Quality analysis of buckwheat (*Fagopyrum esculentum* Moench) genotypes of Assam

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CERTIFICATE – I

This is to certify that the thesis entitled **"Quality analysis of buckwheat(*Fagopyrum esculentum* Moench) genotypes of Assam"** submitted to the Faculty of Agriculture, Assam Agricultural University in partial fulfilment for the degree of **Master of Science (Agriculture)** in **Biochemistry and Agricultural Chemistry** is a record of research work carried out by **Mousumi Thakur** under my personal supervision and guidance.

All helps received by her have been duly acknowledged.

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ABSTRACT

Buckwheat (*F. esculentum)* is a gluten-free pseudocereal with high biological value. It occupies a special place amongst cultivable crops due to its nutritional, dietetic and therapeutic properties. Buckwheat grain is characterized by a high content of starch, protein with an advantageous amino acid composition, a low content of α-gliadin and a high content of dietary fibre. The protein of buckwheat is of excellent quality and is high in the essential amino acid lysine, unlike common cereals. It was a very popular food during the 17^{th}-19^{th} centuries, lost its popularity because of competition from wheat during 20^{th} century, but has recently become popular again because of its health-promoting properties.

In the present investigation, sixteen buckwheat germplasm from RARS, North Lakhimpur were evaluated for biochemical constituents of quality significance. Buckwheat germplasm were found varying significantly in their proximate composition with moisture content ranging from 7.52-9.11%, crude protein from 7.23-9.53%, crude fat 1.97-3.62%, ash 1.83-2.93% and crude fibre from 3.71-4.78% on dry weight basis. Starch, amylose and resistant starch were found in the range of 63.18-72.61%, 22.45-24% and 15.20-20.53% respectively with nitrogen free extract ranging from 71.41-76.97%. Total soluble protein ranged from 4.58% to 7.40% and globulin was the major fraction (2.12-3.53%), followed by glutelin (0.96-1.65%), albumin (0.76-1.35%) and prolamin (0.13-0.24%). Buckwheat contained calcium, iron, phosphorus, potassium and sodium from 144.00-215.33, 2.50-3.50, 242.61-282.00, 237.00-298.27 and 1.56-4.24 mg/100gm respectively. Total phenolic content was found between 378.41 to 652.71 mg/100g and flavonoids between 33.80 to 60.11 mg/100g on dry weight basis. Of the sixteen buckwheat germplasm used in the study, released genotypes Himpriya, VL-7 and PRB-1; local genotypes BWC-1, BWC-2, Jonai and Kharupetia-2; accession genotypes EC-218742 and EC-27242 were found superior over the others in terms of nutritional quality.

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LIST OF ABBREVIATIONS

°C	:	Degree centigrade
%	:	per cent
/	:	per
µg	:	microgram
µl	:	microlitre
AAU	:	Assam Agricultural University
AOAC	:	Association of Official Analytical Chemist
BSA	:	Bovine Serum albumin
CD	:	Critical Difference
conc.	:	Concentrated
et al.	:	et alli (and other)
FAO	:	Food and Agriculture Organisation
Fig.	:	Figure
g/G	:	gram
GAE	:	Gallic Acid Equivalent
hrs.	:	hours
i.e.	:	that is
M	:	Molar (concentration)
mg	:	milligram
min	:	minute
ml	:	millilitre
mM	:	millimolar

N	:	Normal (concentration)
pH	:	log $[H^+]$
ppm	:	Parts per million
QE	:	Quercetin Equivalent
rpm	:	revolution per minute
S.Ed	:	Standard error deviation
Sec.	:	Second
viz.	:	Videlicet (namely)
vol.	:	Volume
w/w	:	weight/weight

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CHAPTER I
INTRODUCTION

Buckwheat (*Fagopyrum esculentum*), or common buckwheat, is a plant cultivated for its grain-like seeds and as a cover crop. Despite its name, buckwheat is not related to wheat and in fact, it isn't a grain at all. The word 'buckwheat' was coined from two Anglo-Saxon terms, *boc* from beechnut and *whoet* from wheat (Robinson, 1980). The word beech was used since the fruit of the plant was similar to that of beechnut. It was called wheat because the grain of buckwheat was used in the same way as wheat. However, buckwheat does not belong to the grass family and is not considered a 'true' cereal. It belongs to a group of foods commonly called pseudocereals, because of the similarity to conventional cereals in its use and chemical composition (Campbell, 1997). Pseudocereals are plants that produce fruits or seeds which are used and consumed as grains, though botanically pseudocereals are neither grasses nor true cereal grains.

Pseudocereals extensively consumed in Middle America during pre-hispanic times were dicotyledonous plant species, which were not closely related to each other or to the monocotyledonous true cereals, their name was derived from their production of small grain like seeds. Presently, the grain amaranth (*Amaranthus* spp. Amaranthaceae), quinoa (*Chenopodium quinoa*, Chenopodiacease), and buckwheat (*Fagopyrum esculentum*, Polygonaceae) are important pseudocereals that have persisted through centuries of civilization and have entered into the agriculture system of the countries where cereals are cultivated. The protein of buckwheat, amaranth and quinoa does not contain gluten therefore it can be used in the diet of patients suffering from celiac diseases. A growing trend for nutraceutical and gluten-free cereal-based products highlights the need for development of new products. Buckwheat can serve as one of the potential candidates for such products.

Buckwheat is an ancient dicotyledonous crop belonging to the Polygonaceae family. Many species of buckwheat are grown around the world; however, only nine have agricultural and nutritional value (Krkoskova & Mrazova 2005). Out of these, only two species are used as food around the world; common buckwheat (*F. esculentum*) and tartary buckwheat (*F. tataricum*). The crop is originating in the southwest China. Russia, China, Ukraine and Kazakhstan are the leading producers of common buckwheat with production also in Slovenia, Poland, Hungary and Brazil (Ahmed *et al.*, 2014). Historically, it was a very popular food during the 17^{th}-19^{th} centuries, although it was later neglected during the 20^{th} century in Western countries because of competition from wheat (Cawoy *et al*., 2008). Yet it has recently been observed to increase because of the health-promoting properties of its grains. In 2016, world production was 2.4 million tonnes, led by Russia with 50% of the world total, followed by China with 17% and Ukraine with 7% (FAOSTAT, 2017).

The estimates for area and production under buckwheat in India are not available separately, since all underutilized grain crops have been dealt with under the term 'coarse cereals'. The area and production of coarse cereals was 26.4 million ha and 42.04 million tons in 2011-12 as compared to 31.8 million ha and 34.1 million tons in 1996-97 respectively as per Economics and Statistics, Ministry of Agriculture, Govt. of India (Anon. 2019). There was about 17% decrease in area under coarse cereals, but production was found to be increased by about 19%. This may be attributed to the development of high yielding varieties in different crops under coarse cereals during this period and improved cultivation practices. Similar trend for buckwheat was also reported (Rana *et al.*, 2012). Although cultivation of buckwheat has shown declining trend, people love to grow and eat buckwheat in several regions of India. The crop is widely grown in the high mountains of Jammu and Kashmir in the west to Arunachal Pradesh in the east. In South India, it is sporadically grown in the Nilgiris and Palani hills. It is a short duration crop (2-3 months), and fits well in the high Himalayas where the growing season of a crop is of limited period because of early winter and snowfall. Buckwheat is widely grown in the Northeastern states

including Sikkim and Arunachal Pradesh and it is also cultivated in Manipur, Nagaland, Meghalaya (West Khasi Hills) and in some areas of the Assam plains (Hore and Rathi, 2002). Cultivation of buckwheat as commercial crops is found in Kokrajhar and Bongaigaon districts and many of the villages of Sadiya region of Assam.https://en.wikipedia.org/wiki/Buckwheat - cite_note-faostat16-38

Buckwheat has a powerful ecological adaptability that allows the plant to grow in almost all kinds of extreme environments (Li and Zhang, 2001). It is a broad-leafed herbaceous annual. Buckwheat seed is a fruit, strictly an achene. The seed, covered by a hull, has a triangular shape. The hull may be glossy or dull and brown, black or gray in colour. The hulled buckwheat seed is called groat and resembles the cereal kernel in its gross chemical composition and structure.

Buckwheat has gained an excellent reputation as a nutritious ingredient in human diet (Kreft *et al.*, 1998). Buckwheat grain is characterized by a high content of starch, protein with an advantageous amino acid composition, a low content of α-gliadin and a high content of dietary fibre (Dziedzic *et al.*, 2011). In comparison to cereals, buckwheat proteins are particularly rich in lysine, arginine, and aspartic acid, but contain less glutamic acid and proline (Zhang *et al.*, 2012). Buckwheat has attracted increasing attention from food scientists for its healing effects over chronic diseases. Many of the health benefits of buckwheat have been due to its high levels of phenolic compounds and bioflavonoids including rutin, which are considered to be effective in prevention or cure of cardiovascular disorders (Park *et al.*, 2004).

The buckwheat grain is generally used as human food and as animal or poultry feed, with the dehulled groats being cooked as porridge and the flour is used in the preparation of pancakes, biscuits, noodles, cereals, etc. All these, coupled with the plant's ability to do well on poorer soils, probably accounts for its widespread usage. With increased interest due to its health benefits, buckwheat has now entered into international trade. This development, in turn has promoted the inclusion of this species into crop improvement programs in several countries.

Faced with acute problem of protein energy malnutrition in present days in many countries, the content and quality of pseudocereal proteins in general and buckwheat in particular have become a pertinent point of consideration. There is a large pool of promising germplasm available in the country which can be utilized for nutritional security of the vulnerable population groups. Despite several reports on the beneficial effects of buckwheat in prevention of human diseases and reports on biochemical studies providing information on buckwheat genotypes are available; little attention has been devoted to the variability of biochemical and physiological traits in different buckwheat genetic resources. Biochemical evaluation of available buckwheat genetic resources will be of great help in the identification of elite genotypes for plant breeding and exploitation. The various types of bioactive compounds present in different varieties provide basic background information needed for the efficient production of buckwheat foods with added value. To achieve this objective, an organized biochemical approach is essential to select nutritionally superior genotypes either to serve as parents or to isolate and identify well established crop varieties with superior nutritional quality.

Considering its various uses, early maturity, good adaptation and suitability for marginal production areas, research work on buckwheat in India was started in 1982 under AICRP on Under-Utilized Crops, now functioning as AICRN on Potential Crops under the umbrella of ICAR, New Delhi. The NBPGR, New Delhi, is coordinating and conducting research on 17 crops of food, fodder and industrial value through 14 main, 9 cooperating and 15 voluntary centers located in diverse agro-climatic zones of India. In India, though the majority of buckwheat varieties grown are farmer's own selection, several buckwheat varieties, such as Himpriya, Himgiri, VL-7, PRB-1, Sangla B1 etc. have so far been developed and released by different institutes of India. Besides, a large number of promising germplasm have also been identified for various agronomic and quality characters (Rana *et al.*, 2012). A total of 40 accessions of two buckwheat species, *F. esculentum* and *F. tataricum* were collected from North Eastern Hill Region of India in the states of Sikkim, Arunachal Pradesh, Assam, Manipur, Nagaland and Meghalaya during the period of

1987-2001 (Hore and Rathi, 2002). NBPGR has conserved 11(eleven) indigenous collections of buckwheat from Assam (Anon, 2015). Assam Agricultural University, Jorhat has also developed a germplasm collection of local buckwheat and emphasized on the development of suitable buckwheat variety for Assam and extensive breeding programme is presently being carried out at Regional Agricultural Research Station, North Lakhimpur.

Consequent upon above considerations, the present investigation on buckwheat has been formulated with the following objective

1. To evaluate and compare buckwheat genotypes for biochemical constituents of quality significance.
2. To evaluate bioactive compounds present in different varieties of buckwheat.

CHAPTER II
REVIEW OF LITERATURE

The study aimed at the biochemical characterization of buckwheat genotypes of Assam. This screening of buckwheat might help in identifying suitable buckwheat genotype with high nutritional quality to be used as parents in nutritional breeding programme on buckwheat. The review of literature related to various aspects of present investigation is presented here under selected heads.

Buckwheat (*Fagopyrum esculentum* Moench) is grown worldwide with special advantages for cultivation such as adaptability to low cultivating temperature and a short growing period. Buckwheat flour based products, such as noodles, pasta, cookies, cake, and bread, are prevalent in Europe, Russia, and Asian countries (Ikeda 2002). This popularity is attributed to nutritional excellence, appetizing flavor and wholesomeness of buckwheat products (Krkošková and Mrazova 2005). The nutritional benefits of buckwheat are manifold, such as a rich concentration of polyphenols that present substantial antioxidant capacity, a nutritionally balanced amino acid content (Tomotake *et al.*, 2000), a great source of dietary fiber (Bonafaccia *et al.*, 2003) and an abundant mineral composition (Ikeda and Yamashita 1994). Additionally, the absence of gluten protein makes buckwheat an ideal dietary supplement for celiac disease patients.

Proximate composition of *Fagopyrum esculentum* genotypes

Proximate analysis is partitioning of compounds in a sample into six categories based on the chemical properties of the compounds. The six categories are: moisture, ash, crude fiber, crude fat, crude protein and soluble carbohydrates. Buckwheat is a rich source of protein, carbohydrates, fibre and most of essential amino acids. Considering the health awareness, craze of healthy foods among the people and the decreased productivity of the buckwheat crop, extensive efforts are being made to increase the production of the buckwheat crop. Studies on chemical

composition of buckwheat indicated that variety had a significant effect on chemical composition. Buckwheat's potential contribution to sustainable agriculture and to nutritional and health benefits of humans should not be underestimated (Izydorczyk *et al.*, 2014). The content and composition of flavonoids in common buckwheat (*F. esculentum*) and tartary buckwheat (*F. tataricum*) seeds vary widely.

Dietryoh-szostak and Ploszynski (1986) reported in vitro protein digestibility in five different fractions of buckwheat groat to range from 78.9 to 88.3 % of dry matter and also evaluated the ash, crude fiber, fat and crude protein content in different fractions of buckwheat groat and reported variation in these parameters from 2.11 to 2.64, 0.24 to 0.60, 3.04 to 4.64 and 12.50 to 13.13%, respectively on dry matter basis. Bonafaccia *et al.* (1994) carried out proximate chemical composition analysis of five samples of buckwheat from the different geographical areas of Italy. The approximate chemical composition of the buckwheat analyzed indicated that varieties cultivated in Italy did not differ much from varieties cultivated in other countries of Europe. The buckwheat samples were found to contain 12.8-13.4% moisture, 11.0 to 13.6% protein, 2.23 to 2.65% ash, 2.93 to 3.37% fat, 5.27 to 6.48% total dietary fibre and 61.0 to 64.9% soluble carbohydrates on dry weight basis.

Mann *et al.* (2012) evaluated the nutritional quality and antioxidant potential of Indian buckwheat grains and demonstrated that grains of *Fagopyrum* species possessed high protein, carbohydrates, phenolic contents and antioxidative potential. Khan *et al.* (2013) reported that buckwheat of high altitude was high in protein, fat and ash and low in nitrogen free extract and fibre, while the buckwheat of lower was high in nitrogen free extract and fibre and low in protein, fat and ash. Raghuvanshi *et al.* (2017) analyzed six indigenous cultivars and one local variety of buckwheat grains. Proximate constituents in different cultivars of sieved buckwheat flour (SBF) were found to be in the range of 0.75 to 2.33, 10.43 to 11.23, 1.82 to 3.10, 3.53 to 4.80 and 66.01 to 72.89% for crude fat, crude protein, total ash, crude fibre and carbohydrate respectively. Alonso-Miravalles and O'Mahony (2018) studied the composition, protein profile and rheological properties of pseudocereal-based protein-

rich ingredients and found 8.75, 1.51, 14.2, 2.77, 72.8, 61.6 and 10.3% moisture, ash, protein, fat, carbohydrate, starch and total dietary fibre in buckwheat dehulled flour.

2.1 Moisture content

Moisture content is one of the properties that are important for nutritional labeling, food quality and microbial stability. It is an important parameter for safe storage and depends on stage of harvesting and storage condition. The status of moisture content roughly indicates the degree of maturity and accumulation of different nutrients in food crops. It is an important criterion contributing towards acceptability of the crop harvest.

Bonafaccia *et al.* (1994) reported 12.8-13.4% moisture in samples of buckwheat from various regions of Italy. Wang *et al.* (1995) studied the nutritional composition of buckwheat and reported moisture 13.0%. Steadman *et al.* (2001) analyzed the general composition of buckwheat groat and observed that buckwheat contained 11.8% moisture. Tang (2007) also reported 13.0% moisture content in buckwheat. While studying the chemical composition of buckwheat plant (*F. esculentum*) and selected buckwheat product, Vojtíšková *et al*. (2012) found 8.3, 11.5 and 11.9% moisture content in groats, flour and whole meal flour. Dogra and Awasthi (2015) evaluated fourteen genotypes of common buckwheat from Himachal Pradesh and found moisture content ranging from 10.2 to10.9%. Sindhu and Khatkar (2016) found 9.92% moisture content in the flour of tartary buckwheat cultivar, Shimla B-1.

2.2 Crude fat content

Fat content plays a major role in determining the overall sensory characteristics, such as flavor, texture, mouth feel and appearance. Total lipid in buckwheat seed/grains was reported to vary between 1.5 to 3.8% (Tahir and Farooq, 1985; Campbell, 1997; Steadman *et al.*, 2001; Li and Zhang, 2001). The content of free lipids in buckwheat flour was found higher than bound lipids (Soral-Śmietana, 1987). Bonafaccia *et al.* (1994) analyzed five buckwheat samples from Italy and found crude fat content from 2.93 to 3.37% of the dry matter. Gupta *et al.* (2002) evaluated the nutritive value of hulled and dehulled buckwheat grains and reported

crude fat content from 4.38 to 16.96 g/100g dry matter basis. Bonafaccia *et al.* (2003) analyzed the chemical composition of grain, bran and flour of common buckwheat and reported the fat content as 2.88% in the grains, 7.2% in the bran and 2.3% in flour on dry weight basis. Katar *et al.* (2016) found crude fat content in the range of 2.04 to 2.45% in three buckwheat germplasm. Crude fat content in the grains of common buckwheat cultivar, VL-7 was found to be 3.16% (Sindhu and Khatkar, 2016).

2.3 Crude protein content

Proteins are the main structural constituent of tissues in a human body and of biologically active compounds, *i.e.*, enzymes, hormones and antibodies. Buckwheat proteins are known to possess a high biological value and to be less susceptible to enzymatic hydrolysis. Buckwheat is a good source of non-gluten protein with high biological value (Biel and Maciorowski, 2013; Kato *et al.*, 2001), relatively low digestibility (Liu *et al.*, 2001), but with the highest amino acid scores of protein in plant foodstuffs (Qin *et al.*, 2010). The biological value of buckwheat protein was reported as 81.4% of egg white protein and 92.3% of that of skimmed milk powder (Wronkowska, *et al*., 2010).

Dietryoh-szostak and Ploszynski (1986) evaluated crude protein content in different fractions of buckwheat groat and reported variation from 12.50 to 13.13% on dry matter basis. In literature, different authorities reported different values for the protein content in buckwheat grains: 12.0-13.0% by Steadman *et al*. (2001); 12.11% by Li and Zhang (2001); 13.30-15.55% by Wei *et al*. (2003) and 8.51–18.87% by Krkoškova and Mrazova (2005). Protein content in the flour of common buckwheat cultivars ranged from 8.06 to 12.44% with an average 10.32% (Qin *et al.*, 2010). Vojtíšková *et al*. (2012) reported 13.1, 12.9 and 14.4% crude protein content in groats, flour and whole meal flour of buckwheat. Dogra and Awasthi (2015) evaluated fourteen varieties/ genotypes of common buckwheat from Himachal Pradesh and reported crude protein in buckwheat ranging from 10.1% to 15%. Katar *et al.* (2016) carried out biochemical characterization of seven buckwheat genotypes from Turkey and reported crude protein content to vary from 10.74 to

13.24%. Grains of common buckwheat cultivar, VL-7 were reported to have 13.57% protein (Sindhu and Khatkar, 2016).

2.4 Ash content

Ash content is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amounts of minerals present within a food. Buckwheat contains 2-2.7% ash (Awasthi and Thakur, 2010; Lin *et al*., 2009; Sato *et al.*, 2001; Wijngaard and Arendt, 2006; Yıldız and Bilgiçli, 2012).

Bonafaccia *et al.* (1994) reported 2.23 to 2.65% ash in buckwheat kernel. Li and Zhang (2001) reported ash content in grains of buckwheat as 2.1g/100g. Bonafaccia *et al.* (2003) analyzed the chemical composition of grain, bran and flour of common buckwheat. They reported ash content as 2.19% in the grains, 4.1% in the bran and 1.8% in flour (on dry weight basis) of buckwheat. Tang (2007) reported ash content in buckwheat as 2.3%. Mann *et al.* (2012) observed that the two buckwheat species *F. esculatum* and *F. tartaricum* contained almost similar levels of ash. The ash content in *F. esculatum* was reported to be 2.23% against 2.4% ash for *F. tartaricum*. Dogra and Awasthi (2015) studied the variation in biochemical composition of promising genotypes of the buckwheat grains and found the ash content to vary between 1.4 to 2.5% on dry weight basis. Sindhu and Khatkar (2016) found 2.07% ash content in the flour of tartary buckwheat cultivar, Shimla B-1. Ash content in three buckwneat germplasm was found to be 1.78 to 2.18% (Katar *et al.*, 2016). Total ash in the improved varieties of buckwheat grains in India was recorded as 1.82-3.10% (Raghuvanshi *et al.*, 2017).

2.5 Crude fibre content

Crude fibre is a measure of the quantity of indigestible cellulose, pentosans, lignin and other components of this type in present foods. Total dietary fiber (TDF) content in buckwheat groats was found comparable to grain crops (Krkoskova and Mrazova, 2005). Dietary fibre is the edible part of a plant or analogous carbohydrates that is resistant to digestion and absorption in the human

small intestine but is partly or completely fermented by microflora in the large intestine. Total dietary fibre (TDF) is classified in view of its affinity to water as either insoluble dietary fibre (IDF) or soluble dietary fibre (SDF). In general, IDF includes cellulose, lignins, and certain non-cellulosic polysaccharides, while SDF includes pectins and some associated non-cellulosic polysaccharides.

Amarowicz and Fornal (1987) analyzed the dietary fibre content and its composition in buckwheat grain and its products *i.e.*, flour, seed coat and groat on % dry matter basis. Buckwheat grain, flour, seed coat and groat contained dietary fibre as 24.75, 3.94, 80.31 and 4.51%, whereas 20.39, 3.98, 61.98 and 2.29% acid detergent fibre, respectively. Steadman *et al.* (2001) indicated that the whole grain buckwheat contained 7% of total dietary fibre, while bran with hull fragments had 40% of total dietary fibre. Gupta *et al.* (2002) evaluated the nutritive value of hulled and dehulled buckwheat grains and reported that crude fibre ranged from 4.38 to 16.96 g/100g dry matter basis. Amelchanka *et al.* (2010) analyzed buckwheat fresh, buckwheat ensiled and buckwheat grain meal for dietary fibre composition and noticed that dry matter, crude protein, neutral detergent fibre, acid detergent fibre and lignin to vary from 142 to 854; 119 to 137; 256 to 555; 154 to 427; 72 to 94.9 g/kg on dry weight basis respectively. The soluble dietary fiber, insoluble dietary fiber and total dietary fiber in groats of ten buckwheat cultivars were found in the range of 1.4-3.4%, 2.3-8.6% and 3.6-10.6% respectively (Lu *et al.*, 2013). Dogra and Awasthi (2015) evaluated fourteen varieties/ genotypes of common buckwheat from Himachal Pradesh and found crude fibre content to vary from 6.0 to 9.3%. Sindhu and Khatkar (2016) found 4.06% crude fibre content in flour of tartary buckwheat.

Raghuvanshi *et al.* (2017) analyzed sieved buckwheat flour (SBF) from six indigenous cultivars and one local variety of buckwheat grains and found crude fibre in the range of 3.53-4.80%. SBF was also observed to be an excellent source of fibre with total dietary fibre observed in the range of 14.52 to 17.77% and soluble dietary fibre 5.11 to 6.84%.

2.6 Carbohydrate content

Starch is the major component of buckwheat endosperm, which plays a significant role in appearance, structure and quality of food products. Buckwheat achene's contain mostly carbohydrate, especially starch (55.8%). In buckwheat, the starch content was 55.8% in grains, 40.7% in bran and 78.4% in flour (Bonafaccia *et al.*, 2003). In the whole grain of buckwheat, starch content varies from 60% to 70% of dry mass, demonstrating fluctuations under variable climatic and cultivation conditions (Vojtíšková *et al.*, 2012). Buckwheat starch is composed 25% amylose and 75% amylopectin (Qin *et al.*, 2010). Vojtíšková *et al*. (2012) reported 69.5, 67.9 and 61.6% starch content in groats, flour and whole meal flour of buckwheat. Raghuvanshi *et al.* (2017) reported 66.01 to 72.89% carbohydrate in sieved buckwheat flour (SBF) from India. Soral-Smietana *et al.* (1984) reported relatively high amylose content (42-52% of starch) was reported in buckwheat samples. Yoshimoto *et al.* (2004) found the apparent amylose content in buckwheat starch is 26 to 27%. Buckwheat grains also contain 0.65 to 0.76% reducing sugars (Campbell, 1997).

2.7 Resistant starch content

The nutritional property of starch is related to its rate and extent of digestion and absorption in the small intestine. For nutritional purposes, starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RS has assumed great importance owing to its unique functional properties and health benefits. The beneficial effects of RS include glycemic control and control of fasting plasma triglyceride and cholesterol levels and absorption of minerals. Resistant starch is not absorbed in the small intestine and is partly or completely available for fermentation by microflora in the large intestine. The amount of RS in a given food will vary for different individuals, as the exact time of the transition from small to large intestine will fluctuate depending on an individual's gastrointestinal functioning, the concentrations of endogenous carbohydrate digestion enzymes, the viscosity of the gastric fluid, as well as the food matrix itself. Nigudkar

(2014) estimated and reported the RS content of typical traditional Indian starchy cereal and legume preparations and opined that RS content of food preparation was influenced by many factors such as cooking method, processing technique, storage etc. In a study on resistant starch content in starchy foods from Thailand, it was found that green bananas contained highest amount of resistant starch ranging between 35.14 and 45.87%, 2.33 to 10.63% in legumes, 3.19 to 23.25% in tuber crops and 1.16 to 4.85% in cereal grains (Moongngarm, 2013.)

Skrabanja and Kreft (1998) found 33.5% resistant starch in raw buckwheat groats. Skrabanja *et al*. (2004) reported 7-37% of resistant starch in buckwheat seeds. Resistant starch in buckwheat grains of three Polish varieties constituted from 16% to over 18% d.m. (Stempińska and Soral-Śmietana 2006). Raw buckwheat grain may contain 33-38% of resistant starch; however cooking reduces its content three-fold (Christa and Soral-Smietana, 2008). Wronkowska and Soral-Śmietana (2008) reported 6.56% resistant starch in buckwheat flour.

2.8 Mineral composition of buckwheat

Minerals are those elements that remain largely as ash when plant or animal tissues are burnt. Mineral elements are present in relatively low concentrations in foods. Nonetheless, they play key functional roles in both living systems and foods. They are usually required in small amounts from less than 1 to 2500 mg per day, depending on the mineral. Although they yield no energy, they have important roles to play in many activities in the body. Minerals are essential for hundreds of enzymatic reactions in the body, they are key players in the regulation of metabolism, constituents of enzymes, regulate the permeability of cell membranes, the osmotic pressure and water balance between intracellular and extracellular compartments, the response of nerve stimuli, the contraction of muscles and the maintenance of acid-base balance. Buckwheat is reported to contain appreciable amounts of minerals. Therefore, buckwheat and its products appear to be a key source of minerals for people who consume them. Despite of the importance of buckwheat as a food,

minerals in buckwheat and its products are still not fully characterized in view of nutrition and functionality.

In general, the content of minerals in buckwheat grains and their morphological fractions (on dry weight basis) is : 2-2.5% in whole grains, 1.8-2.0% in kernel, 2.2-3.5% in dehulled grains, about 0.9% in flour and 3.4-4.2% in hulls (Li and Zhang 2001). Buckwheat is rich in potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na). P, K and Mg are most concentrated in bran, particularly in the bran from which the hulls were removed before milling the grains. Buckwheat may be an important nutritional source of such microelements as iron (Fe), manganese (Mn) and zinc (Zn) (Wei *et al*., 1995). As in other plant material, mineral content is highly influenced by the presence of these elements in soil where the crop is grown. Marshall and Pomeranz (1982) reported values for of calcium, iron, magnesium, phosphorus, potassium, copper, manganese and zinc content in buckwheat as 110, 4, 390, 330, 450, 0.95, 3.37 and 0.87 mg/100g, respectively.

Amarowicz and Fornal (1987) determined various mineral elements in buckwheat grains and reported potassium, magnesium, manganese, iron, zinc and copper content as 244.1, 168.6, 5.44, 4.82, 3.40 and 0.59 mg/100 g respectively. Lin and Jia (1988) evaluated the common buckwheat flour for mineral composition and reported potassium, sodium, calcium, magnesium and iron content as 0.29, 0.29, 0.03, 0.14. and 0.014% respectively.

Ikeda and Yamashita (1994) determined that there were variations in the mineral element contents of buckwheat flour. Wang *et al.* (1995) reported mineral composition comprising of potassium, sodium, calcium, magnesium, iron, manganese, zinc, copper and phosphorus content in buckwheat in the order 320, 2.3, 39, 94, 4.4, 1.31, 2.02, 0.89 and 244 mg/100g. Ikeda *et al*. (1999) stated that buckwheat flour contained high levels of magnesium, phosphorus and potassium. Calcium, magnesium, phosphorus, potassium, zinc, copper and manganese content in flour were noticed as 14.5, 248, 379, 411, 2.79, 0.63 and 0.89 mg/100g, whereas in the hull the values for the these parameters were found as 97.4, 112, 127, 1267, 1.24, 0.61 and

9.16 mg/100g, respectively. Gupta *et al.* (2002) analyzed the nutritive value of hulled and dehulled buckwheat grain and reported calcium and phosphorus content to vary from 0.20 to 0.28 and 0.30 to 0.36 g/100g on dry matter basis.

Wei *et al.* (2003) analyzed the mineral content in five different fractions of three common buckwheat and one tartary buckwheat varieties. The buckwheat kernel was found to be rich in potassium and zinc in albumin; calcium, magnesium and manganese in globulin and sodium in prolamin and glutelin fractions. Ikeda *et al.* (2005) studied the composition of seven important minerals, namely zinc, copper, manganese, calcium, magnesium, potassium and phosphorus in various buckwheat groats. The zinc content per 100g dry matter of buckwheat groats ranged from 1.29 to 2.61 mg; copper 0.31 to 0.63 mg; manganese 0.79 to 2.53 mg; calcium 6.7 to 16.9 mg; magnesium 141 to 217 mg; potassium 322 to 518 mg and phosphorus 265 to 510 mg.

Siener *et al.* (2006) found 484, 214, 308, 9.0 and 3.7 mg/100g of potassium, calcium, magnesium, iron and zinc content in edible portion of buckwheat nuts.

2.9 Soluble protein content and protein fractionation

Buckwheat is one of the best sources of high biological value protein in the plant kingdom, reaching 92.3% of the biological value of skimmed milk powder proteins and 81.4% of that of egg white proteins (Pomeranz, 1975). Eggum *et al.* (1981) determined chemical composition and protein quality of buckwheat and reported that due to high contents of crude fibre and tannin, the true protein digestibility of buckwheat grains was slightly below 80 % Seed storage proteins of common buckwheat have been characterized by several researchers (Skerritt, 1986; Radovic *et al*., 1999; Fujino *et al*., 2001).

Based on solubility characteristics, proteins are usually characterized as: albumins (soluble in water and dilute buffers at neutral pH); globulins (soluble in salt solutions but insoluble in water); glutelins (soluble in dilute acid or alkali solutions); and prolamins (soluble in aqueous alcohols of 70-90%). Separation of

proteins according to their solubility is widely used for the characterization of seed proteins and this characterization could also provide useful information about protein quality. The major solubility fractions of buckwheat protein are water-soluble and salt-soluble albumins and globulins representing almost one-half of all buckwheat proteins. Imai and Shibata (1978) fractionated the wheat and buckwheat flour proteins and observed the range of 40 to 77% albumins and globulins, 0.7 to 2% prolamins, 23 to 59% glutelins and some residual protein in commercial buckwheat flour, whereas values for these proteins varied from 24 to 32; 25 to 33; 41 to 44% in wheat flour in that order.

Pomeranz (1983) reported about 80% buckwheat protein was composed by albumins and globulins. Tahir and Farooq (1985) observed the ratios of albumins plus globulins: prolamins: glutelins: residual proteins as 38-44: 2-5: 21-29: 28-37 in four buckwheat species. Buckwheat proteins are composed of 12.5-18.2% albumins, 43.3-64.5% globulins, 0.8-2.9% prolamins, 8.0-22.7% glutelins, and 15.0% residual protein (Ikeda *et al.*, 1991). Wei *et al.* (1993) reported that buckwheat flours were rich in albumin and globulin and very poor in prolamin, glutelin.

Buckwheat has a very low content of prolamins and, based on chemical and immunological studies; it may be a valuable source of dietary protein for gluten-sensitive individuals (Skerritt, 1986). Bonafaccia *et al.* (1994) evaluated the protein fractions in buckwheat cultivated in Italy. The study indicated that almost half of the protein content in the five samples analyzed was constitued by globulin with its values over 44%, while prolamin represented the smallest fraction (0.7%). The albumin (18%) and glutenin (22%) contents were constant in all the samples. A low amount of prolamin fraction (2%) was also reported by Ikeda (2002) in the buckwheat flour. Phiarais *et al.* (2008) observed that the globulin fraction accounted for 28.5% of the total protein extract which was lower than the 43% reported by Javornik *et al.* (1981).

2.10 Total phenolic content

Buckwheat is associated with the antioxidant properties due to the phenolic compounds. In literature, buckwheat is characterized as functional food with antioxidant and anti-inflammatory properties (Zhang *et al.*, 2012), where the antioxidant potential of buckwheat is determined mainly by phenolic compounds (Holasova *et al.*, 2002). Ikeda *et al.* (2001) also reported high levels of polyphenols in buckwheat groats and suggested that the polyphenol content of buckwheat sample might be associated their anti-oxidative characteristics. Buckwheat has a total phenolic content ranging from 29 to 1371 mg/100 g, depending on the method of extraction, and calibration standard used *i.e*. gallic acid or ferulic acid. (Oomah *et al.*, 1996; Holasova *et al.*, 2002; Zielinska *et al.*, 2007; Cao *et al.*, 2008).

Tahir and Farooq (1985) reported phenolics content 0.73, 0.79 and 0.77 % on dry weight basis in hull, groat and whole grain of four buckwheat cultivars. Oomah *et al.* (1996) determined phenolic acid content in grains from five buckwheat cultivars of Canada and reported 12-16 g/kg of total phenolic acids, about 3 g/kg of esterified phenolic acids, and 8-13 g/kg of etherified phenolic acids. They observed that growing location had no significant effect on phenolic acid contents of buckwheat and was independent of seed color and protein content. Holasova *et al.* (2002) reported total phenolics content as 3303 mg/kg in buckwheat grains and 3903 mg/kg in dehulled grains, respectively, on dry matter basis. Amelchanka *et al.* (2010) analyzed total phenol content in buckwheat fresh, buckwheat ensiled and buckwheat grain meal and reported their values as 35.7; 34.5 and 7.3 g/kg respectively on dry matter basis. The total phenolic contents varied from 5,150 to 9,660 μmol of gallic acid equivalents per 100 gram of dry weight (DW) of tartary buckwheat (Guo *et al.*, 2011). The free phenolics and total phenolics contents in the groats of ten Canadian cultivars of buckwheat were found to be 4.5-17.1 mg of gallic acid equivalent [GA]/g and 6.8-20.7 mg of GA/g respectively (Lu *et al.*, 2013).

Common buckwheat and tartary buckwheat cultivars originating in different world countries were investigated and compared for their quantitative and

qualitative abundance of phenolics and flavonoids. Mikulajeva *et al*. (2016) analysed 22 common buckwheat cultivars, in which total phenolic ranged between 0.897 to 4.226 mg GAE g^{-1}dw.

2.11 Total flavonoid content

Flavonoids are polyphenolic compounds that are present ubiquitously in foods of plant origin. Buckwheat has a high level of antioxidant activity compared to other cereal crops, and this has been attributed to its high levels of flavonoid compounds (Holasova *et al*., 2002). Buckwheat contains several flavonoids, like rutin, quercetin, kaempferol, orientin/isoorientin, and vitexin/isovitexin, rutin being the major flavonoid compound. They all have demonstrated antioxidant, antimicrobial and anti-inflammatory properties (Cai *et al*., 2004).The content and composition of flavonoids in common buckwheat (*F. esculentum*) and tartary buckwheat (*F. tataricum*) seeds vary widely. Generally, flavonoids content of *F. tartaricum* (about 40 mg/g) is higher than that of *F. esculentum* (10 mg/g) (Oomah & Mazza 1996; Li & Zhang, 2001; Chao *et al.*, 2002).

Buckwheat contained an average of 387 and 1314 mg/100 g of flavonoid and 47 and 77 mg/100 g of rutin in the seed and hull, respectively. Location was the main source of variation for flavonoid and rutin contents of the seed, while growing season had significant influence on the flavonoid content of the hulls (Oomah and Mazza, 1996). Dietrych-Szostak and Oleszek (1999) isolated and identified flavonoid content in buckwheat and found 18.8 and 74 mg total flavonoid per 100g dry matter in the seeds and in the hulls respectively.

Buckwheat is one of the richest sources of polyphenols and flavonoids. These are concentrated mainly in outer layers of buckwheat seed (Krkošková and Mrázová, 2005). Quercetin concentration in buckwheat is several times lower than that of rutin (Fabjan *et al.*, 2003). Rutin and quercetin concentration changes depending on technological parameters applied in seeds processing (Bonafaccia *et al.*, 2003) reported that both rutin and total flavonoid content in buckwheat species varied significantly with values of 0.02 and 0.04% in *F. esculentum*, 0.10 and 0.35% in *F.*

homotropicum and 1.67 and 2.04% in *F. tataricum*, respectively. Compared to most grain crops, buckwheat contains more rutin, which is reported to be the most abundant flavonoid providing natural antioxidant, anti inflammatory and anti-carcinogenic properties, *i.e*. rutin may inhibit lipid peroxidation within food (Oomah and Mazza, 1996; Lin *et al.*, 2009; Wron-kowska *et al.*, 2015).

Jindal and Saxena (2016) evaluated total phenolic content and total flavonoid content of whole buckwheat flour and buckwheat groat flour. The whole buckwheat flour was found to be better with regard to total phenolic content (12.60 mg GAE/g against 8.57 mg GAE/g for buckwheat groat flour) and total flavonoid content (24.7 mg RUE/g against 19.3 mg RUE/g for buckwheat groat flour).

CHAPTER III
MATERIALS AND METHODS

3.0 Materials

The present investigation was designed to evaluate the biochemical composition of buckwheat (*Fagopyrum esculentum* Moench) seeds grown in Assam. The grain samples of sixteen varieties/ genotypes of common buckwheat were collected from Regional Agricultural Research Station (RARS), North Lakhimpur, Assam Agricultural University, Assam (Table 1).

Table 1. Buckwheat varieties/ genotypes used in the study

Buckwheat varieties/ genotypes	Description
BWC-1	Local collection from Biswanath Chariali
BWC-2	Local collection from Biswanath Chariali
Jonai	Local collection from Jonai
Kharupetia-1	Local collection from Kharupetia
Kharupetia -2	Local collection from Kharupetia
NLC	Local collection from North Lakhimpur
EC-218740	Buckwheat germplasm accession
EC-218742	Buckwheat germplasm accession
EC-27242	Buckwheat germplasm accession
IC-109728	Buckwheat germplasm accession
IC-258233	Buckwheat germplasm accession
IC-329456	Buckwheat germplasm accession
Himpriya	Released variety
PRB-1	Released variety
SHIMLA B-1	Released variety
VL-7	Released variety

The grains were cleaned and dried in hot air oven and converted to powder using stainless steel grinder and kept in desiccators for further analytical works.

3.1 Analytical Methods

The proximate analysis of different buckwheat germplasm was done by using the following standard methods. All the estimations were done in triplicate for each sample and the mean of the estimations was recorded for interpretation of the result.

3.2.1 Determination of moisture content

Moisture content was determined by following the method of AOAC (1970). For this 5g of powdered sample was weighed in aluminum moisture boxes and dried in an oven at 70°C (±2°C) for 16 hours, cooled in desiccators and weighed again. The experiment was conducted in triplicate and the mean was recorded.

Calculation

$$\text{Moisture content (g/100g sample)} = \frac{\text{Initial weight (g) - final weight (g)}}{\text{Weight of the sample taken (g)}} \times 100$$

3.2.2 Determination of Ash content

Ash (total mineral) content was determined as per the procedure of AOAC (2000).

For this, 5 g moisture free powdered sample was taken in a silica crucible, charred with low Bunsen flame and finally ignited at 600°C for 6 hours in the muffle furnace. The ash percentage is calculated by dividing the weight of the ash by the weight of the sample taken and multiplied by 100.

Calculation

$$\text{Ash content (g/100g sample)} = \frac{\text{Weight of the ash (g)}}{\text{Weight of the sample taken (g)}} \times 100$$

The ash was moistened with a small amount of distilled water and added 5 ml of distilled HCl. The mixture was evaporated to dryness on a boiling water bath. Another 5 ml of HCl was added again and the solution evaporated to dryness as before. 4ml of HCl and few ml of water were then added and the solution warmed

over a boiling water bath and filtered into a 100ml volumetric flask using Whatman No. 40 filter paper. After cooling, the volume was made up to 100 ml and suitable aliquots were used for mineral estimation.

3.2.3 Estimation of crude protein content

Crude protein content of the sample was determined by micro Kjeldahl method (AOAC, 2000)

In determining the total nitrogen, the organic form of nitrogen in the sample was first converted into inorganic form (ammonium sulphate) by wet digestion with conc. sulphuric acid in the presence of catalysts, copper sulphate and potassium sulphate and subsequent decomposition of ammonium sulphate by excess alkali (40% NaOH). The librated ammonia was collected in 4% boric acid solution and was then titrated with standard hydrochloric acid (0.1 N). An amount of 500mg of powdered sample, 1g catalyst mixture and 10 ml of concentrated sulphuric acid were taken in a digestion tube and kept overnight for pre-digestion. Next day the pre digested sample was digested in automatic digestion unit (KEL-PLUS, model KES O4L, Pelican, India) till a clear solution was obtained.

The digested sample were distillated using an automatic distillation unit (KEL PLUS, model ELITE EX, Pelican, India) in presence of 40% sodium hydroxide solution and liberated ammonia was trapped in 4% boric acid solution. Distilled sample in the conical flask mixed with indicator was titrated against 0.1N HCl till the colour changes from bluish green to pale pink.

Calculation

$$\text{Nitrogen content (g/100g sample)} = \frac{(a - b) \times \text{Normality of HCl} \times 14 \times 100}{\text{g of sample} \times 100}$$

Where,

a = ml standard acid of sample

b = ml standard acid of blank

If total nitrogen value is X, protein contain in 100 g = X × 6.25

The estimate was done in triplicate for each sample and the mean of the estimation was recorded for interpretation of the result. The protein content was expressed as percentage on moisture free basis.

3.2.4. Estimation of crude fat

Crude fat or ether extract were determined from oven dried sample using a Soxhlet extraction apparatus (AOAC, 1970).

In a thimble, 5g dried and powdered sample was taken. The top of the thimble was plugged with fat free cotton. The thimble was placed in the fat extraction tube of Soxhlet extraction apparatus. The bottom of the extraction tube was attached to the empty, dried extraction flask which is previously weighed (W_1). Approximately 75ml or more anhydrous petroleum ether (boiling point 60 to 80°C) was poured through the sample into the extraction tube, which came down to the flask. The top of the fat extraction tube was connected with the condenser. Extraction was started by heating the petroleum ether by a regulated heater. The temperature should be regulated in such a way that volatilized ether could condense and then dropped continuously upon the sample without any appreciable loss. The extraction was continued for about 16 hr when petroleum ether in the Soxhlet became totally colourless. The thimble was removed from the apparatus and most of the ether was distilled off and was collected in the extraction tube to be used for next sample. When a small portion of ether is present in the extraction flask, the thimble was removed from the apparatus, dried over a water bath at low temperature, cooled and weighed. This was repeated until a constant weight (W_2) was obtained. The difference in weight (W_2-W_1) gives the amount of fat soluble material present in the sample.

Calculation

$$\text{Percentage of crude fat (on dry weight basis)} = \frac{\text{Weight (g) of fat soluble material}}{\text{Weight of dried sample (g)}} \times 100$$

The estimation was done in triplicate and their mean was recorded as percentage of crude fat content in moisture free sample.

3.2.5. Estimation of crude fibre

Crude fibre was determined as per the procedure of AOAC (2000).

Powdered seed sample (2g) was extracted with petroleum ether to remove the fat. After fat extraction, the material was boiled with 200ml of H_2SO_4 for 30min with bumping chips, filtered through muslin cloth and washed with the boiling water until washings were no longer acidic. Then again boiled with 200ml of sodium hydroxide solution for 30 min, filter through muslin cloth and washed with 25ml of boiling 1.25% H_2SO_4, then 50ml portions of water and 25ml alcohol. Removed the residue and transferred to ashing dish (pre-weighed dish W_1). The residue was dried for 2h at 130±2°C. Cooled the dish in desiccator and weighed (W_2), and finally ignite at 600±15°C for 30 min. Cooled in desiccators and weighed again.

Calculation:

$$\text{Percentage of crude fiber in ground sample} = \frac{(W_2 - W_1)(W_3 - W_1)}{\text{Weight of the sample}} \times 100$$

The estimation was done in triplicate and their mean was recorded as percentage of crude fibre content in moisture free sample.

3.2.6 Estimation of Nitrogen free Extract/ Total carbohydrate content

Total carbohydrate content was determined by following the method of AOAC 942.05. Total carbohydrate was calculated by subtraction of the sum of weight of crude protein (CP), crude fat (CF), crude fibre (CF), moisture (M) and ash (A) from the total weight of the sample used.

Total carbohydrate (%) = 100-(% crude protein + % crude fat + % crude fibre + % moisture + % ash)

3.2.7 Estimation of starch content

The starch content was estimated by the method as described by Chopra and Konwar (1976).

For extraction of starch, the buckwheat powder was first extracted with 80% ethanol to make the samples free of sugars. Starch was extracted with 52% perchloric acid from the sugar free samples by constant stirring for 30 minutes. This was then centrifuged and the supernatant was collected in a volumetric flask (50 ml). The process was repeated for 2-3 times and the volume of the combined extract was made up to the mark.

To 1ml of aliquot of the starch extract, 10ml of anthrone reagent was added slowly by the side of the test tube, placed in ice cold water and kept for 10 min. The tubes were then placed in boiling water bath and kept for 10 min and cooled immediately to room temperature. The intensity of the colour was measured in a spectrophotometer at 650nm against a reagent blank. The sugar content was estimated from a standard curve was multiplied by a factor 0.9 to get the starch content. The estimation was carried on triplicate and mean was expressed as gram of starch per 100g moisture free sample.

3.2.8 Estimation of amylose content

Amylose content was determined by the method describe by Juliano (1971). Defatted moisture free powdered sample (100mg) was taken in a stoppered conical flask. One ml of distilled ethanol was added to wet the powder, followed by 9 ml of 1 N NaOH gently added by side. The contents were heated in a boiling water bath for 10 minutes. After cooling, volume was made up to 100ml with distilled water.

To a 100ml volumetric flask 5 ml of above dispersion was transferred and 50 ml of water was added to it followed by addition of 1ml of 1N acetic acid and 2ml of iodine (0.2%) solution. The volume was made up to the mark with distilled water. 1ml of standard amylose (100mg/100ml) solution was taken and treated using

the same procedure. A reagent blank was also prepared in the same way but without amylose. After 20 minutes, the intensity of colour developed was measured in a spectrophotometer at 620 nm against the reagent blank. Amylose content was calculated using the following relationship.

$$\text{Amylose content} = \frac{R}{A} \times \frac{a}{r} \times \frac{1}{5} \times 100$$

Where,

R = 620 nm reading for sample dispersion.

A = 620 nm reading for standard amylose solution.

a = Amount of the standard amylose taken.

r = Amount of sample taken.

The estimation was done in triplicate and their mean was recorded as a gram of amylose per 100g on dry weight basis.

3.2.9 Estimation of Resistant starch content

The resistant starch was estimated by the method of Goni *et al.* (1996).

100mg of dried milled sample was weighed out and kept into a 50 ml centrifuge tube. 10ml of KCL-HCL buffer was added, pH 1.5(pH was adjust with 2M HCL or 0.5M NaOH) and it was homogenized into the centrifuge tube. 0.2ml of the pepsin solution (1g pepsin/10ml KCL-HCL) was added and mixed well and left in a water bath at 40^0 C for 60 min with constant shaking. The sample was taken out of the water bath and cooled to room temperature. 9ml of 0.1M Tris-maleate buffer of pH 6.9 (pH adjustment with 2M HCL or 0.5 NaOH) and 1ml of alpha-amylase solution (40mg alpha amylase per ml tris-maleate buffer) were added to the solution. The content was mixed well and incubated for 16h in a water bath at 37°C with constant shaking. The sample was centrifuged for 15 min at 3000g and the supernatant was discarded. The solution was washed at least once with 10ml distilled water, centrifuged again and supernatant was discarded. 3ml of distilled water was added to

the residue carefully to moisten the sample. 3ml of 4M KOH was added, mixed and left for 30min at room temperature with constant shaking, then 5ml of 2M HCL and 3 ml of 0.4M sodium acetate buffer, pH 4.75 (pH adjustment with 2M HCL or 0.5 NaOH) were added to the solution. 80 microlitre of amyloglucosidase was added, mixed well and left for 45 min in a water bath at 60°C with constant shaking. The solution was centrifuged for 15min at 3000g and the supernatant was saved in a volumetric flask. The residue was washed at least once with 10ml of distilled water, centrifuged again and combined the supernatant with that obtained previously. The volume was made up to 100ml. In this extract the resistant starch content in terms of glucose was determined using anthrone reagent.

Calculation

The resistant starch content of the test samples were calculated by multiplying the glucose content by 0.9 and it was finally expressed as percentage on moisture free basis.

3.3 Extraction of seed storage protein

Defatting

250mg of powdered material was defatted in eppendorf tube using Chloroform: acetone: methanol (2:1:1) with occasional vortexing for 12 hours and the contents were air dried after decantation.

3.3.1 Extraction of total soluble protein

Defatted sample (0.5g) was soaked overnight at 4°C in 2ml of 50mM Tris-Cl buffer (pH 7.6) containing 6mM β-mercaptoethanol (β-ME) and subsequently grinding and homogenizing in a pestle and mortar. The cell paste suspension was centrifuged at 12000g for 10 minutes at 4°C and the supernatant was obtained for the further analysis.

3.3.2 Extraction of albumin and globulin by modified Osberne's method (Juliano and Boulter, 1976)

Albumin and globulin were extracted twice from 250mg defatted flour with 1.0ml of distilled water and 0.5M NaCl respectively (each containing 0.6% β-ME). The supernatant obtained after centrifuging at 10000g for 10 minutes were mixed with double volume of 10% chilled TCA and kept overnight in refrigerator at 4°C. Protein was pelleted down by centrifugation at 10000g for 10 minutes. The pellet so obtained was dissolved with 2 N NaOH and used for protein estimation.

3.3.3 Extraction of prolamin and glutelin by modified Osberne's method (Juliano and Boulter, 1976)

Prolamin and glutelin fractions were extracted from the residue after extraction of albumin and globulin. Prolamin was extracted twice with 1.0ml of 70% ethanol containing 0.6% β- ME. Then glutelin was extracted with 1.0ml of 0.1M NaOH. Both supernatant obtained after centrifuging at 10000g for 10 minutes were mixed with double volume of 10% chilled TCA separately and kept overnight in refrigerator at 4°C. Protein was pelleted down by centrifugation at 10000 g for 10 minutes. The pellet so obtained was dissolved with 2N NaOH and used for further study.

The sequence of extraction of different fractions of soluble protein is schematically represented below:

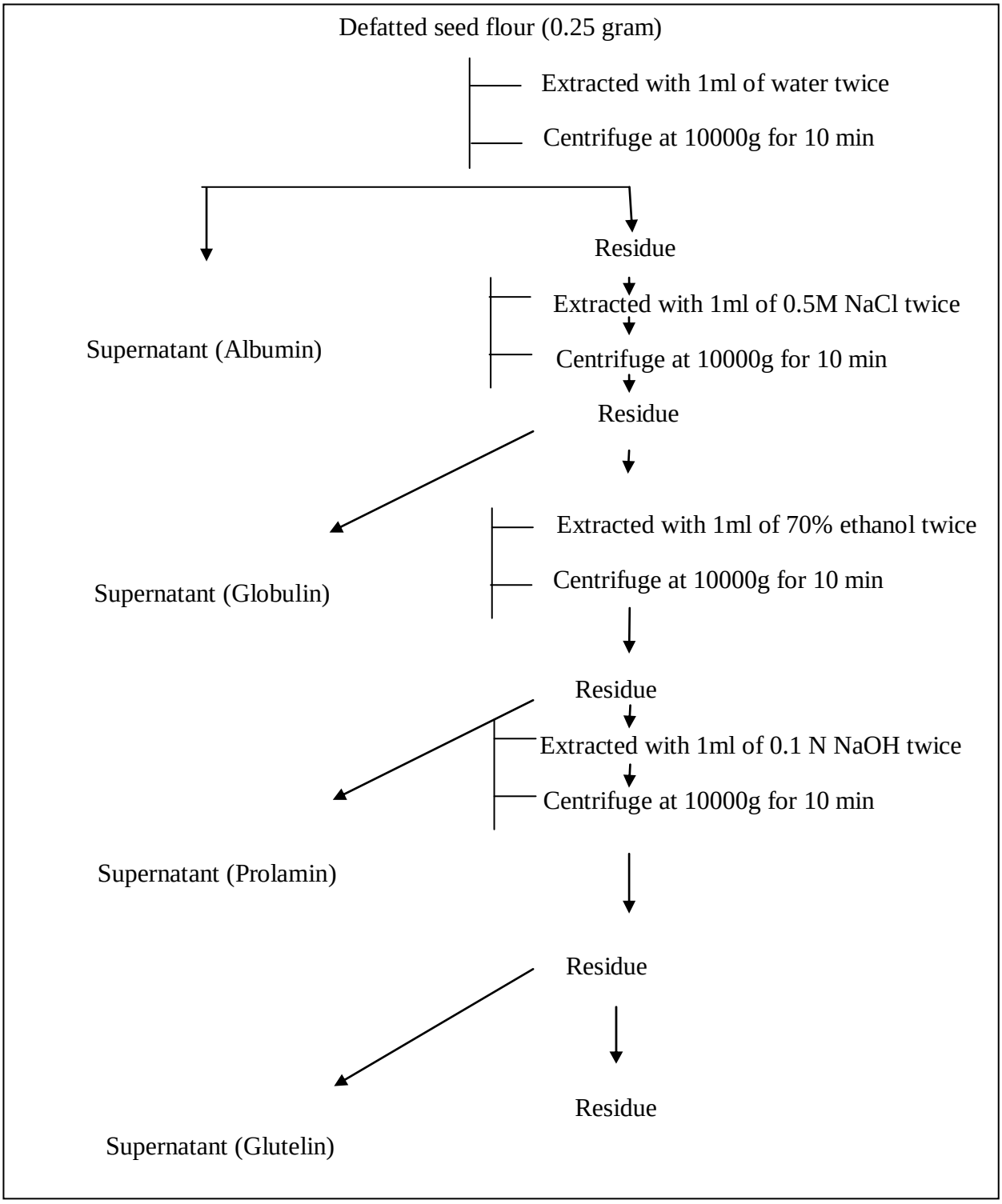

Fig.1: Flow chart for extraction of different fraction of total soluble protein

3.3.4 Protein estimation by Lowry's method

Total soluble protein and all four fraction extracted were estimated by the method of Lowry's *et al*. (1951)

Reagent A

2% Na_2CO_3 in 0.1N NaOH

Reagent B

Stock A: 0.5% $CuSO_4.5H_2O$

Stock B: 1% sodium potassium tartarate

Working solution of reagents B was prepared fresh just before use by mixing equal volume of stock A and stock B.

Reagent C

50ml of reagent A and 1ml of working solution of reagent B was mixed just prior to use.

Reagent D

1N Folin-ciocalteau reagent

Protein solution (Stock Standard)

50mg of BSA (Bovine Serum Albumin) fraction V was dissolved in water and the volume was made up to 50ml.

Working standard

Diluted 10ml of the stock to 50ml with distilled water (1ml = 200 µg of protein)

Procedure

Different volumes of working standards and sample extracts, less than or equal to 1ml were taken in different test tube and volume was made up to ml. A tube with 1ml of water was taken as blank. An amount of 5ml of reagent C was added

to each test tube including the blank and allowed to stand for 10 minutes after mixing well. Then 0.5ml of reagent D was added, mixed well and incubated at room temperature in the dark for 30 minutes. Absorbance was measured at 660nm and protein content in the sample was calculated from the standard graph.

3.4.1 Estimation of total phenolic content

Dried moisture free 0.5g of sample was ground with a morter in 10 times (10ml) volumes of 80% ethanol and centrifuged at 10,000 rpm for 20 minutes and supernatant was collected. The residue was re-extracted with 5 times the volume of 80% ethanol, centrifuged and the supernatant was collected together and evaporated to dryness. The residue was dissolved in 5 ml of distilled water and was used for further analysis. A modified version of the Folin-Ciocalteu assay (Slinkard and Singleton, 1977) was used to determine the total phenolic content in the extracts from the buckwheat samples. Gallic acid standard curve was made with appropriate concentrations of aqueous gallic acid solution. For the analysis, 20 microlitre each of extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 ml of distilled water was added, followed by 100 microlitre of Folin-Ciocalteu reagent(1N), mixed well and within 8 min, 300 microlitre of sodium carbonate was added. The samples were vortexed immediately and allowed to incubate in dark for 30 min at 40^{o}C. The absorbance was measured at 765 nm. The phenolic content was expressed in mg GAE/100g.

3.4.2 Estimation of total flavonoid content

Dried 0.5g of sample powder was mixed with 5ml of 80% ethanol in 100ml of conical flask and put on a shaker at 200rpm for 24 hrs. After 24hrs, the extracts were filtered through Whatman no. 42 filter paper. The volume of the filtrate was adjusted to 5ml with 80% ethanol and was used for further analysis. The total flavonoid content was determined according to the method as described by Woisky and Salatino (1998). In a test tube, 0.5ml of extracts of each sample was taken followed by addition 1.5ml of 95% ethanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. After incubation for 30 min at

room temperature, the absorbance was measured at 415nm in a UV-VIS spectrophotometer. Quercetin (100µg/ml) was used as the standard.

From 100µg/ml quercetin solution 0.2, 0.4, 0.6, 0.8 and 1ml of quercetin were taken in 5 test tubes corresponding to concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively. The volume of each tube was made up to 3ml by adding distilled water, followed by addition of 0.1ml 10% aluminum chloride, 0.1ml of 1M of potassium acetate and 1.5ml 95% ethanol respectively. After incubation at room temperature for 30min, absorbance was measured at 415nm in a UV-VIS spectrophotometer. The blank was prepared in the same way by taking water in place of quercetin.

Concentration of total flavonoid content was calculated from the standard curve and expressed as mg quercetin equivalent (mg QE/100g) of sample.

3.5 Preparation of mineral solution

The mineral solution was prepared according to the method as described by AOAC (1970). Ash was dissolved in HCl (1:1) on a water bath at 100°C and the solution was evaporated to dryness. After that 4ml HCl and 2ml glass distilled water were added, warmed and acid soluble portion obtained after filtration was made up to 100 ml in a volumetric flask with glass distilled water. This solution was used for the estimation of phosphorus (P), potassium (K), sodium (Na), calcium (Ca), and iron (Fe).

3.5.1 Determination of iron content

Iron content was determined by spectro-photometric method (Wong, 1925).

To an aliquot (6.5ml or less) of mineral (water was added to the final volume of 6.5ml) 1ml of 30% sulfuric acid (H_2SO_4), 1ml of 7% potassium persulphate ($K_2S_2O_8$) solution and 1 ml of potassium thiocyanate (KCNS) solution in this order , mixed well after each addition. After sometime, the absorbance of the solution was measured in a spectrophotometer at 540nm against a reagent blank. The

amount of iron was calculated out from the standard curve. The estimation was done in triplicate and mean was recorded as miligram of iron in per 100g of sample.

3.5.2 Determination of phosphorus content

Phosphorus content was determined colorimetrically by the method described by Fiske and Subbarow (1925).

To an aliquot (0.1ml) of mineral, 1 ml of ammonium molybdate (5g in 15ml conc. H_2SO_4, made volume to 100ml), one ml of 0.5% hydroquinone and 1 ml of 20% sodium sulphate solution in this order, mixed well after each addition. The volume was then made up to 15 ml with water and the solution was thoroughly mixed. After 30 minutes, the absorbance of the solution was measured in a spectrophotometer at 660 nm against a reagent blank. The amount of phosphorus was calculated out from standard curve prepared by using potassium dihydrogen phosphate (KH_2PO_4) solution. The estimation was done in triplicate and mean was recorded as miligram of phosphorus in per 100g of moisture free sample.

3.5.3 Determination of K, Na and Ca content

The three minerals (K, Na and Ca) were estimated using atomic absorption spectrophotometer (Chemito, AA203D, Double beam atomic absorption spectrophotometer). The working range for different minerals was prepared and those were listed below:

Minerals	Working range
Sodium(Na)	0.1-0.6 ppm
Potassium(K)	0.5-2.0 ppm
Calcium(Ca)	1-4.00 ppm

Statistical analysis

- Analysis of variance in factorials completely randomized design with three replications was used to interpret the results.

Significance or non significance of the variations due to effects was determined by calculating respective 'T' value.

The critical difference (C.D.) was calculated to find out the significance or non significance of mean difference between and amongst treatments. C.D was calculated by using the following expression.

$C.D. = S.Ed \times t_{0.05}$

Where, t = tabulated of 't' at 5 per cent level of probability for appropriate degree of freedom.

CHAPTER V
DISCUSSION

Buckwheat is grown worldwide with special advantages for cultivation such as adaptability to low cultivating temperature and a short growing period (Lorenz and Dilsaver 1982). Buckwheat flour based products, such as noodles, pasta, cookies, cake, and bread, are prevalent in Europe, Russia, and Asian countries (Ikeda 2002). This popularity is attributed to nutritional excellence, appetizing flavor, and wholesomeness of buckwheat products (Lorenz and Dilsaver 1982; Krkošková and Mrazova 2005; Janeš *et al.,* 2009). The nutritional benefits of buckwheat are manifold, such as a rich concentration of polyphenols that present substantial antioxidant capacity (Luthar 1992; Zdunczyk *et al*., 2006), a nutritionally balanced amino acid content (Tomotake *et al*., 2000), a great source of dietary fiber (Bonafaccia *et al*., 2003), and an abundant mineral composition (e.g., zinc, copper, manganese) (Ikeda and Yamashita 1994). Additionally, the absence of gluten protein makes buckwheat an ideal dietary supplement for celiac disease patients (Kupper 2005).

5.1 Proximate analyses

5.1.1. Moisture content

The moisture content is a measure of yield and quantity of solid matters and its distribution is an important factor for storage and preservation of germplasm. The moisture content of sixteen *Fagopyrum esculentum* germplasm were found to be in the range of 7.52% to 9.11% with an average of 8.18%. Different values for moisture content in different germplasm had been reported by other workers. Wang *et al*. (1995) reported the moisture content of the whole seeds of buckwheat as 13% where as Steadman *et al*. (2001) reported average moisture content to be 11.8%. Dogra and Awasthi (2015) reported the moisture content range from

10.2% to 10.9% in buckwheat. Thus the finding of the present study was found almost similar to the values reported earlier. The varietal effect on moisture content was found significant and such variation in the moisture contain in sixteen different *Fagopyrum esculentum* germplasm might be due to their stage of harvest, climate from where the sample was collected, genetic makeup, as well as method used for moisture determination.

5.1.2. Ash content

Ash content is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amounts of minerals present within a food. The minerals constituents of plants and their products vary in amounts according to the stage of maturity, condition of growth, fertilization and the nature of soil.

In the present study the ash contents in *Fagopyrum esculentum* germplasm were found to be in range of 1.83 to 2.93% on dry weight basis with the average of 2.32%. Dietryoh-szostak and Ploszynski (1986) reported higher ash content in *F. esculentum* seeds is 8.54 per cent on dry matter basis. Li and Zhang (2001) reported ash content in grains of buckwheat as 2.1g/100g which was almost similar to the present finding. Bonafaccia *et al*. (2003) analysed the chemical composition of grain, bran and flour of common buckwheat. They reported ash content as 2.19 per cent in the grains, 4.1 per cent in the bran and 1.8 per cent in flour (on dry weight basis) of buckwheat. These results were in agreement with the results obtained in the present study.

5.1.3. Crude protein

Protein quality or nutritional value of a protein means the usefulness of the proteins for specific vital purposes such as growth, replacement of metabolic losses of damage tissues, reproduction, location and general well being. Buckwheat is a rich source of protein, carbohydrates, fibre and most of essential amino acids.

In the present study crude protein content was found to vary from 7.23% to 9.53% with the average of 8.40%. Dogra and Awasthi (2015) evaluated fourteen varieties/ genotypes of common buckwheat from Himachal Pradesh and a wide variations in crude protein observed in buckwheat ranging from 10.1% to 15%. Dietryoh-szostak and Ploszynski (1986) reported crude protein content from 12.50 to 13.13%. Such variations in crude protein content might be due to genetical difference, location, stage of maturity and also by level of fertilization and nature of soil.

5.1.4. Crude fat

Fat content plays a major role in determining the overall sensory characteristics, such as flavor, texture, mouth feel and appearance. Crude fat or lipids are the group of heterogeneous compounds which are classified together because of their solubility in organic solvents such as chloroform, ether or benzene. This solubility differentiates them from constituents such as protein, carbohydrates and nucleic acids of cells and tissues. They include free fatty acids, mono, di or triacylglycerols, phospholipids and lipoproteins. They are important as a source of essential fatty acids and a concentrated source of energy.

In the present investigation the crude fat contents in buckwheat germplasm were found to be in range of 1.97% to 3.62% on dry weight basis with the average of 2.72% Tahir and Farooq (1985) reported crude fat 2.31 per cent in the grains in *Fagopyrum esculentum Moench*. which was almost similar to the present finding. According to Campbell (1997), the buckwheat seed contains 1.5 to 3.7% total lipids which was quite similar to the present findings. Li and Zhang (2001) reported fat content in grains of buckwheat as 2.3% wherase Steadman *et al*. (2001) analysed the general composition of buckwheat groat and observed that buckwheat contained 3.8 per cent lipids. Gupta *et al.* (2002) evaluated the nutritive value of hulled and dehulled buckwheat grains and reported crude fat ranged from 2.11 to 2.62%. Bonafaccia *et al.* (2003) analyzed the chemical composition of grain, bran and flour of common buckwheat and reported fat content a 2.88 per cent in the grains. Thus the finding of the present study was found similar to the values reported earlier. The

varietal effect on crude fat content was found significant. Such a little variation in the fat content might be due to the genetic composition of cultivar/variety used for the analysis as well as maturity and harvesting stage and the agro-climatic condition.

5.1.5. Crude fibre

Crude fibre is a measure of the quantity of indigestible cellulose, pentosans, lignin, and other components of this type in present foods. Crude fibre represents the ash less material that remains after vigorous digestion with hot sulphuric acid and hot sodium hydroxide whereas, the dietary fibre include all plant constituents that are not digested by human digestive system, mainly cellulose, hemicelluloses, pectin and gums and non carbohydrate like lignin.

In the present study the crude fibre contents in *Fagopyrum esculentum* germplasm were found to be in the range of 3.71% to 4.78% on dry weight basis with an average of 4.28%. Amarowicz and Fornal (1987) analysed the dietary fibre content and flour contained dietary fibre as 3.94%. Gupta *et al.* (2002) evaluated the nutritive value of hulled and dehulled buckwheat grains and reported that crude fibre ranged from 4.38 to 16.96g/100g dry matter basis, respectively. Dogra and Awasthi (2015) evaluated fourteen varieties/ genotypes of common buckwheat from Himachal Pradesh and reported crude fibre content from 6.9 to 9.3%. Raghuvanshi *et al.* (2017) analyzed six indigenous cultivars and one local variety of buckwheat grains. Proximate constituents in different cultivars of sieved buckwheat flour (SBF) were found to be in the range from 3.53 to 4.80 per cent for crude fibre. The variation in crude fibre content in buckwheat may be attributed to the differences in genetic makeup as well as maturity stage.

5.1.6. Nitrogen Free Extracts (NFE)

Nitrogen free extracts is the total soluble carbohydrate content (by difference). It represents soluble non fibrous carbohydrates like sugar and starch and other digestible and easily utilized non- nitrogenous substances in feed stuff. When moisture content, ash content, crude protein, crude fat and crude fibre content are

added and sum is subtracted from 100, the difference is called as nitrogen free extracts (NFE).

The estimation of Nitrogen free extracts (NFE) content in sixteen *Fagopyrum esculentum* germplasm by difference method revealed the average NFE content as 78.36%. The range of NFE was found to be 76.02% to 80.87%. Raghuvanshi *et al.* (2017) analyzed six indigenous cultivars and one local variety of buckwheat grains. Sieved buckwheat flour (SBF) was found to be in the range of 66.01 to 72.89 per cent for carbohydrate which was found to be less than the present value. Such variation in NFE content in buckwheat germplasm might be attributed to the genetic make up of the materials used as well as the seed maturity at harvest.

5.2 Total soluble protein

Proteins are polymers of amino acids that are major source of energy. Seed storage proteins of common buckwheat have been characterized by several researchers (Fujino, Funatsuki, Inada, Shimono and Kikuta, 2001; Radovic, Maksimovic, Brkljacic, Varkonji-Gasic and Savic, 1999; Radovic, Maksimovic and Varkonji-Gasic, 1996; Skerritt, 1986).

Soluble protein level reflects the proteins available in diet. The total soluble protein content ranged from 4.8% to 7.4% with an average of 6.29% in all sixteen *F.esculentum* germplasm under study. These results are in agreement with those reported by other workers. Marshal and Pomeranz (1982) reported protein content in whole buckwheat and buckwheat groats as 13.80 and 16.40 per cent, respectively which was quite higher than the reported value.

Wang *et al.* (1995) studied the nutritional composition of buckwheat and reported protein content 9.30% which is almost similar to the present value. Ikeda *et al.* (2005) reported protein content to range from 4.2 to13.6g/100g in buckwheat groats grown in Japan, Europe and Canada, so the given result was in agreement with the results obtained in the present study.

Bonafaccia *et al*. (2003) analysed the chemical composition of grain and flour of common buckwheat. They reported protein content as 11.7 per cent in the grains and 10.6per cent in the flour, in that order (on dry weight basis) of buckwheat. Li and Zhang (2001) reported protein contents in grains of buckwheat as 12.30% and Steadman *et al*. (2001) analysed the general composition of buckwheat groat and observed that buckwheat contained 12.3 per cent protein. So, from the above reference the differences in the protein contents in buckwheat might be because of their genetic makeup and environmental factors such as application of fertilizers, season of growth, degree of maturity and cultural practices influence the protein content in buckwheat. The quality of protein is also influenced by the location.

5.2.1 Protein profile

Buckwheat grains proteins have been fractionated into albumins, glutelins, globulins and prolamins based on their solubility in different solvents. In the present study globulin was found as the major storage protein in *F. esculentum* followed by al glutelin, albumin and prolamin.

Wei *et al*. (1993) studied that buckwheat flours were rich in albumin and globulin and very poor in prolamin and glutelin. Bonafaccia *et al*. (1994) evaluated the protein fractions in buckwheat cultivated in Italy. The study indicated that almost half of the protein content in the five samples analysed was constitued by globulin with its values over 44 per cent, while prolamins represented the smallest fraction (0.7%).

Globulin was found as the major storage protein in *F. esculentum* followed by al glutelin, albumin and prolamin. With an average extraction efficiency of 88.14%, the average globulin, glutelin, albumin and prolamin contents were found to be 2.98, 1.35, 1.02 and 0.18% respectively

In present study, the albumin fraction was found 18.48% of the total protein extract, the amount is almost similar to the 12.5-18.2% which was reported by Ikeda *et al.* in 1991 and 18% reported by Bonafaccia *et al*. in 1994.

In present study, the globulin fraction was found 53.83% of the total protein extract, this amount is higher than the 43% reported by Javornik *et al*. (1981) and 30% reported (Nic Phiarais *et al.*, 2008) but similar to the 43.3-64.5% reported by Ikeda *et al.* in 1991. Milisavljevic *et al*. in 2004 reported that in buckwheat, storage proteins belong to the albumin and globulin fractions, forming 70% of total seed proteins which was quite true in the present study of buckwheat.

In present study, the prolamin fraction was found 3.27% of the total protein extract, the amount is slightly higher than the 0.8-2.9% which was reported by Ikeda *et al.* in 1991**.** But very low amount of the prolamin fraction was found in the buckwheat flour (2%), as previously reported by Ikeda in 2002. Wei *et al*. (1993) studied that buckwheat flours contained very poor amount of prolamin and glutelin.

In present study, the glutelin fraction was found 24.42% of the total protein extract, the amount is lower than the 43.6% which was reported by Bejosano and Corke in 1999 but slightly higher than the 22% reported by Bonafaccia *et al*. in 1994 and 8.0-22.7% which was reported by Ikeda *et al.* in 1991.

5.3 Starch content

Starch is the major storage polysaccharide in plants. It is the most common carbohydrate in human diets. In the food industry the main role of starch is its inclusion in the diet as a high-caloric food source and it is also used as gelling and pasting component in sauces, soups, dressings and spreads (Wang *et al.*, 1998). In the present study the varietal effect on starch content was found significant. The average starch content for all germplasm was found 69.22%.

The starch content in buckwheat was ranged from 63.18% to 72.61%. According to Bonafaccia *et al.* in 2003, they reported the starch content was 55.8% in grains, 40.7% in bran and 78.4% in flour which was quite higher than the present value *i.e*. 69.22% but almost similar to 60% to 70% which was reported by Vojtíšková *et al.* in 2012. Such differences might arise due to factors such as plant genetics, soil composition and growing conditions, state of maturity and post-harvest conditions as well as methods used for estimation and expression.

5.4 Amylose content

It is one of the two components of starch, making up approximately 20-30%. Some early investigations on the physicochemical properties of buckwheat starch were carried out by Kim et al. (1977). The values for amylose were 25% in isolated starch (*i.e*., 28.4% of amylose in dry matter of starch).

In the present study the varietal effect on amylose content was found significant. The average starch content for all germplasm was found 23.22%. The amylase content in buckwheat was ranged from 22.45% to 24%. According to Soral-Smietana *et al.* in 1984, they reported the amylose content was 42-52% which was quite higher than the present value *i.e*. 23.22% but almost similar to 26-27% which was reported by Yoshimoto et al. in 2004. Buckwheat starch is composed 25% amylose and 75% amylopectin (Qin *et al.*, 2010).

5.5 Resistant starch content

Resistant starch is a carbohydrate that resist digestion in the small intestine and ferments in the large intestine. As it is resistant to human digestive enzymes, the slow release of glucose results in a reduced energy intake by the intestinal cells, which is evident by the low glycemic index of the non-digested starch. This can help to improve glucose regulation in diabetes and facilitate better weight control for the obese (Higgins *et al.,* 2004).

The resistant starch content in buckwheat varies from 15.2% to 20.53%. The average resistant starch content of all germplasm was found 17.64% which was almost similar to 16-18% in polished buckwheat which was reported by Wronkowska *et al.* (2008) but quite lower than the 33.5% which was reported by Skrabanja and Kreft in 1998.

5.6 Phytochemicals

5.6.1 Phenol

Buckwheat is associated with the antioxidant properties due to the phenolic compounds. Phenolic compounds may lower the blood sugar and lipid levels

and contribute to the hypocholesterolaemic effect. In the present investigation, total phenol content ranged between 378.41 to 652.71mg/100g with an average content of 518.68mg/100g.

Tahir and Farooq (1985) evaluated chemical composition of grains in four buckwheat cultivars and reported phenolics content 0.73, 0.79 and 0.77 per cent on dry weight basis in hull, groat and whole grain in that order. Oomah *et al*. (1996) also determined phenolic acid content in grains from five buckwheat cultivars grown at three locations in western Canada for four years and reported 12-16 g/kg of total phenolic acids. Buckwheat has a total phenolic content ranging from 29 to 1371mg/100 g, depending on the method of extraction and calibration standard used *i.e*. gallic acid or ferulic acid (Holasova *et al.,* 2002; Alvarez-Jubete *et al.,* 2010; Gorinstein *et al.,* 2007; Velioglu *et al.,* 1998; Oomah *et al.,* 1996; Cao *et al.,* 2008; Zielinska *et al.,* 2007).

Holasova *et al*. (2002) pointed out that the antioxidant potential of buckwheat is determinedmainly by phenolic compounds and noticed total that phenolics content was 3303mg/kg in buckwheat grains and 3903mg/kg in dehulled grains, respectively, on dry matter basis.

So, from the above reference the differences in the phenolic contents in buckwheat might be because of their genetic makeup as well the methods used for the estimations.

5.6.2 Flavonoids

Flavonoids are a group of plant metabolites thought to provide health benefits through cell signalling pathways and antioxidant effects**.** Buckwheat has high levels of flavonoids and other bioactive com-pounds (Krkoskova and Mrazova, 2005) with a potential to inhibit lipoprotein oxidation and to reduce the risk of cardiovascular diseases (Jiang *et al.,* 2007). Buckwheat contains several flavonoids, like rutin, quercetin, kaempferol, orientin/isoorientin, and vitexin/isovitexin, which have demonstrated antioxidant, antimicrobial and anti-inflammatory properties (Cai *et*

al., 2004). Chao *et al*. (2002) have suggested a protective role of flavonoids in buckwheat seeds helping to prevent coronary heart diseases and possibly cancers.

This is the largest group of phytonutrients, with more than 6000 types. Flavonoids are well known for the red, purple and brown pigmentation they give to flowers, fruits, and seeds. Flavonoids have attracted considerable attention because of their potential beneficial health effects and they are also recognized as potent antioxidant due to their phenolic hydroxyl group constitution. They can delay or inhibit oxidation process.

In the present study, total flavonoids content ranged between 33.80 to 60.11mg/100g with an average content of 46.57mg/100g. The flavonoids contents in present study were found to be slightly higher than the reported value 40mg/100g by Jiang *et al*. (2007). Compared to most grain crops, buckwheat contains more rutin, which is reported to be the most abundant flavonoid providing natural antioxidant, antiinflammatory and anti-carcinogenic properties. Li and Zhang (2001) reported that the total flavonoid content in common buckwheat was 10 mg/g which was lower than the present investigation

Krkoskova *et al*. (2004) reported that the flavonoids level in buckwheat grains decreased significantly by dehulling process. The flavonoid content of whole seeds of buckwheat was 6.20mg/100g which decreased to 4.60mg/100g whereas in dehulled seeds it increased from 2.20mg/100g to 2.98mg/100g. Such a wide variation might be mainly because of the methods used for estimating flavonoid contents and the genetic makeup of the materials.

5.7 Mineral content

Buckwheat is a rich source of minerals phosphorus, potassium, calcium, sodium and iron. The range of variability in different minerals among various genotypes was evaluated in buckwheat in view of the dietary significance of these dietary constituents in human nutrition. The data relating to extent of variation in minerals among different genotypes included in the study and discussed as under -

Variability in the potassium content in grains of buckwheat genotypes was studied and data relating to this attribute are depicted in Table 4.19.

The calcium content in different varieties/genotypes differed significantly from 144.00 to 215.30mg/100g. The average calcium content of all germplasm was found 177.58mg/100g which is lower than the 214mg/100g which was reported by Siener *et al*. in 2006 and 20.1 mg/kg by Bonafaccia *et al*. in 2003. These results were in agreement with the results obtained in the present study.

The iron content in different varieties/genotypes differed significantly from 2.50mg/100g to 3.50mg/100g. The average iron content of all germplasm was found 3.14mg/100g which is lower than the 4 mg/100g which was reported by Pomeranz in 1983, 4.82mg/100g which was reported by Amarowicz and Fornal in 1987 and 4.4 mg/100g by Wang *et al*. in 1995. But in 1988 Lin and Jia evaluated the common buckwheat flour for mineral composition and reported iron content as 0.014 per cent (14mg/100g) which was quite lower than the present value *i.e*. 3.14mg/100g.

The phosphorus content in different varieties/genotypes differed significantly from 242.6 to 282mg/100g. The average phosphorus content of all germplasm was found 264.08mg/100g which is lower than the 330mg/100g which was reported by Pomeranz in 1982. But in 1995 Wang *et al*. reported phosphorus content in buckwheat 244 mg/100g which was lower than the presented value *i.e*. 264.08mg/100g. Ikeda *et al*. in 1999 reported mineral content of buckwheat grains *i.e*., flour and hull on dry weight basis and found phosphorus content 379mg/100g and again Ikeda *et al*. in 2005 reported phosphorus content in buckwheat flour from 265 to 510mg/100g.

The potassium content in different varieties/genotypes differed significantly from 237.00mg/100g to 298.27mg/100g. The average potassium content of all germplasm was found 257.7 mg/100g which is lower than the 290mg/100g which was reported by Lin and Jia in 1988. Amarowicz and Fornal (1987) determined various mineral elements in buckwheat grains and reported potassium 244.1mg/100g which was lower than the presented value. Ikeda *et al*. (2005) evaluated phosphorus

content in various buckwheat groats and they reported potassium content from 322 to 518 mg/100g which is also much more higher than the presented value.

The sodium content in different varieties/genotypes differed significantly from 1.56 to 4.24mg/100g. The average sodium content of all germplasm was found 2.54mg/100g. Kusano *et al*. (1983) determined the mineral composition of tetraploid and two diploid varieties of the original (Kataoka) and the traditional (Ina) grains. The range of variation for sodium was from 2.5 to 4.0mg/100g. Wang *et al*. (1995) reported mineral composition of buckwheat and reported sodium content in buckwheat was 2.3mg/100g which was quite lower than the present investigation.

CHAPTER VI
SUMMARY AND CONCLUSION

Buckwheat (*F. esculentum*), or common buckwheat, is a plant cultivated for its grain-like seeds and as a cover crop. Buckwheat has gained an excellent reputation as a nutritious ingredient in human diet. Buckwheat grain is characterized by a high content of starch, high content of protein with an advantageous amino acid composition, high content of dietary fibre and polyphenols. Absence of gluten protein makes buckwheat an ideal dietary supplement for celiac disease patients.

The present investigation attempted to analyze the nutritional quality of sixteen different buckwheat genotypes collected from Regional Agricultural Research Station, Assam Agricultural University, North Lakhimpur. The findings of the present study have been summarized below:

- ✓ Moisture content in buckwheat genotypes ranged from 7.52% to 9.11% with an average of 8.18%.
- ✓ Highest crude fat was recorded as 3.62% and the lowest as 1.97 %.
- ✓ The crude protein content varied between 7.23 – 9.53% with the average of 8.40% for the sixteen buckwheat genotypes tested.
- ✓ The ash content in buckwheat genotypes was 2.32%.
- ✓ Buckwheat genotypes were found to contain 177.58, 3.14, 264.08, 257.70 and 2.54 mg/100g of Ca, Fe, P, K and Na
- ✓ Crude fibre content in buckwheat genotypes ranged from 3.71 to 4.78%.
- ✓ Nitrogen free extracts was found within the range of 71.41-76.97%.
- ✓ Starch content in buckwheat genotypes was found within the range of 63.18-72.61%.
- ✓ The average amylose content for all the genotypes was found 23.22%.

- ✓ The resistant starch content in buckwheat genotypes ranged from 15.2% to 20.53% with an average content of 17.64%.
- ✓ Total soluble protein content ranged from 4.58% to 7.4% with an average of 6.29% for all the sixteen buckwheat genotypes tested.
- ✓ Globulin was found as the major storage protein followed by glutelin, albumin and prolamin in the buckwheat genotypes.
- ✓ Average globulin, glutelin, albumin and prolamin contents were found to be 53.83, 24.42, 18.48 and 3.27% respectively.
- ✓ Total phenol content ranged from 378.41 to 652.71 mg GAE/100g with an average content of 518.68 mg GAE/100g.
- ✓ Total flavonoids content ranged between 33.80 to 60.11 mg QE/100g with an average content of 46.57 mg/100g in the sixteen buckwheat genotypes analyzed.

✓ Conclusion

- ✓ Wide variations among genotypes for quality attributes have shown ample potential to be exploited for further quality improvement programme.
- ✓ Thegenotypes superior in individual quality trait i.e., total soluble protein, crude protein, crude fibre, total carbohydrate content, starch, resistant starch, minerals, total phenolic acid and flavonoid content in buckwheat grains were identified.
- ✓ Thesoluble protein fractionation studies revealed globulin was found as the major protein fraction in *F. esculentum* followed by glutelin, albumin and prolamin.
- ✓ The cumulative genotypic grading for desirable quality traitstaken together showed that released genotype Himpriya, VL-7 and PRB-1; local genotype BWC-1, BWC-2, Jonai and Kharupetia-2; accession genotype EC-218742 and

EC-27242 emerged over all superior versatile genotypes for further value addition /quality enrichment.

✓

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Table 4.1 Proximate composition of *F. esculentum* genotypes (% dry weight)

Germplasm	Moisture	Crude fat	Crude Protein	Ash	Crude fibre
BWC-1	8.26	2.26	9.38	2.15	4.49
BWC-2	8.05	3.34	8.39	1.87	4.78
Jonai	7.87	1.98	7.33	1.93	3.90
Kharupetia-1	8.65	3.13	9.11	2.93	3.88
Kharupetia-2	8.27	2.37	8.99	2.75	3.78
NLC	7.90	2.89	9.29	1.93	4.60
EC-218740	9.03	1.99	8.38	2.00	4.70
EC-218742	8.50	3.27	9.53	2.67	4.61
EC-27242	7.55	2.33	8.58	1.83	4.56
IC-109728	8.00	2.41	7.98	2.57	4.19
IC-258233	7.88	2.12	8.30	2.23	4.09
IC-329456	8.10	3.40	7.85	2.88	4.30
Himpriya	8.72	3.62	7.30	1.88	3.71
PRB-1	7.52	3.41	7.23	2.39	4.74
Shimla B-1	9.11	1.97	8.09	2.73	3.91
VL-7	7.59	2.99	8.79	2.32	4.16
Mean	8.18	2.72	8.40	2.32	4.28
$CD_{0.05}$	0.171	0.173	0.156	0.193	0.0325
S.Ed(±)	0.084	0.085	0.077	0.095	0.016

Values represented in the table are mean of three replications

Table 4.2 Proximate composition of *F. esculentum* genotypes (% dry weight)

Germplasm	Total carbohydrate	Starch	Amylose	Resistant starch
BWC-1	73.44	68.27	22.51	18.70
BWC-2	73.56	63.18	22.96	18.69
Jonai	76.97	70.25	23.57	15.65
Kharupetia-1	72.29	69.28	23.49	16.40
Kharupetia-2	73.82	69.44	23.20	15.59
NLC	73.38	68.40	23.04	15.20
EC-218740	73.89	71.93	23.90	18.32
EC-218742	71.41	72.61	24.00	15.71
EC-27242	75.12	72.48	23.02	20.03
IC-109728	74.84	68.42	23.09	16.99
IC-258233	75.37	65.62	22.45	20.53
IC-329456	73.46	68.90	23.73	16.72
Himpriya	74.77	69.35	23.26	19.89
PRB-1	74.70	70.34	23.60	19.07
Shimla B-1	74.17	68.49	22.95	19.27
VL-7	74.13	70.55	22.61	15.40
Mean	74.08	69.22	23.21	17.64
$CD_{0.05}$	0.428	0.242	0.0936	0.545
S.Ed	0.210	0.119	0.046	0.268

Values represented in the table are mean of three replications

Table 4.3 Total soluble protein and soluble protein fractions of *F. esculentum* genotypes (% dry weight)

Germplasm	Total protein	Albumin	Globulin	Prolamin	Glutelin	Total	Extraction efficiency* (%)
BWC-1	7.10	1.11 (17.96)	3.37 (54.53)	0.22 (3.56)	1.48 (23.95)	6.18	87.04
BWC-2	6.80	1.07 (18.26)	3.21 (54.78)	0.18 (3.07)	1.40 (23.89)	5.86	86.18
Jonai	4.80	0.76 (17.88)	2.30 (54.12)	0.15 (3.53)	1.04 (24.47)	4.25	88.54
Kharupetia-1	5.40	0.82 (17.05)	2.66 (55.30)	0.17 (3.53)	1.16 (24.12)	4.81	89.07
Kharupetia-2	7.30	1.15 (17.86)	3.53 (54.81)	0.24 (3.73)	1.52 (23.60)	6.44	88.18
NLC	6.16	1.03 (18.86)	2.93 (53.66)	0.13 (2.38)	1.37 (25.09)	5.46	88.64
EC-218740	5.62	0.89 (17.91)	2.68 (53.92)	0.17 (3.42)	1.23 (24.75)	4.97	88.43
EC-218742	7.40	1.10 (17.38)	3.47 (54.82)	0.14 (2.21)	1.62 (25.59)	6.33	85.54
EC-27242	7.15	1.12 (17.83)	3.45 (54.94)	0.22 (3.50)	1.49 (23.73)	6.28	87.83
IC-109728	6.46	1.13 (20.07)	3.06 (54.35)	0.19 (3.37)	1.25 (22.20)	5.63	87.15
IC-258233	6.16	0.97 (17.73)	2.97 (54.30)	0.14 (2.56)	1.39 (25.41)	5.47	88.80
IC-329456	6.42	1.01 (17.69)	3.12 (54.64)	0.20 (3.50)	1.38 (24.17)	5.71	88.94
Himpriya	4.58	0.89 (21.55)	2.12 (51.33)	0.16 (3.87)	0.96 (23.24)	4.13	90.17
PRB-1	5.34	0.92 (19.09)	2.63 (54.56)	0.18 (3.73)	1.09 (22.61)	4.82	90.26
Shimla B-1	6.56	1.01 (17.32)	2.96 (50.77)	0.23 (3.95)	1.63 (27.96)	5.83	88.87
VL-7	7.35	1.35 (21.23)	3.21 (50.47)	0.15 (2.36)	1.65 (25.94)	6.36	88.53
Mean	6.29	1.02 (18.48)	2.98 (53.83)	0.18 (3.27)	1.35 (24.42)	5.53	88.14
$CD_{0.05}$	0.2709	0.055	0.178	0.0203	0.067		
S.Ed(±)	**0.133**	**0.027**	**0.087**	**0.010**	**0.033**		

Values represented in the table are mean of three replications

*Extraction efficiency is the percentage extracted protein fraction from total soluble protein

Values shown in the parentheses indicate relative percentage of protein fraction

Table 4.4 Phytochemical analysis of *F. esculentum* genotypes (mg/100g dry weight)

Germplasm	Total phenol content (mg GAE/100g)	Total flavonoid content (mg QE/100g)
BWC-1	540.19	48.84
BWC-2	523.15	44.13
Jonai	640.33	60.11
Kharupetia-1	442.61	40.22
Kharupetia-2	582.10	43.51
NLC	461.33	43.20
EC-218740	378.41	33.80
EC-218742	538.19	51.92
EC-27242	425.77	40.28
IC-109728	533.00	51.97
IC-258233	577.81	54.42
IC-329456	523.08	51.90
Himpriya	652.71	49.62
PRB-1	546.67	44.91
Shimla B-1	554.33	51.30
VL-7	379.20	36.06
Mean	518.68	46.57
$CD_{0.05}$	27.721	4.271
S.Ed (±)	13.609	2.097

GAE - Gallic acid equivalent (mg/100g)

QE - Quercetin equivalent (mg/100g)

Values represented in the table are mean of three replications

Table 4.5 Mineral content of *Fagopyrum esculentum* germplasm (mg/100g dry weight)

Germplasm	Calcium	Phosphorus	Iron	Potassium	Sodium
BWC-1	148.33	281.00	3.31	248.00	2.22
BWC-2	144.00	282.00	3.49	247.67	2.56
Jonai	169.00	243.00	3.50	251.67	4.07
Kharupetia-1	156.00	263.00	3.29	248.33	1.97
Kharupetia-2	152.64	265.60	2.80	237.00	1.89
NLC	210.32	274.00	3.14	298.27	3.52
EC-218740	181.67	271.00	2.50	256.67	2.06
EC-218742	179.30	265.00	2.84	250.11	2.00
EC-27242	176.00	261.60	2.93	245.00	1.88
IC-109728	187.60	260.30	3.43	246.50	1.93
IC-258233	205.60	262.70	3.28	242.00	1.76
IC-329456	192.31	259.30	3.30	254.00	2.23
Himpriya	174.00	266.00	3.15	267.33	4.24
PRB-1	184.00	255.00	2.83	284.60	3.53
Shimla B-1	165.00	272.60	3.37	280.33	1.56
VL-7	215.33	242.61	3.03	265.33	3.36
Mean	177.57	264.07	3.14	257.68	2.55
$CD_{0.05}$	7.487	14.543	0.145	6.652	0.305
S.Ed	3.676	7.140	0.071	3.266	0.150

Values represented in the table are mean of three replications

Table 4.5

Germplasm	Total protein (%)	Albumin (%)	Globulin (%)	Prolamin (%)	Glutelin (%)	Total (%)	Extraction efficiency*(%)
BWC-1	7.10	1.11 (17.96)	3.37 (54.53)	0.22 (3.56)	1.48 (23.95)	6.18	87.04
BWC-2	6.80	1.07 (18.26)	3.21 (54.78)	0.18 (3.07)	1.40 (23.89)	5.86	86.18
EC-218740	5.62	0.89 (17.91)	2.68 (53.92)	0.17 (3.42)	1.23 (24.75)	4.97	88.43
EC-218742	7.40	1.10 (17.38)	3.47 (54.82)	0.14 (2.21)	1.62 (25.59)	6.33	85.54
EC-27242	7.15	1.12 (17.83)	3.45 (54.94)	0.22 (3.50)	1.49 (23.73)	6.28	87.83
HIMPRIYA-9	4.58	0.89 (21.55)	2.12 (51.33)	0.16 (3.87)	0.96 (23.24)	4.13	90.17
IC-109728	6.46	1.13 (20.07)	3.06 (54.35)	0.19 (3.37)	1.25 (22.20)	5.63	87.15
IC-258233	6.16	0.97 (17.73)	2.97 (54.30)	0.14 (2.56)	1.39 (25.41)	5.47	88.80
IC-329456	6.42	1.01 (17.69)	3.12 (54.64)	0.20 (3.50)	1.38 (24.17)	5.71	88.94
JONAI	4.80	0.76 (17.88)	2.30 (54.12)	0.15 (3.53)	1.04 (24.47)	4.25	88.54
KHARUPETIA-1	5.40	0.82 (17.05)	2.66 (55.30)	0.17 (3.53)	1.16 (24.12)	4.81	89.07
KHARUPETIA-2	7.30	1.15 (17.86)	3.53 (54.81)	0.24 (3.73)	1.52 (23.60)	6.44	88.18
NLC	6.16	1.03 (18.86)	2.93 (53.66)	0.13 (2.38)	1.37 (25.09)	5.46	88.64
PRB-1	5.34	0.92 (19.09)	2.63 (54.56)	0.18 (3.73)	1.09 (22.61)	4.82	90.26
SHIMLA B-1	6.56	1.01 (17.32)	2.96 (50.77)	0.23 (3.95)	1.63 (27.96)	5.83	88.87
VL-7	7.35	1.35 (21.23)	3.21 (50.47)	0.15 (2.36)	1.65 (25.94)	6.36	88.53
Mean	6.29	1.02 (18.48)	**2.98 (53.83)**	**0.18 (3.27)**	**1.35 (24.42)**	**5.53**	**88.14**
$CD_{0.05}$	**0.2709**	**0.055**	**0.178**	**0.0203**	**0.067**		
S.Ed(±)	**0.133**	**0.027**	**0.087**	**0.010**	**0.033**		

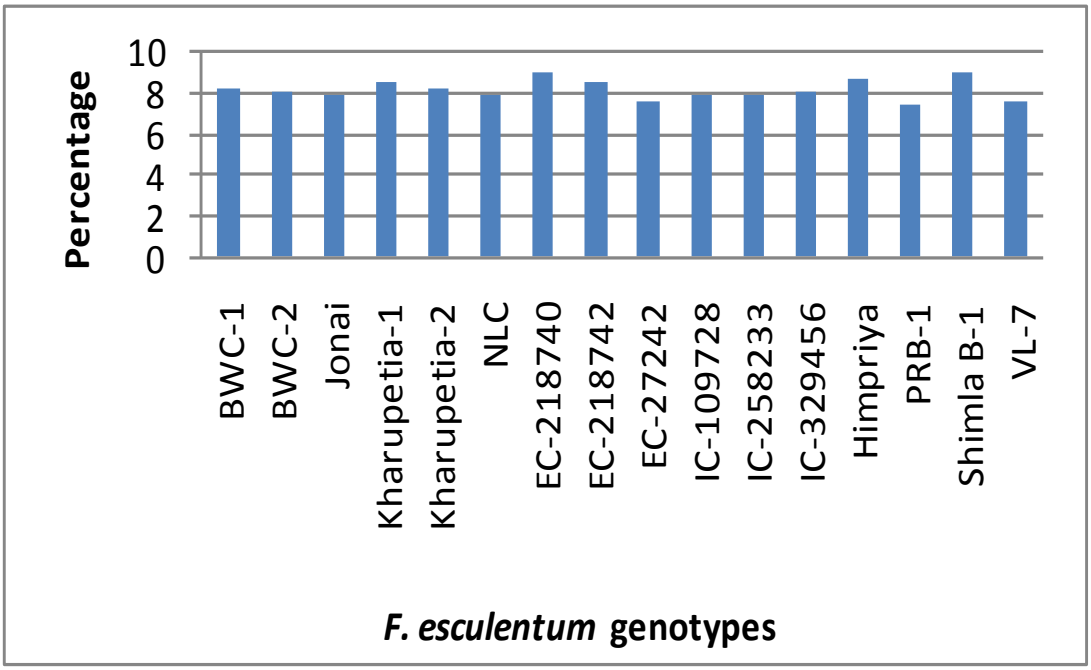


Fig. 1: Moisture content (%) of sixteen *F. esculentum* germplasm

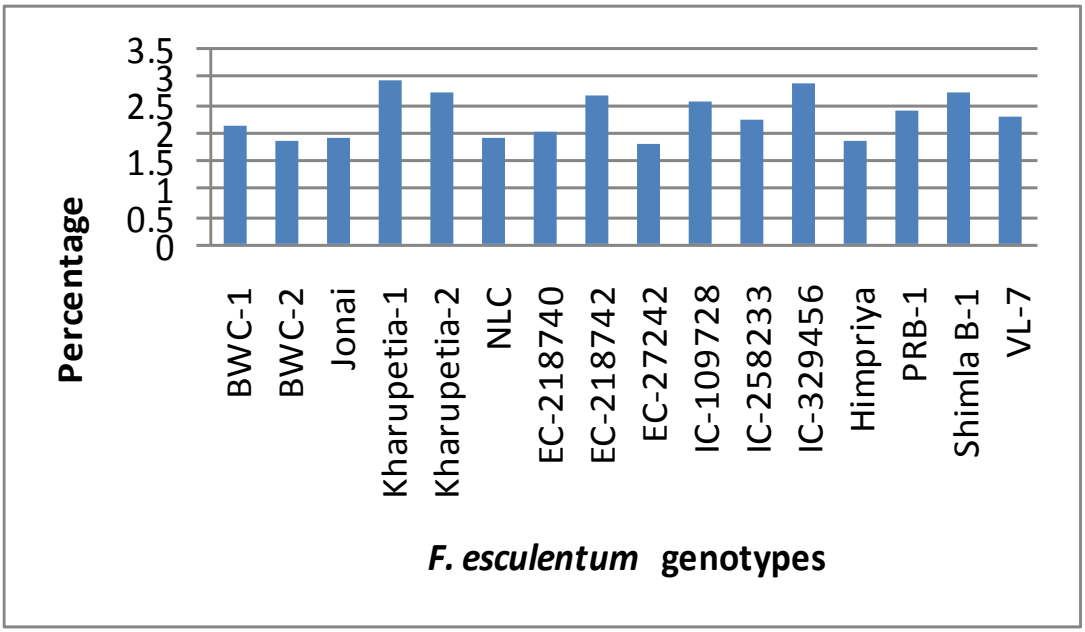


Fig. 2: Ash content (%) of sixteen *F. esculentum* germplasm

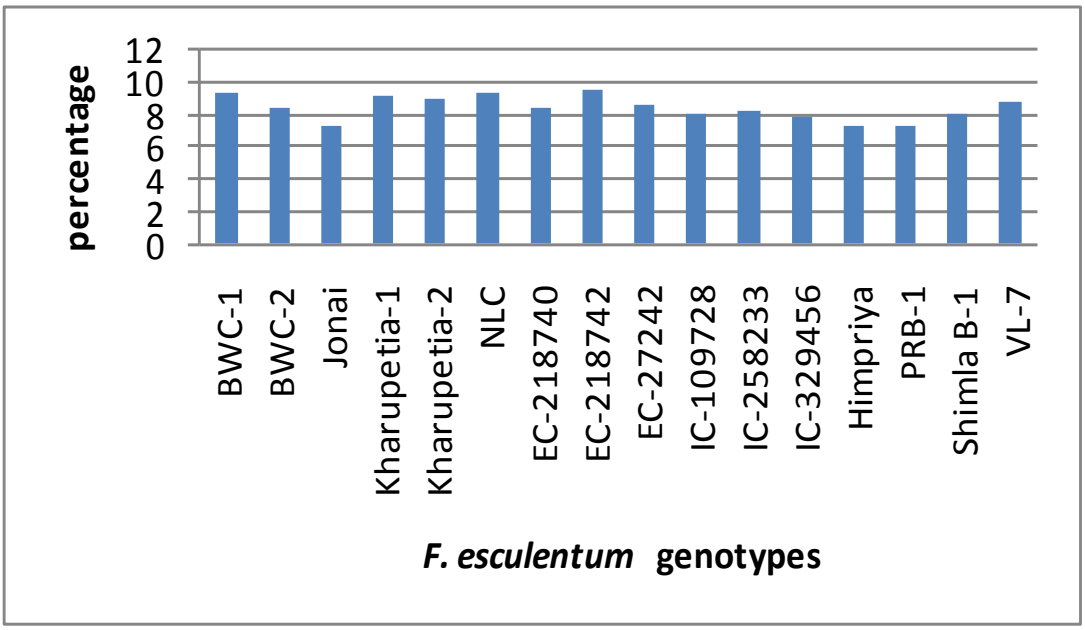


Fig. 3: Crude protein content (%) of sixteen *F. esculentum* germplasm

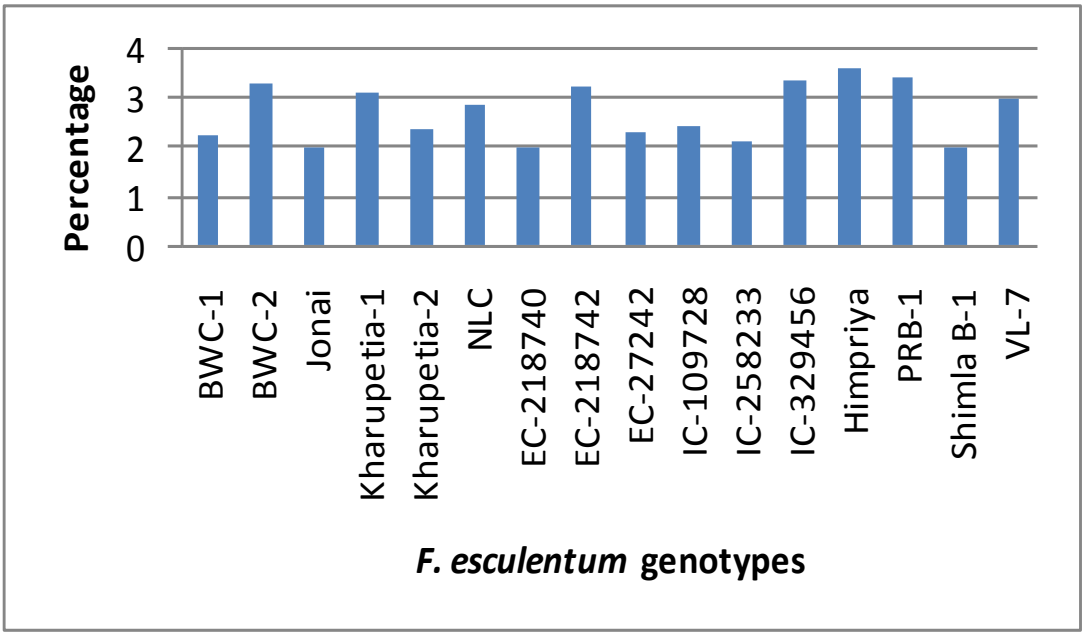


Fig. 4: Crude fat (%) of sixteen *F. esculentum* germplasm

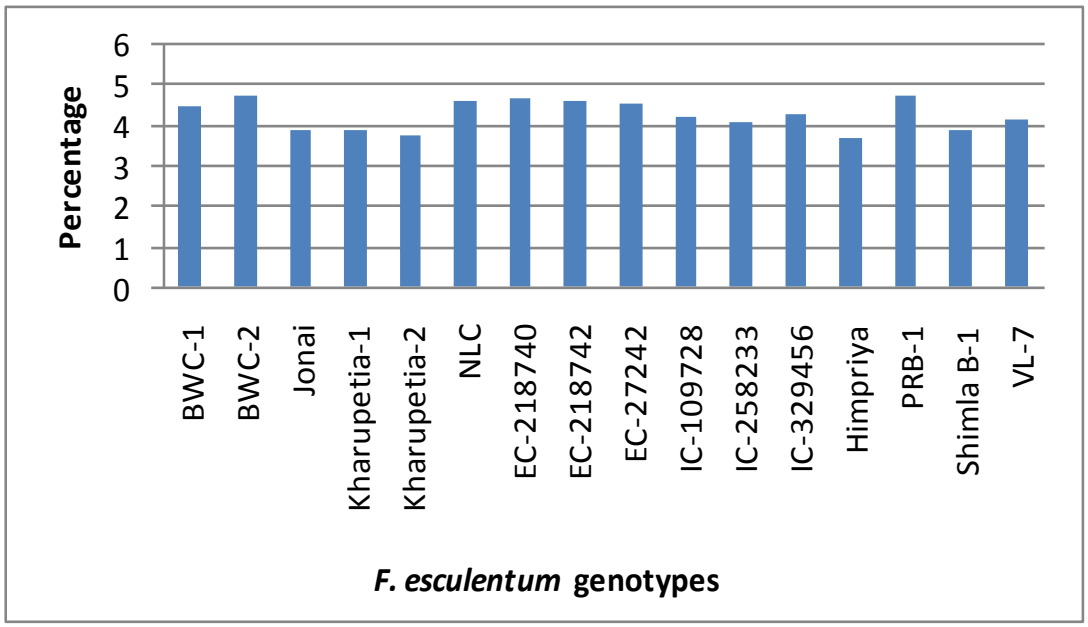
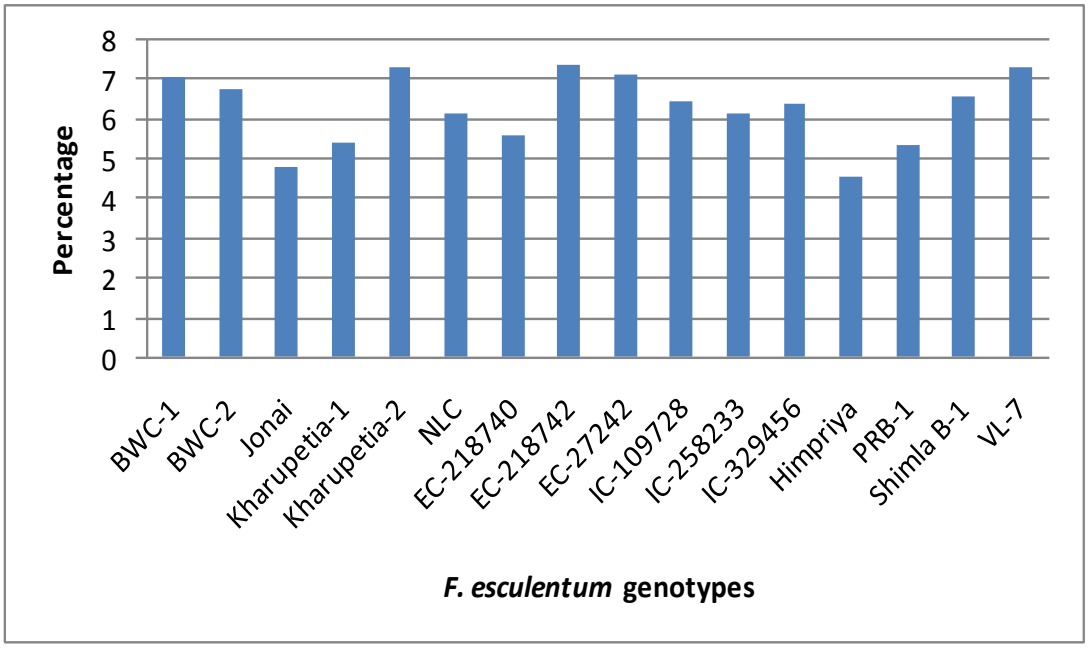


Fig.5: Crude fibre (%) of sixteen *F. esculentum* germplasm



.5: Total soluble protein (%) of sixteen *F. esculentum* germplasm

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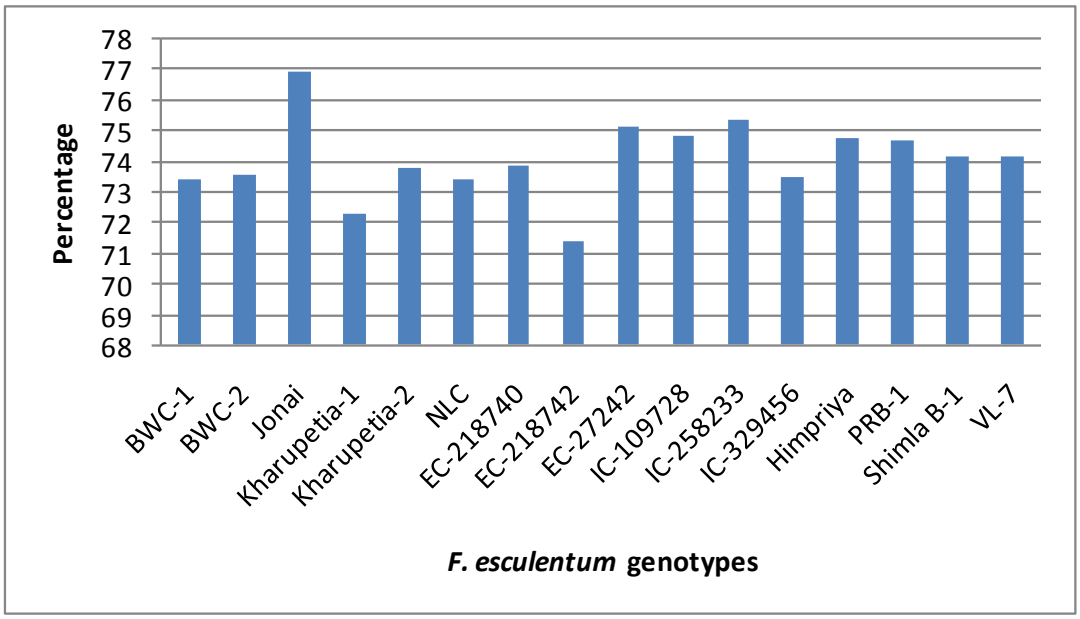

Fig. 6: Nitrogen free extract (%) of sixteen *F. esculentum* germplasm

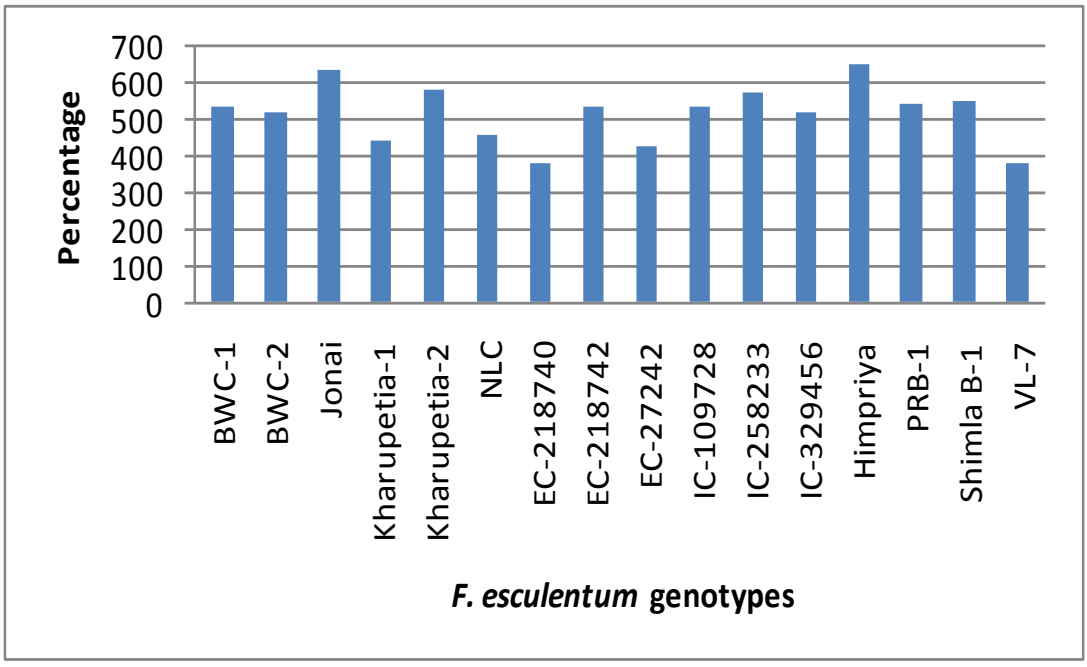

Fig. 7: Total phenol (mg/100g) of sixteen *F. esculentum* germplasm

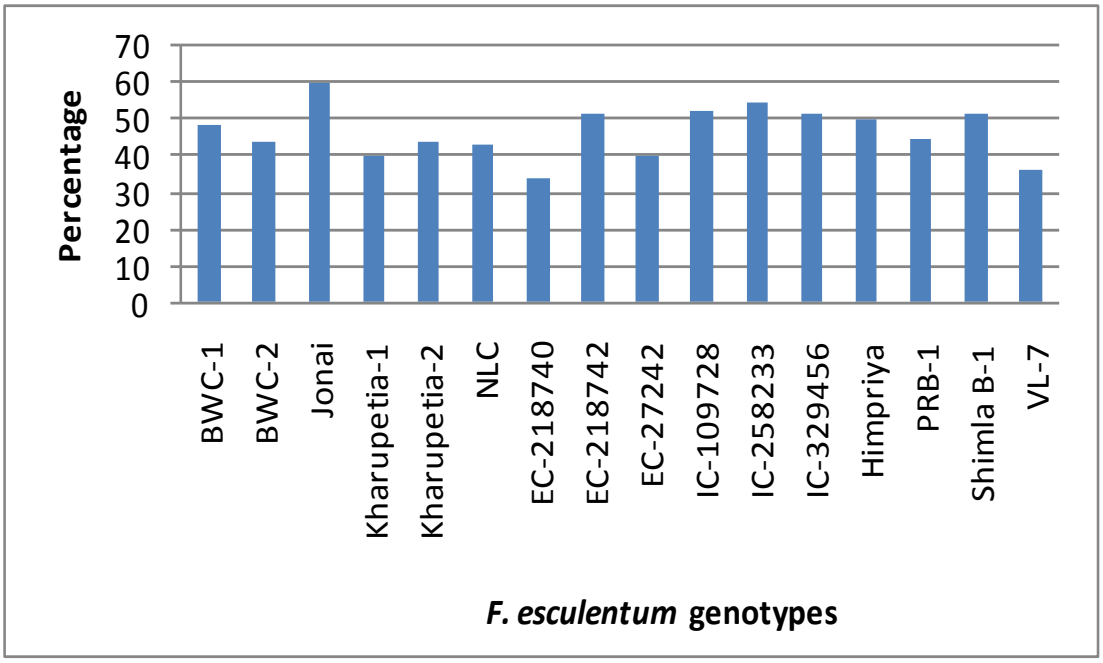

Fig. 8: Total flavonoids (mg/100g) of sixteen *F. esculentum* germplasm

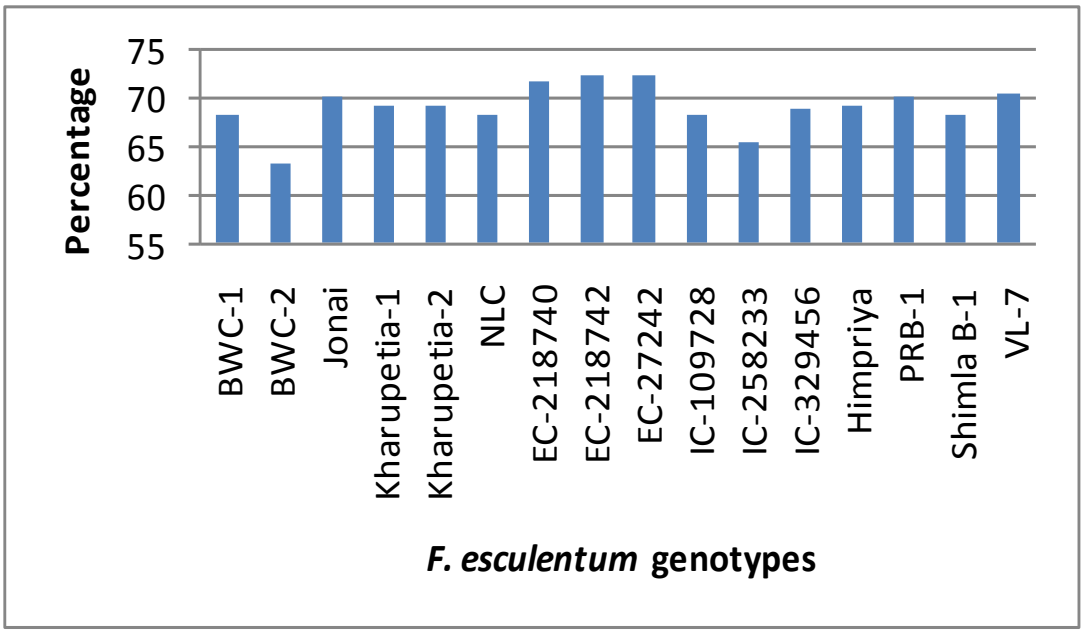

Fig. 9: Starch (%) of sixteen *F. esculentum* germplasm

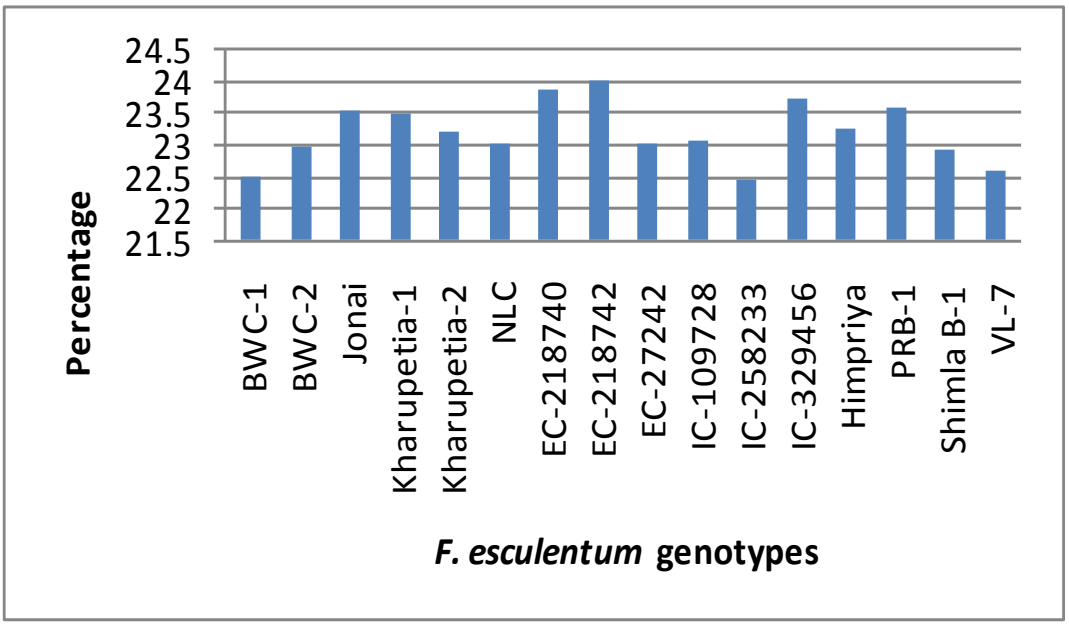

Fig. 10: Amylose (%) of sixteen *F. esculentum* germplasm

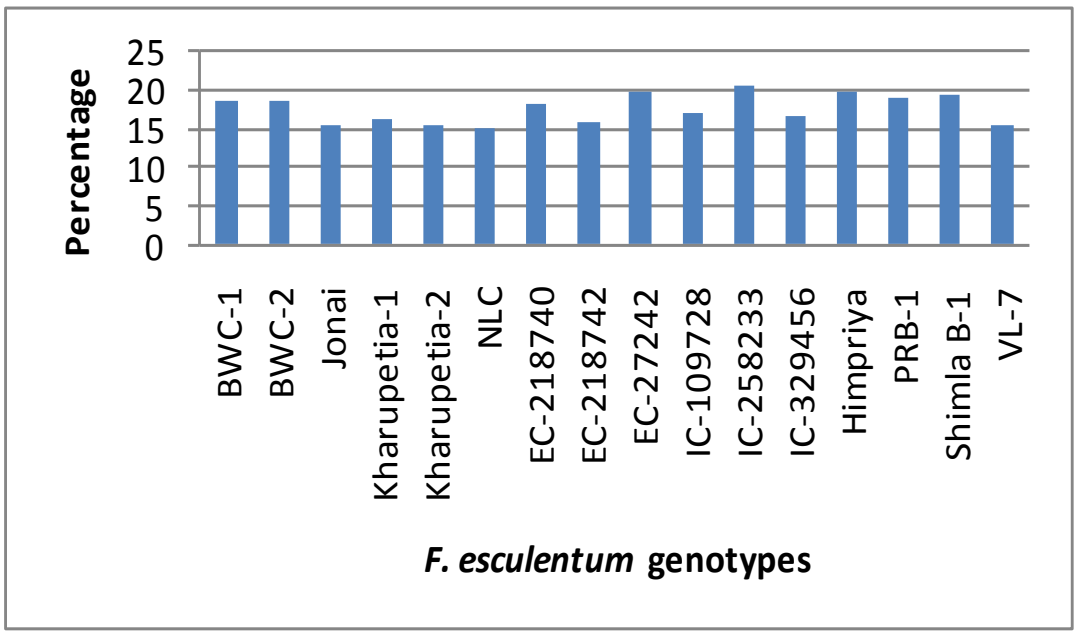

Fig. 11: Resistant starch (%) of sixteen *F. esculentum* germplasm

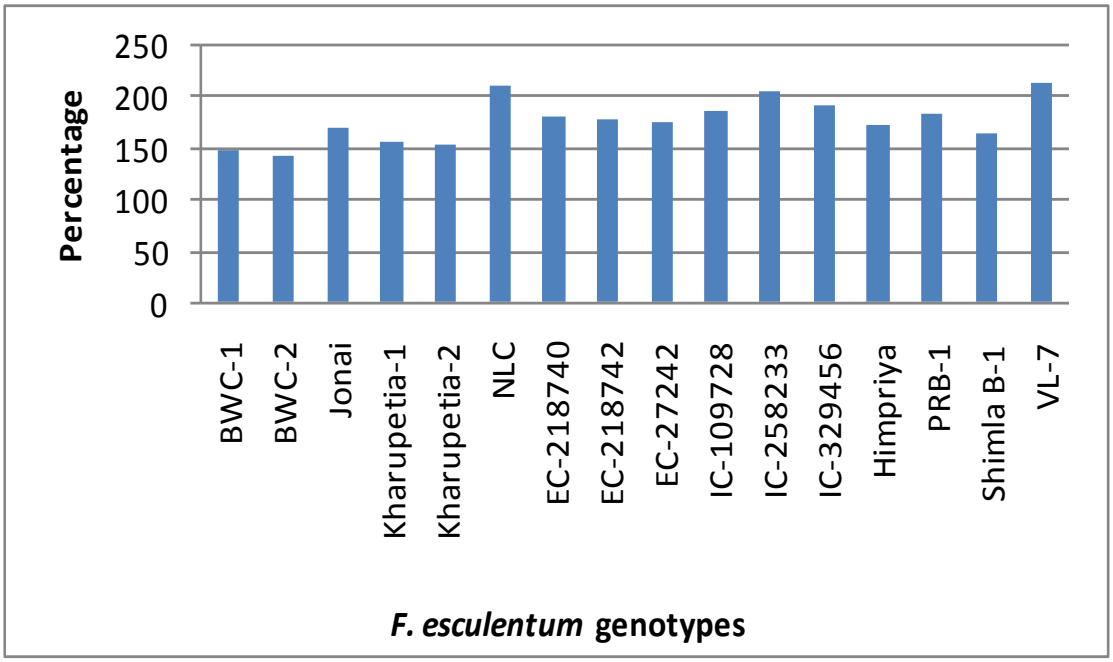

Fig. 11: Calcium (mg/100g) of sixteen *F. esculentum* germplasm

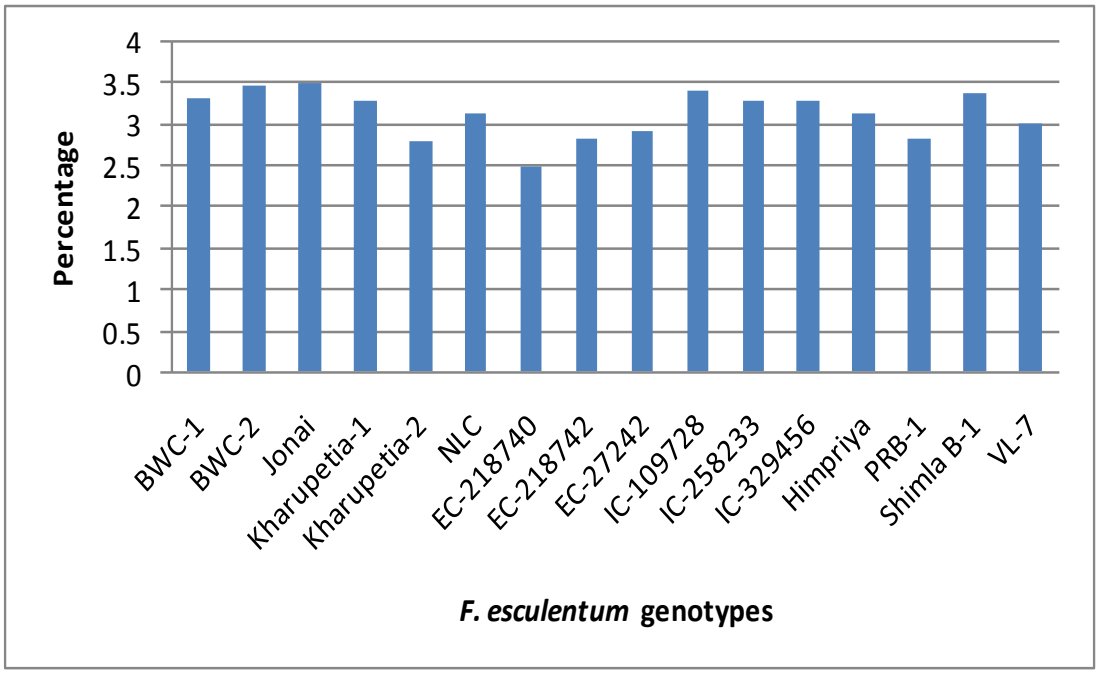

Fig. 11: Iron (mg/100g) of sixteen *F. esculentum* germplasm

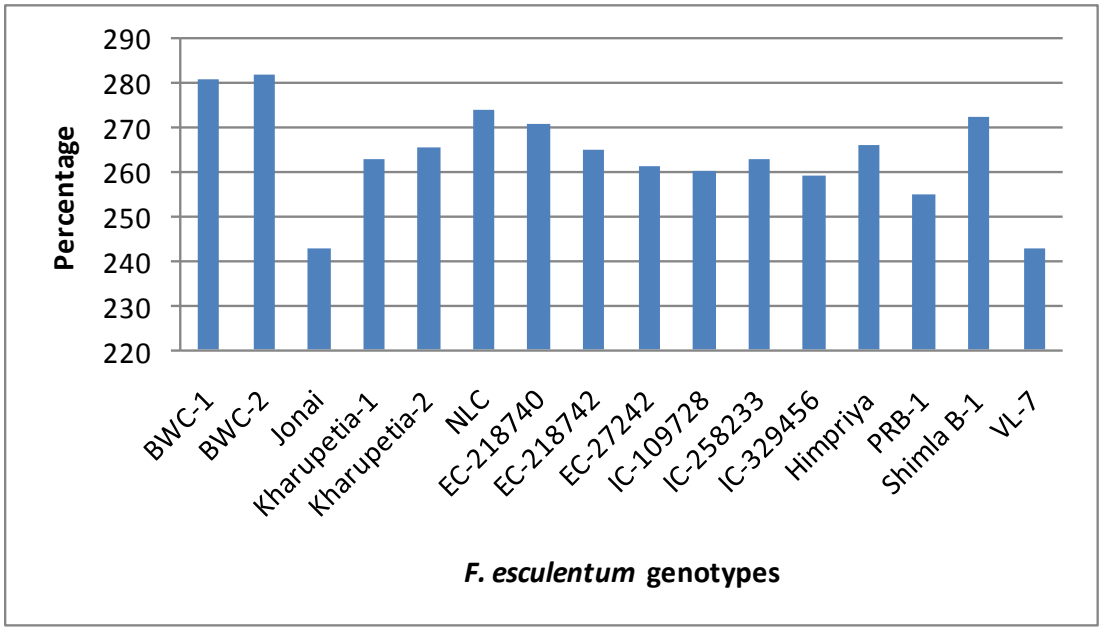

Fig. 11: Phosphorus (mg/100g) of sixteen *F. esculentum* germplasm

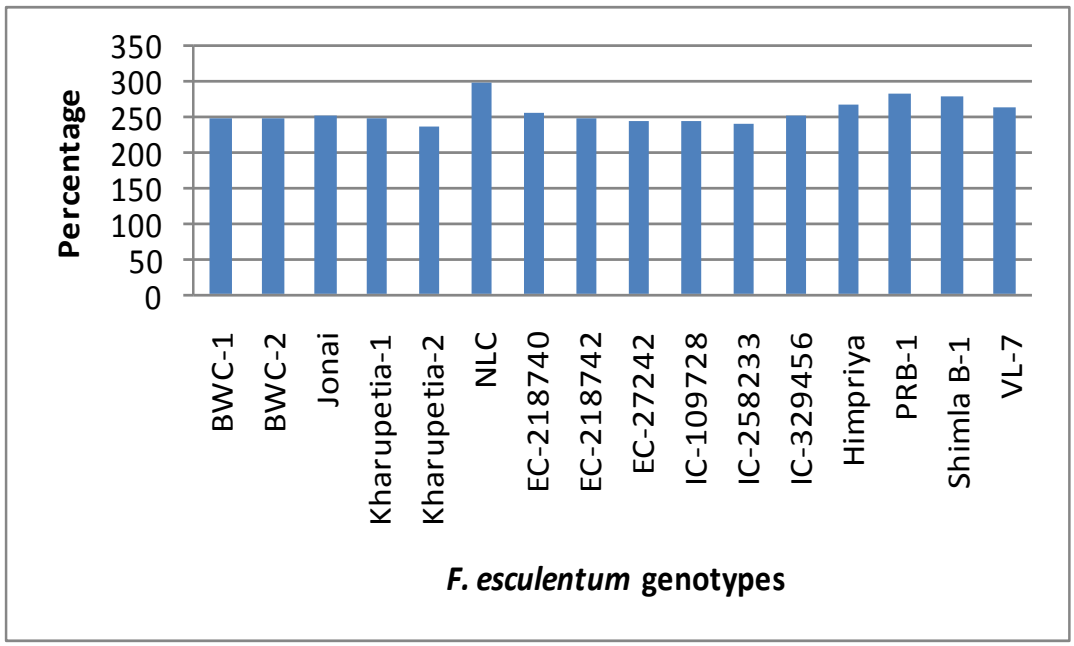

Fig. 11: Potassium (mg/100g) of sixteen *F. esculentum* germplasm

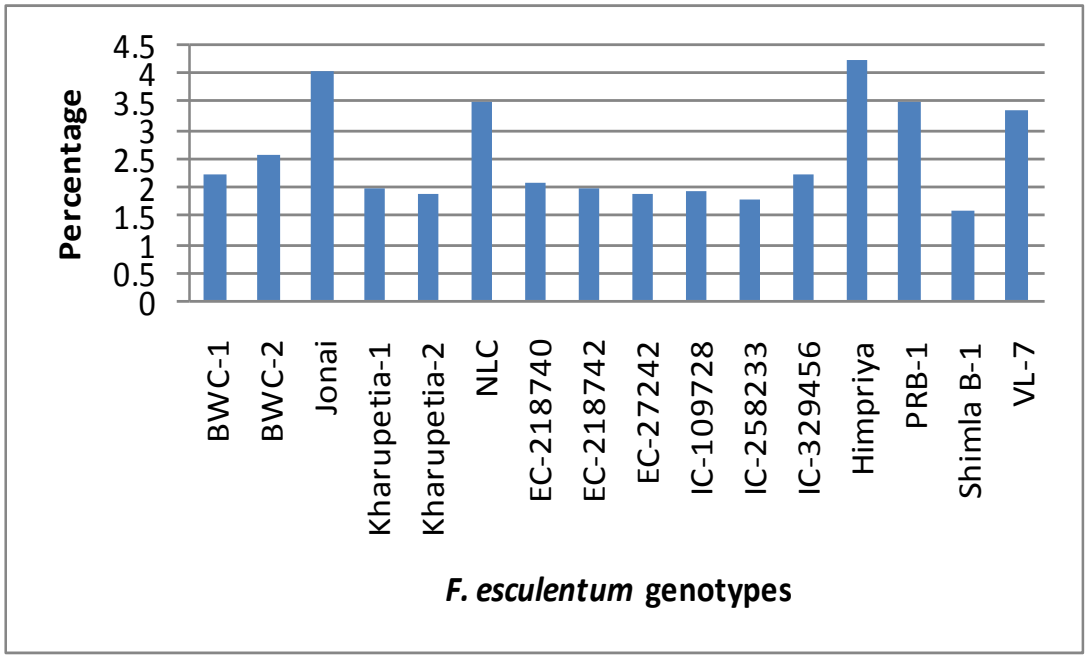

Fig. 11: Sodium(mg/100g) of sixteen *F. esculentum* germplasm

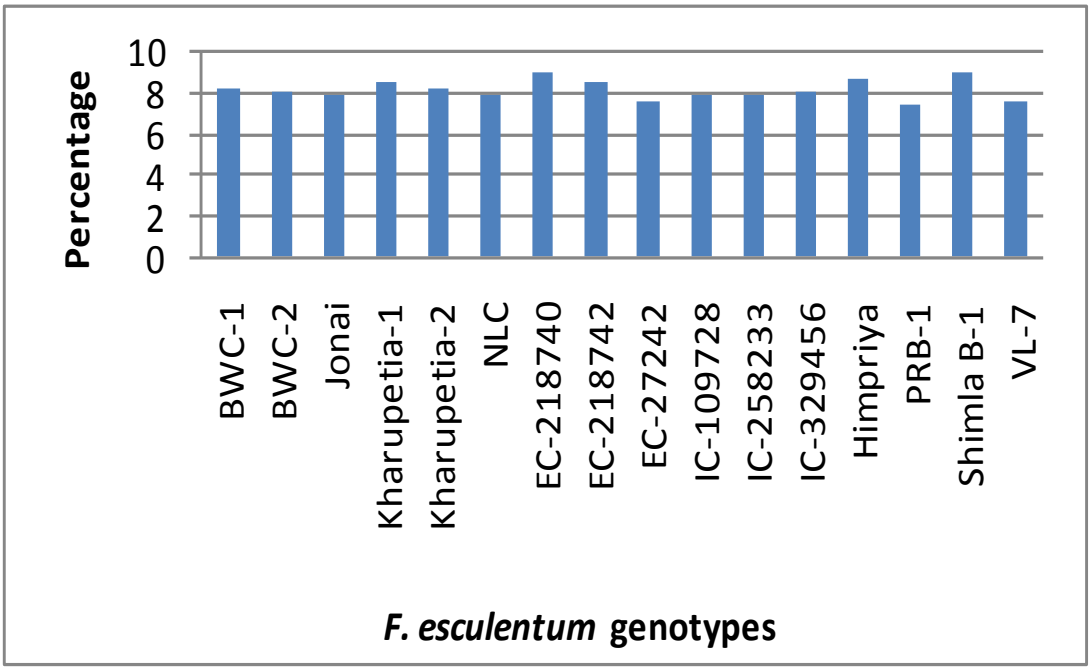

Fig. 1: Moisture content (%) of sixteen *F. esculentum* genotypes

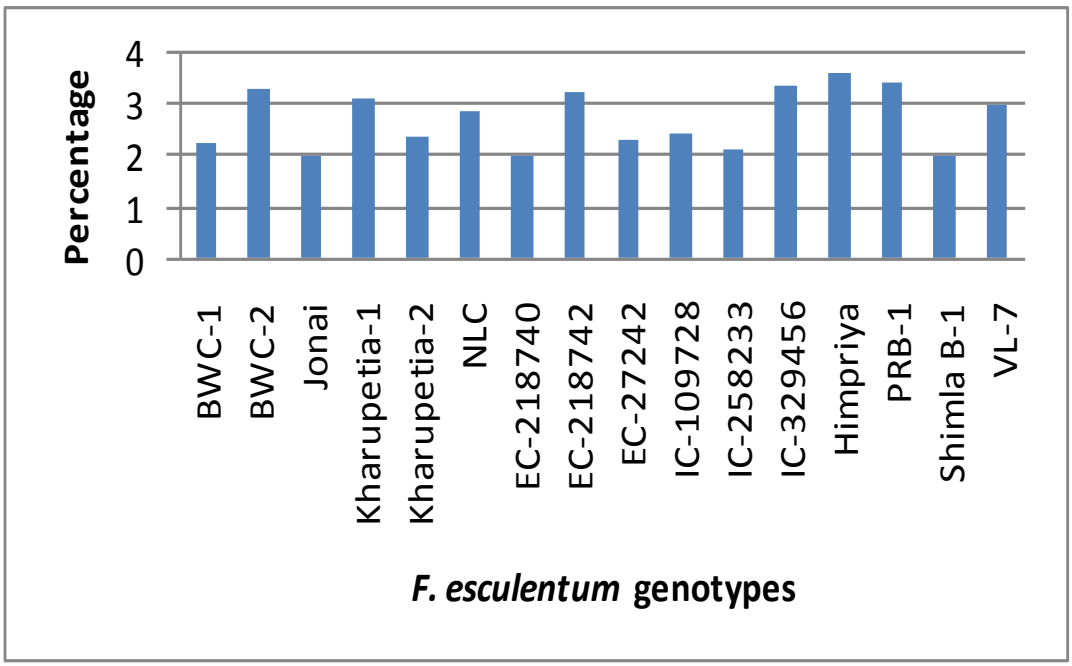

Fig. 2: Crude fat (%) of sixteen *F. esculentum* genotypes

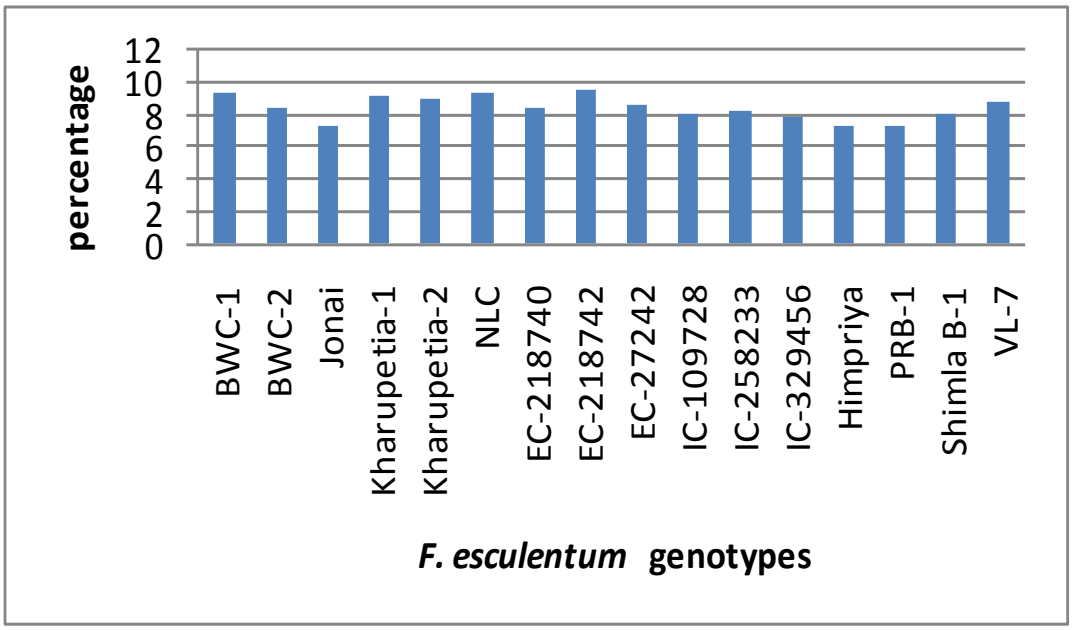

Fig. 3: Crude protein content (%) of sixteen *F. esculentum* genotypes

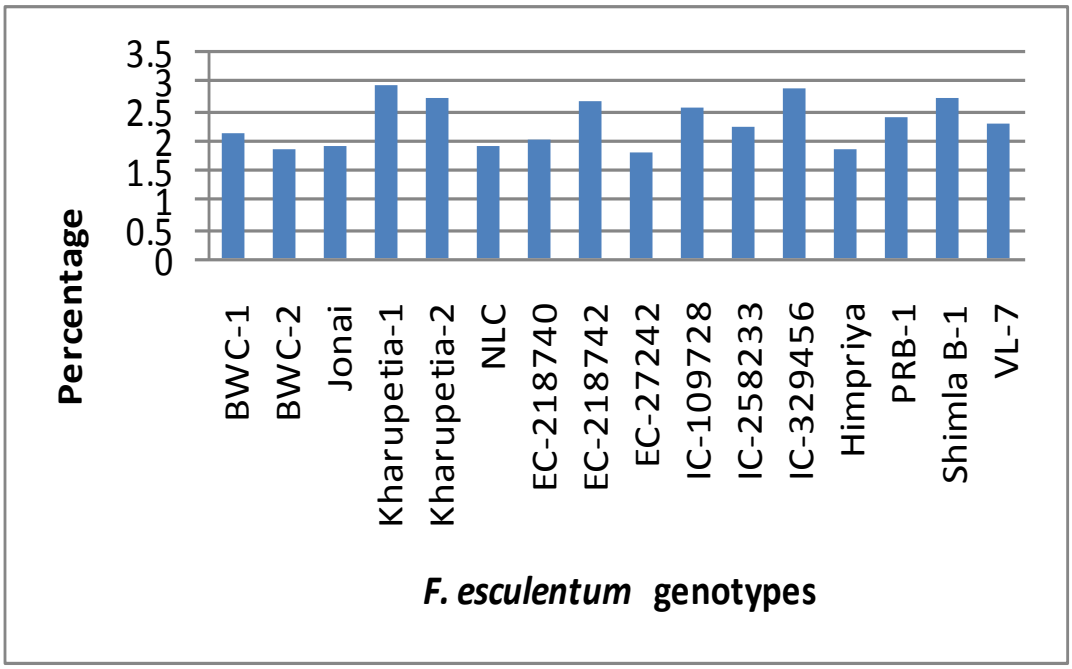

Fig. 4: Ash content (%) of sixteen *F. esculentum* genotypes

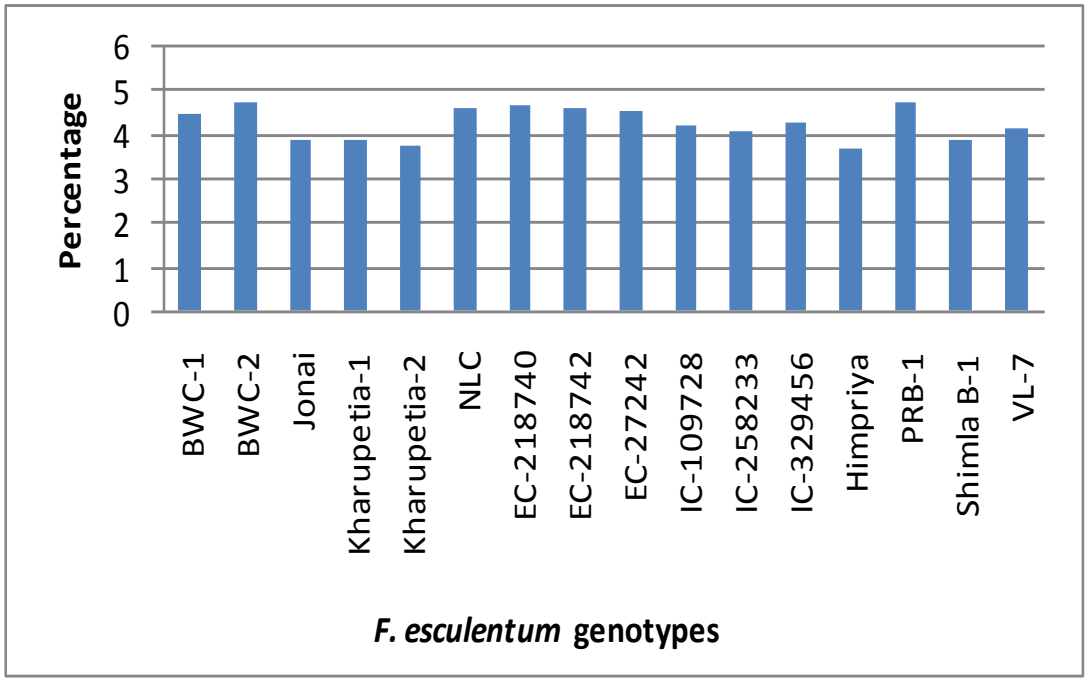

Fig.5: Crude fibre (%) of sixteen *F. esculentum* genotypes

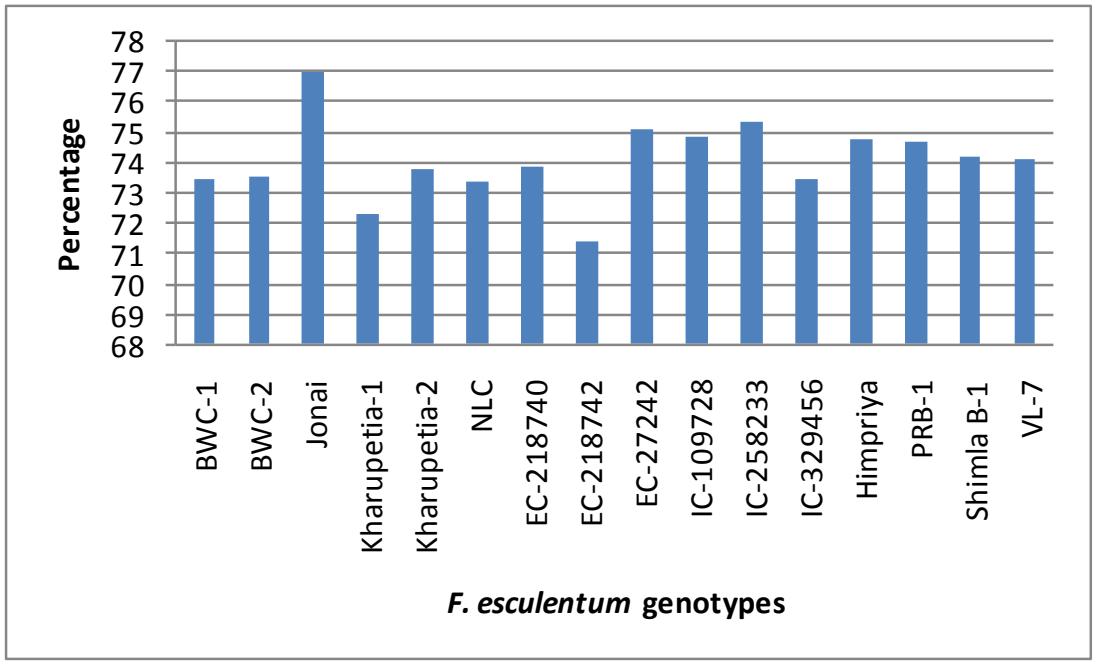

Fig. 6: Nitrogen free extract (%) of sixteen *F. esculentum* genotypes

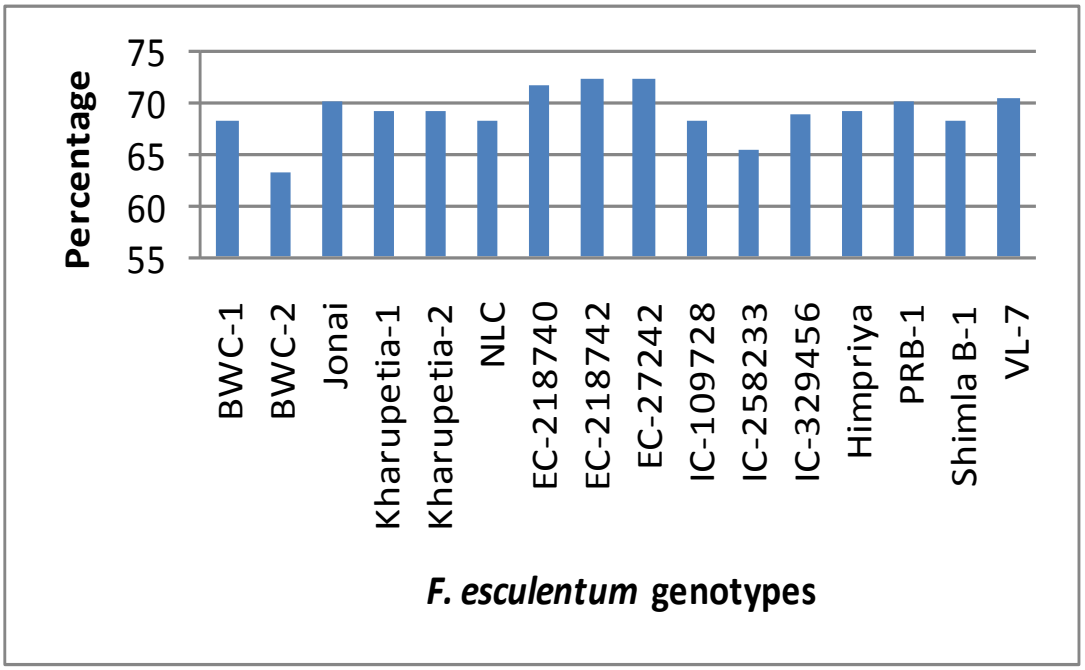

Fig. 7: Starch (%) of sixteen *F. esculentum* genotypes

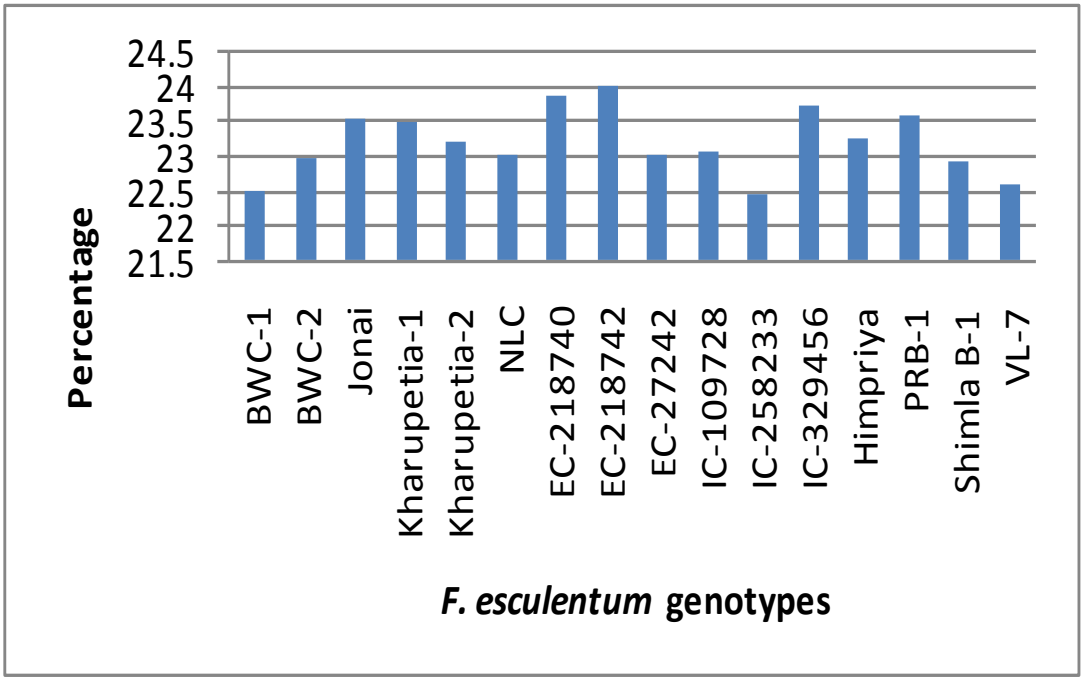

Fig. 8: Amylose (%) of sixteen *F. esculentum* genotypes

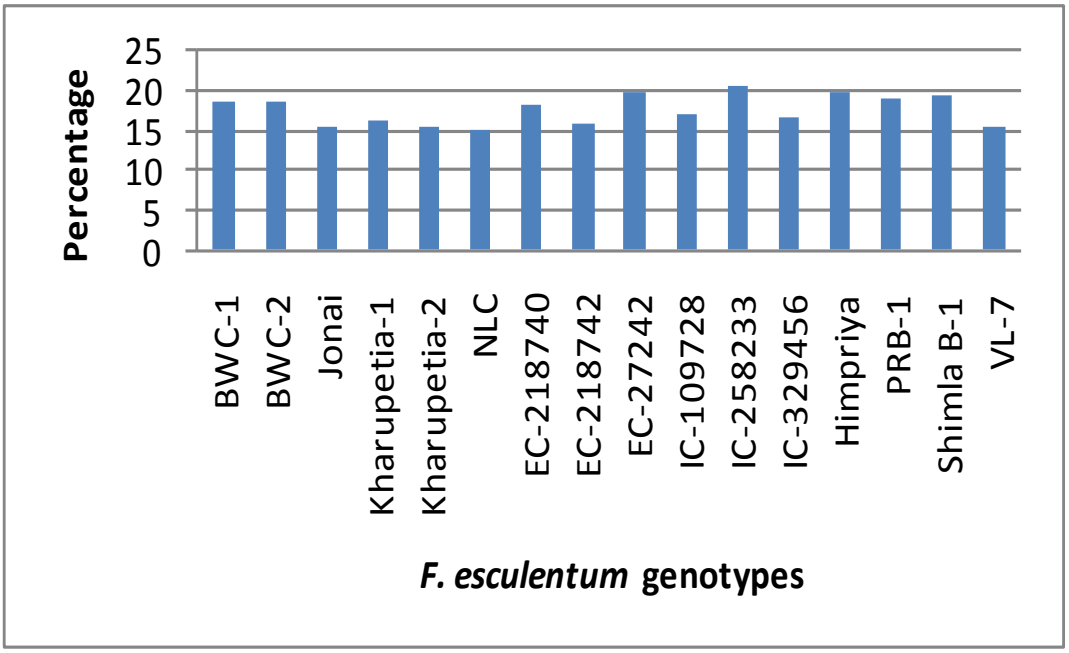

Fig. 9: Resistant starch (%) of sixteen *F. esculentum* genotypes

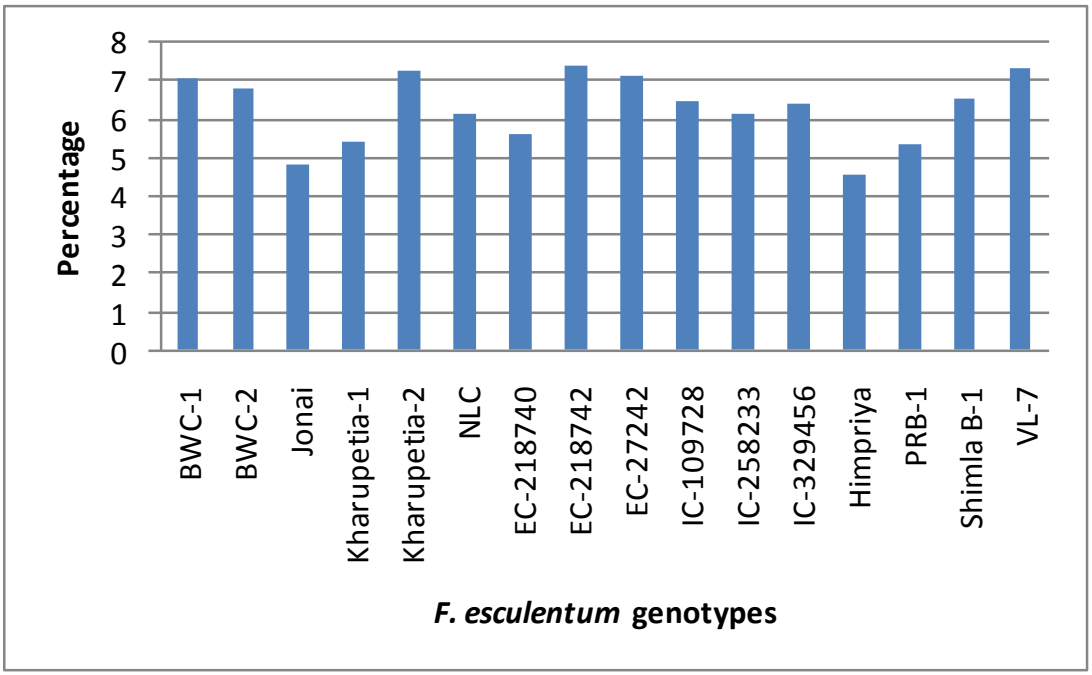

Fig. 10: Total Soluble protein (%) of sixteen *F. esculentum* genotypes

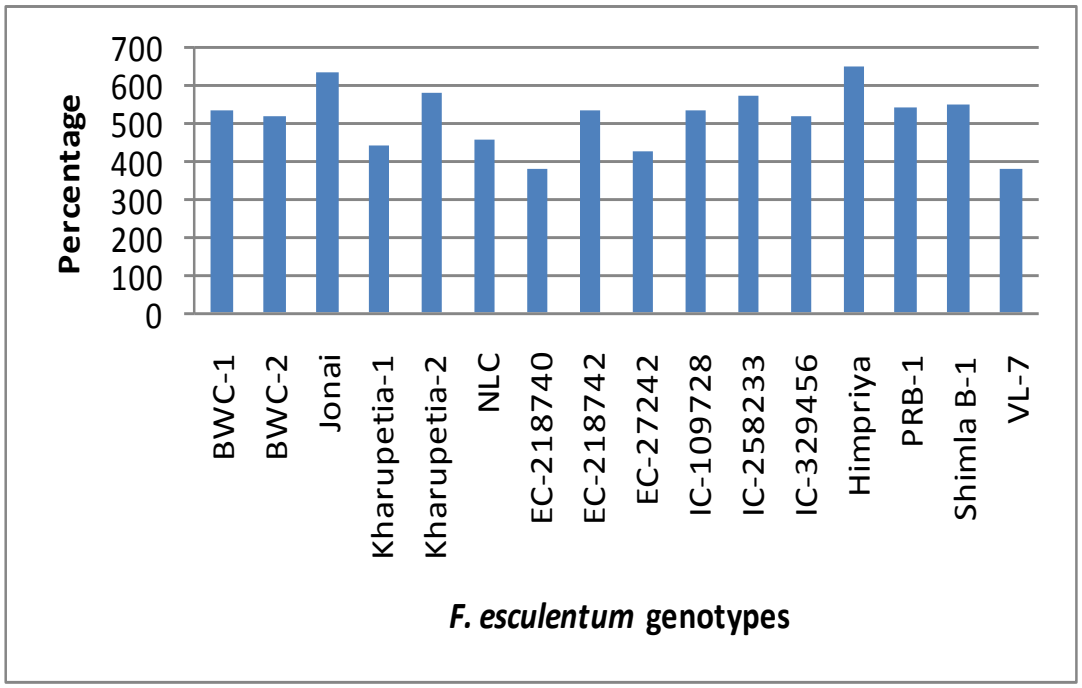

Fig. 11: Total phenol (mg/100g) of sixteen *F. esculentum* genotypes

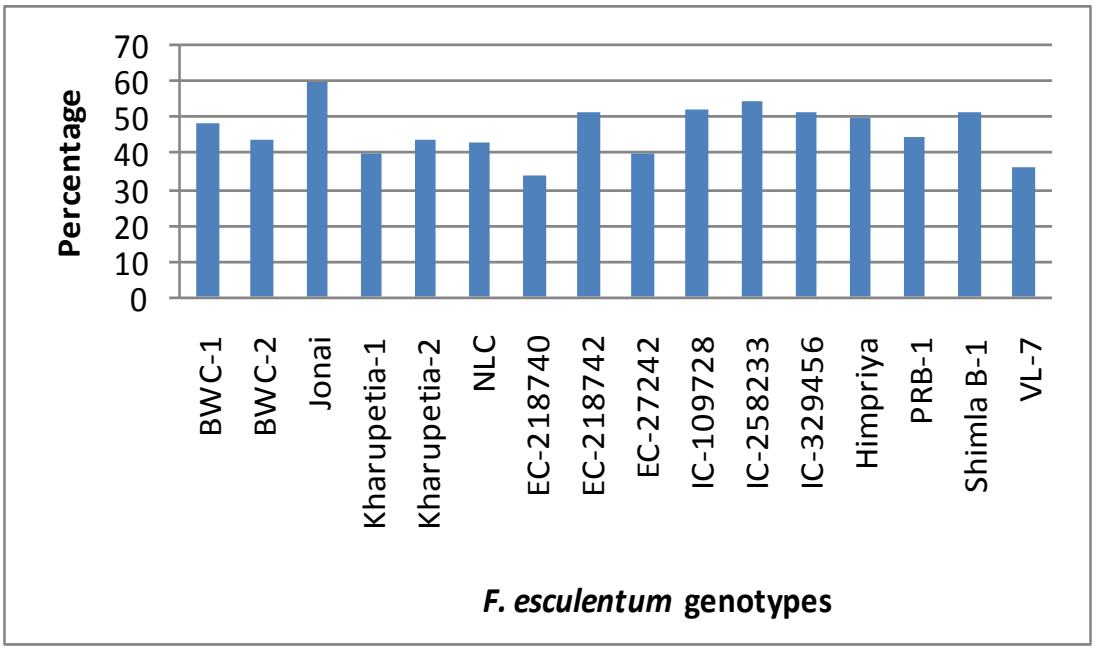

Fig. 12: Total flavonoids (mg/100g) of sixteen *F. esculentum* genotypes

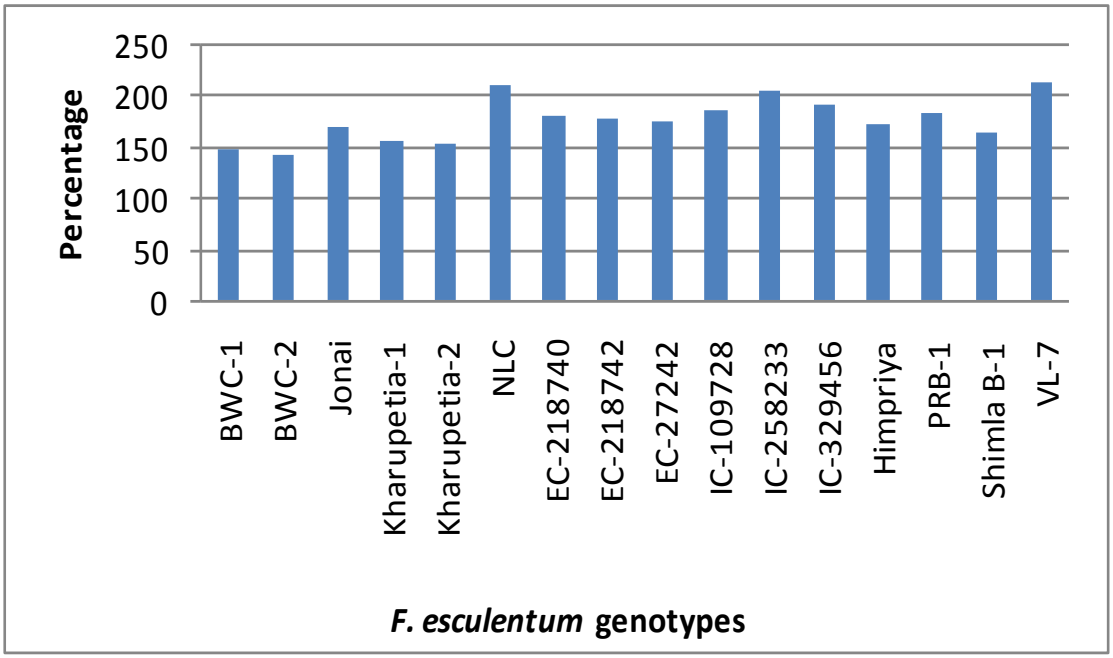

Fig. 13: Calcium (mg/100g) of sixteen *F. esculentum* genotypes

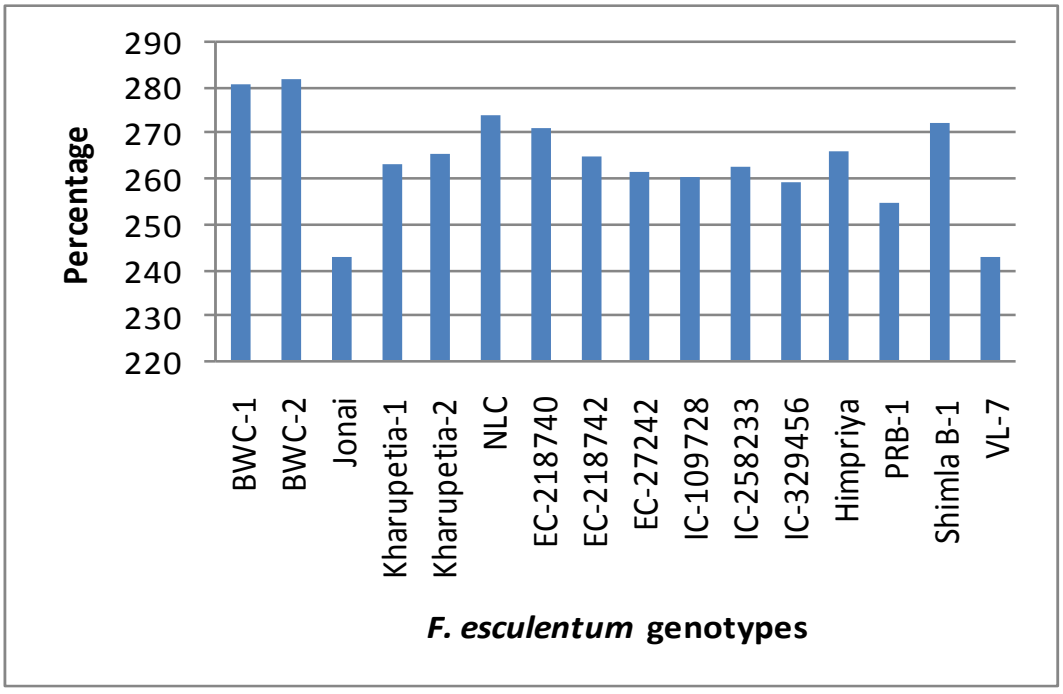

Fig. 14: Phosphorus (mg/100g) of sixteen *F. esculentum* genotypes

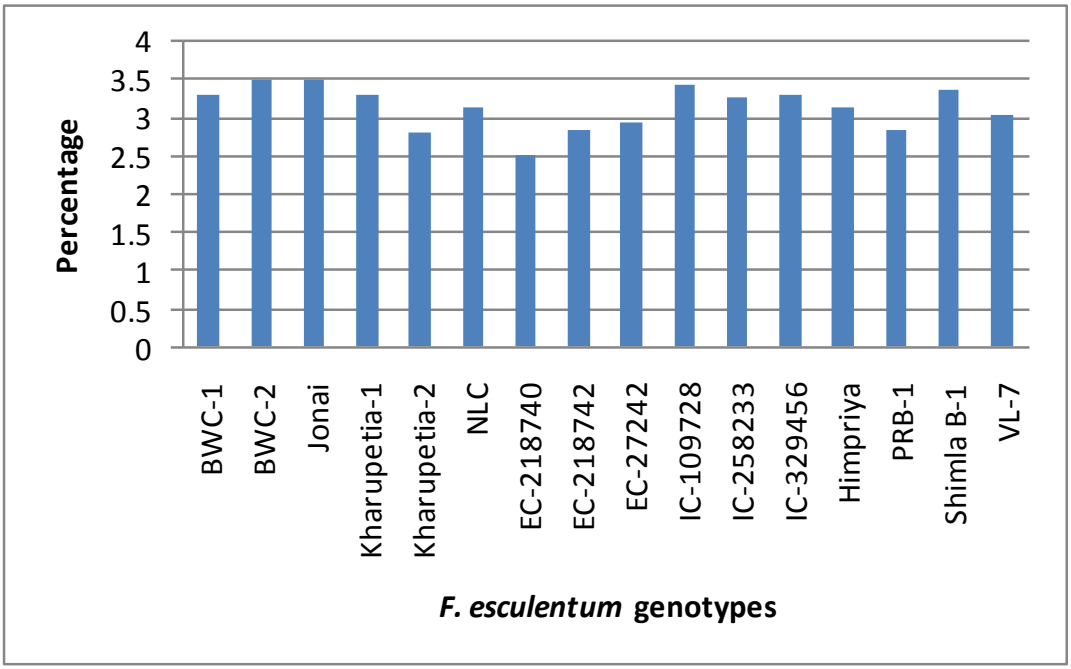

Fig. 15: Iron (mg/100g) of sixteen *F. esculentum* genotypes

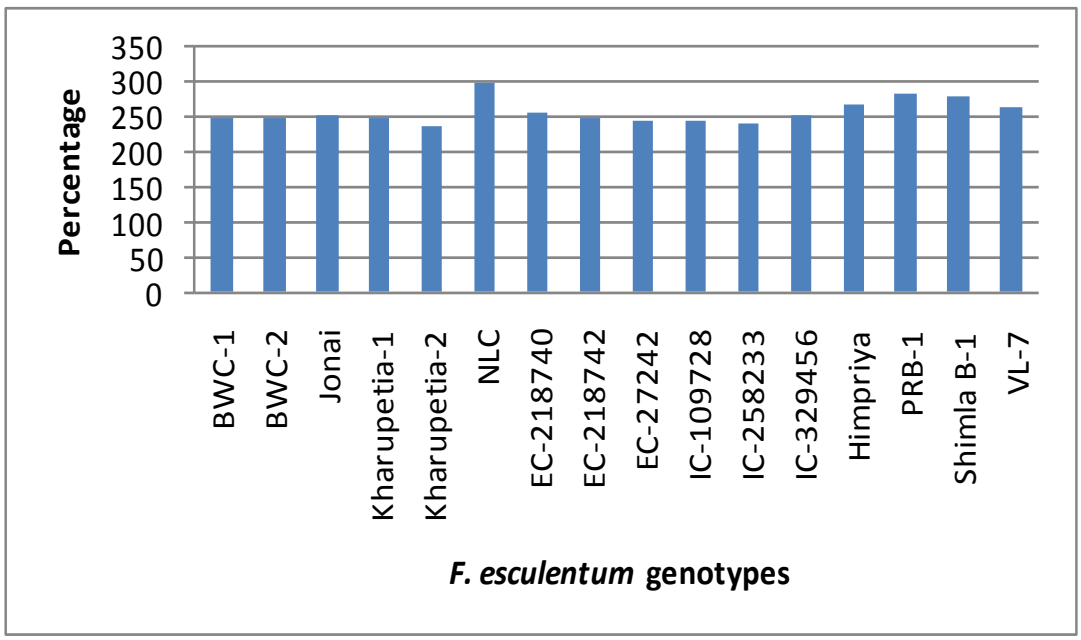

Fig. 16: Potassium (mg/100g) of sixteen *F. esculentum* genotypes

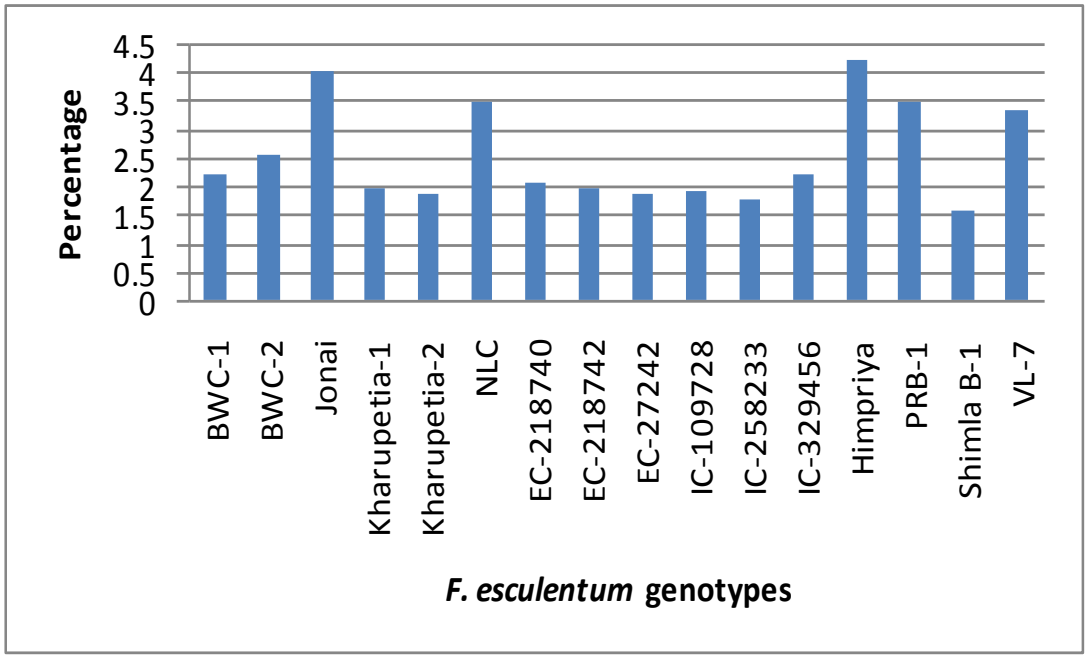

Fig. 17: Sodium (mg/100g) of sixteen *F. esculentum* genotypes

BWC-1

BWC-2

Jonai

Kharupetia-1

Kharupetia-2

NLC

EC-218740

EC-218742

EC-27242

IC-109728

IC-258233

IC-329456

Himpriya

PRB-1

Shimla B-1

VL-7

Fig. 3.1. Sixteen *F. esculentum* genotypes