with grooves	Ranikhet, Uttarakhand

Figure-I: *Fagopyrum*seed samples collected from different agro-ecological zones of India representing *F. esculentum* (F1, F2 and F3) and *F. tataricum* (F4 and F5).

Germination paper and petriplate method

The seed germination parameters were recorded by germination paper and petriplate method. In germination paper method, nine seeds were kept in wet germination paper for ten days under controlled conditions and recorded for parameters such as percent seed germination, hard seeds, fungal infected seeds etc. The results showed highest germination rate in F5 seed sample (89%) followed by F4 seed sample (33%) and F2 seed sample (25%). Percent germination was found to be zero in F1 and F3. Seedling length of *Fagopyrum spp.* was also measured by germination paper method (Figure-II).The length of the seedlings obtainedafter ten days of germination was found to be the highest in F4 seedling (16.3±6.11 cm)followed by F5 seedling(12.25±5.7cm) and

F2seedling measuring 11±5.6 cm (Table-IIa), indicating F4 seed sample to be a promising sample which can tolerate seasonal variation and possess high germination potential in variable environment.

In petriplate method, total number of seeds kept for seed germination was 17 seeds per petriplate. The results were observed after ten days of germination and recorded for growth parameters(Figure-II). The results obtained for petriplate method indicated F4 seed sample with highest germination rate (82%) followed by F5 seed sample (59%) compared to the other three seed samples (Table IIb). The length of the seedlings obtainedafter ten days of germination was found to be the highest in F5seedling (14.30±2.1cm)followed by F4seedling(12.0±3.5) and F2seedling measuring 10.5±2.0 cm. Of the five seed samples, F1 seed was found to be highly susceptible to fungal infection.

Phytochemical screening of secondary metabolites

In the present study, two different extracts, *viz* aqueous and ethanol extractswere screened for presence of various phytochemicals alkaloids, saponins, terpenoids, phenolics and tannins, flavonoids, phytosterols, coumarins, lignins, diterpenes, oil and fats and carbohydrates (Reducing sugars, Non-reducing sugars and glycosides) . The screening of phytochemicals was done in all the five samples used in the present study (Table-III). It was observed that in aqueous extract, F4 seed sample showed the highest amount of alkaloids followed by F2 seed sample. F3 and F5 had equal amounts of alkaloids and this amount was lesser than that of F2. F1 showed absence of alkaloids. Screening of carbohydrates, including reducing sugars, non-reducing sugars and glycosides showed F5 seed sample with highest amount of reducing sugars followed by F4 and F2. F3 and F1 had low amounts of reducing sugars. However, F1 and F2 showed absence of non-reducing sugars and F3, F4 and F5 showed equal amounts of non-reducing sugars. All the five seed samples showed presence of glycosides in low amounts. It was observed that phytosterols, free phenolics, tannins and flavonoids were absent in aqueous extract. The aqueous extract showed the presence of saponins in F2 and F3 seed samples with equal amounts and was absent in F1, F4 and F5 (Table-III). Terpenoids were detected only in F4 and absent in all the other four seed samples. Oils and fats did not test positive in aqueous extract of the five seed samples. F1 showed the highest amounts of coumarins, followed by F2, F3 and F5 with equal amounts. F4 seed sample showed low amount of coumarins, Phytochemicals, such as diterpenes were present in all seed samples but F2 and F5 had high amounts of diterpenes followed by F4. F1 and F3 showed low amount of diterpenes. Lignins were found to be absent in all the five seeds.

Ethanolic extract showed extraction of more phytochemicals than aqueous extract of buckwheat seeds (Table-IV). For alkaloids, F3 seed sample showed the highest amount of alkaloids followed by F1 and F2 which showed equal amounts. F4 and F5 showed absence of alkaloids.Screening of carbohydrates revealed absence of reducing and non-reducing sugars in all five seed samples.However, all the five seed samples showed presence of glycosides but in low amounts.In ethanolic extract, only F3 and F4 seed samples showed presence of phytosterolsandin low amounts. Ethanolic extract also showed absence of free phenolics and tannins. Interestingly, flavonoids, category of polyphenolic compounds were found in high amounts in F2 and F5 seeds followed by F4, F1 and F3 seed samples.All the five seed samples showed absence of saponins.Terpenoids were detected only in F1 and absent in all the other four seed samples (TableIV).All the five seed samplesshowed presence of oils and fats in low amounts. Coumarins were found in low amounts in ethanolic extract of F4 and F5 seeds only.Phytochemicals, such as diterpenes were found to be extracted in both aqueous and ethanolic extract with F4 and F5

showinghigh amounts of diterpenes followed by F1 and F2. F3 seed sample showed low amount of diterpenes. Lignins were absent in all the five seeds.

Table-II: Study of seed germination parameters by a) germination paper and b) petriplate method. The experiment was set up in replicates.

Germination paper method	**Total number of seeds**	**Number of seeds germinated**	**Number of hard seeds**	**% seed germination**	**Fungal infected seeds**	**Seedling length (cm)**
FI	9	0	4	0	1	0
F2	9	1	3	25±3.5	0	11±5.6
F3	9	0	0	0	0	0
F4	9	3	2	33±2.2	0	16.3±6.11
F5	9	8	1	89±2.5	0	12.25±5.7
b) Petriplate method	**Total number of seeds**	**Number of seeds germinated**	**Number of hard seeds**	**% seed germination**	**Fungal infected seeds**	**Seedling length (cm)**
F1	17	0	2	0	7	0
F2	17	4	7	24±2.5	2	10.5±2.0
F3	17	0	0	0	0	0
F4	17	14	2	82±2.0	1	12.0±3.5
F5	17	10	4	59±2.2	0	14.30±2.1

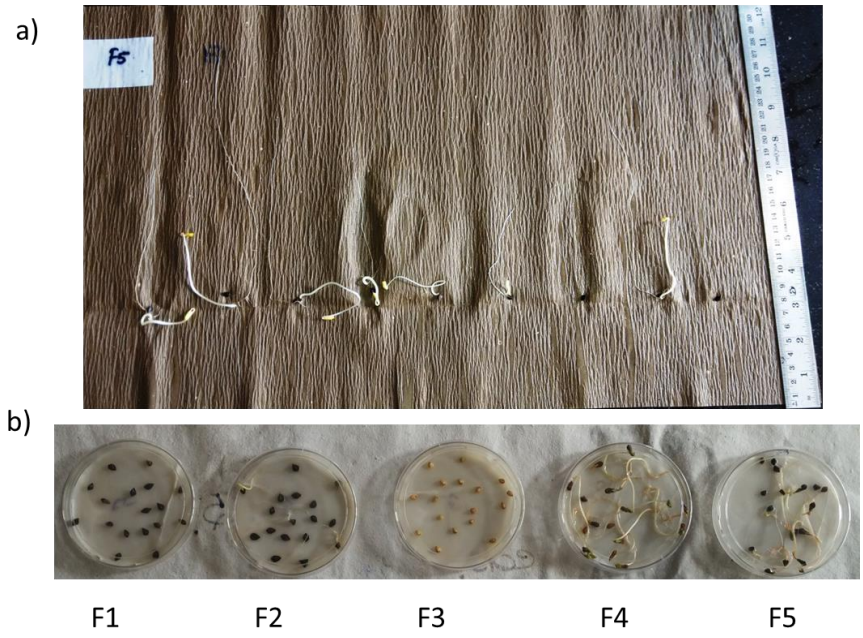

Figure-II: Figure depicting seed germination parameters recorded in five seed samples by a) germination paper and b) petriplate method.

Table-III:Preliminary screening of bioactive components in aqueous extract of buckwheat seeds.

S.No.	Bioactive components	Buckwheat seed samples				
		F1	**F2**	**F3**	**F4**	**F5**
1	Alkaloids	-	++++	++	+++++	++
2	Carbohydrates					
(a)	Reducing	+	++	+	+++	++++
(b)	Non reducing sugars	-	-	+	+	+
(c)	Glycosides	+	+	+	+	+
3	Phytosterols	-	-	-	-	-
4	Tannins and Phenolics	-	-	-	-	-
5	Flavonoids	-	-	-	-	-
6	Saponins	-	+	+	-	-
7	Terpenoids	-	-	-	+	-
8	Oils and Fats	-	-	-	-	-
9	Coumarins	++++	+++	+++	+	+++
10	Diterpenes	+	+++	+	++	+++
11	Lignins	-	-	-	-	-

DISCUSSION

The seed germination results indicated high germination percentage and germination rate in F4 and F5 seed samples collected from Patan valley, Lahaul and Ranikhet, Uttarakhand belonging to *Fagopyrum tataricum* followed by seed sample from Chattisgarh representing *F. esculentum*. Similar studies on seed germination parameters has been reported (8), which suggested similar germination percentage values (90%) for Ukraine buckwheat seeds and concluded that buckwheat seeds carry good genetic and physiological properties which impart high percentage of seed germination.

The results of phytochemical screening of secondary metabolites revealed ethanolic extract as efficient solvent compared to aqueous extract. The accumulation of secondary metabolites is region and climate specific and this variation in amounts of these bioactive components was observed in this study. The preliminary screening of secondary metabolites present in buckwheat seeds collected from different agro-ecological zones of India revealed presence of alkaloids, glycosides, phytosterols, flavonoids, terpenoids, coumarins and diterpenes (9). Of all, flavonoids and alkaloids were present in high amounts in buckwheat seeds of both the species. Earlier studies have reported presence of six major flavonoids in buckwheat namely rutin, quercetin, orientin, homoorientin, vitexin, and isovitexin (10, 11).

Table IV: Preliminary screening of bioactive components in ethanolic extract of buckwheat seeds.

S.No.	Bioactive components	Buckwheat seed samples				
		F1	**F2**	**F3**	**F4**	**F5**
1	Alkaloids	+	+	++++	-	-
2	Carbohydrates					
(a)	Reducing	-	-	-	-	-
(b)	Non reducing sugars	-	-	-	-	-
(c)	Glycosides	+	+	+	+	+
3	Phytosterols	-	-	+	+	-
4	Tannins and Phenolics	-	-	-	-	-
5	Flavonoids	++	+++++	+	+++	++++
6	Saponins	-	+	+	-	-
7	Terpenoids	+	-	-	-	-
8	Oils and Fats	+	+	+	+	+
9	Coumarins	-	-	-	++	+
10	Diterpenes	++	++	+	+++	+++
11	Lignins	-	-	-	-	-

DISCUSSION

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Among these compounds, rutin, a flavonol glycoside, has been recognized as a major antioxidant component that accounts for about 85%–90% of the total antioxidant activity (11). Rutin is a

natural flavonoid with antihyperglycemic, antihypertensive and antioxidant properties (12). In addition, fagopyritols belonging to category of soluble carbohydrates, present in buckwheat seed play an important role in treatment of type II diabetes (13).

CONCLUSIONS

The study pertaining to seed germination parameters and phytochemical screening of secondary metabolites revealed *F. tataricum* to be the prospective species with high adapting ability to new environment and nutraceutical value. *Fagopyrum tataricum* could act as a valuable raw material to be used for the production of functional foods. Buckwheat flour may be a valuable and important introduction in staple diets, taking into consideration its nutritive value and potential promotion of human health.The screening of the phytochemical constituents would be helpful in formulation of new drugs.

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