ABSTRACT DIGITIZED

SCREENING OF PROMISING BLACK GRAM (*Vigna mungo* (L.) CULTIVARS FOR CHEMICAL COMPOSITION

By

Mahesh Nivrutti Kawale
Reg. No. 93140

A Thesis Submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI 413 722 DIST.-AHMEDNAGAR
Maharashtra State (India)

in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI, DIST- AHMEDNAGAR, M. S.(INDIA)

1995

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1995

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma

Place : Rahuri

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CERTIFICATE

This is to certify that the thesis entitled "Screening of Promising black gram (Vigna mungo (L.) cultivars for chemical compostion". submitted to Mahatma Phule Krishi vidyapeet, Rahuri for the award of the Degree of Master of Science in Biochemistry, embodies the results of a piece of bonafide research work carried out by Mr. M. N. Kawale, under my guidance and supervision and that no part of the thesis has been submitted for any degree or diploma.

The assistance and help received during the course of this investigation have been acknowledged.

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CERTIFICATE

This is to certify that the thesis entitled "Screening of Promising black gram (Vigna mungo (L.) cultivars for chemical compostion". submitted to Mahatma Phule Krishi vidyapeet, Rahuri in partial fulfilment of requirement for the Degree of Master of Science in Biochemistry, embodies the results of a piece of bonafide research work carried out by Mr. M. N. Kawale, under the guidance and supervision of Dr. B. B. Desai, Head Department of Biochemistry, and no part of the thesis has been submitted for any degree or diploma.

Place : Rahuri

Date : 15/7/95

(Dr. N.K. Umrani.)

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Place : Rahuri

Date :

(Mahesh N. Kawale)

TABLE OF CONTENTS

	Page
CANDIDATE'S DECLARATION	ii
CERTIFICATES : i) Research Guide	iii
ii) Associate Dean (PGI), MPKV, Rahuri	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	vii
LIST OF FIGURES	ix
ABSTRACT	x
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1 Proximate compostion	
2.1.1 Moisture	4
2.1.2 Ash	4
2.1.3 Crude Protein	5
2.1.4 Lipids	6
2.1.5 Crude fibre	6
2.1.6 Carbohydrates	7
2.2 Minerals	7
2.3 Limiting amino acids	8
2.4 Polyphenols	9
2.5 Protein Fractionation	11
3. MATERIAL AND METHODS	12
3.1 Materials	12
3.1.1 Seeds	12
3.1.2 Chemicals	13
3.2 Methods	13
3.2.1 Proximate composition	13
3.2.2 Minerals	13
3.2.2.1 Phosphorus	13
3.2.2.2 Iron and Zinc	14
3.2.3 Limiting amino acids	14
3.2.3.1 Methionine	14
3.2.3.2 Tryptophan	16
3.2.4 Polyphenols	18
3.2.5 Protein Fractionation	20
3.2.6 Statistical analysis	20

4. RESULT AND DISCUSSION 21

4.1 Proximate composition 21
4.1.1 Moisture 21
4.1.2 Ash 22
4.1.3 Crude Protein 22
4.1.4 Crude Fat 23
4.1.5 Crude Fibre 24
4.1.6 Carbohydrates 24

4.2 Minerals 25
4.2.1 Phosphorus 25
4.2.2 Iron and Zinc 26

4.3 Limiting amino acids 27
4.3.1 Methionine 27
4.3.2 Tryptophan 28

4.4 Polyphenols 28

4.5 Protein Fractionation 29

5. SUMMARY AND CONCLUSIONS 30

6. LITERATURE CITED 32

7. VITA 46

LIST OF TABLES

Page

1. Proximate compostion of black gram cultivars. 21(a)
2. Phosphorus, Iron, Zinc content of black gram cultivars. 25(a)
3. Methionine and tryptophan contents of black gram cultivars. 27(a)
4. Polyphenols contents of black gram cultivars. 28(a)
5. Distribution of protein in various Solubility Fractions of black gram (T g). (at dry mature stage) 29(a)
6. Distribution of Protein in various solubility fractions of black gram (UL- 338). (at dry mature stage) 29(b)

LIST OF FIGURES

		Between page
1.	Protein content of black gram cultivars.	21(a) - 22
2.	Phosphorus content of black gram cultivars.	25(a) - 26
3.	Methionine content of black gram cultivars.	27(a) - 28
4.	Tryptophan content of black gram cultivars.	27(a) - 28
5.	Polyphenol content of black gram cultivars.	28(a) - 29

ABSTRACT

SCREENING OF PROMISING BLACK GRAM (*Vigna mungo* (L.) CULTIVARS FOR CHEMICAL COMPOSITION

By

M.N. KAWALE
Mahatma Phule Krishi Vidyapeeth
Rahuri - 413 722
1995

Major professor : Dr. B. B. Desai
Department : Biochemistry

The present investigation was undertaken to study proximate composition, minerals, limiting amino acids, polyphenals and distribution of storage proteins into various solubility fractions of black gram.

The nutritional composition of black gram cultivars ranged from :protein from 22.29 to 27.30 % , crude fat, 1.0 to 1.8 %, crude fibre, 2.6 to 3.25 % and carbohydrate for 54.09 to 59.95 %. The cultivars T9 had higher protein content (27.30 %) than other cultivars.

The mineral composition of black gram cultivars ranged in mg/100g for : phosphorus, 215.5 to 329.2 iron, 6.61 to 12 and zinc 4.12 to 5.4.

The methionine and tryptophan content of black gram cultivars ranged from 0.69 to 0.95 and 0.49 to 0.85 g/16 g N, respectively.

The polyphenol content of the whole seeds of black gram cultivars varied from 950 - 1390 mg/100 g.

The major protein fractions of the black gram cultivar, T9 were : albumin , 19.04%, globulin, 57.21 %, prolamin 2.14 %, glutelin 3.37 %, protein(defatted) 27.3 %, and the recovery of protein was 81.76 % and for cultivar (UL - 338). The values were : albumin, 16.50 %, globulin 59.49 %, prolamin 2.31 %, glutelin 4.25%, protein (defatted) 22.29%, and recovery of protein 82.50 %. The globulin appeared to be the major storage protein fraction contributing 57.21 % and 59.49 % of the protein in T9 and UL-338 cultivars respectively.

Total Pages :- 1 to 46

Chapter Opener Page

Introduction

.1. INTRODUCTION

Food legumes, also called pulses are grown throughout the world and their dietary importance has been globally appreciated and recognized. They are economic sources of proteins, carbohydrates, minerals and B group vitamins particularly to the vegetarian diets (Salunkhe et al., 1985). However, compared to animal proteins, utilization of food legumes in human diet is rather low in the developed countries, whereas these are used extensively in developing countries.

Legume seeds are second only to cereals as a source of human food. Nutritionally, they are 2 to 3 times richer in protein than the cereal grains. The protein quality of most grain legumes however, is considered to be poor due to the deficiency of essential amino acids like methionine and tryptophan (Salunkhe 1982). It is well established that in most food legumes, the methionine and tryptophan are negatively correlated with each other, while the non-protein nitrogen is positively correlated with the protein content (Singh and Jambunathan, 1981). Hence, there is a need to develop cultivars which are rich in methionine, tryptophan and at the same time they are low in non-protein nitrogen to meet the needs of high quality proteins for vegetarian population. These cultivars can also serve as good breeding material for further improvement with respect to quality, yield and other agroclimatic characters.

The importance of pulses in agriculture has been well recognised. They fix atmospheric nitrogen with the help of bacteria and improve soil fertility. The crops belonging to the family Leguminosae are good for crop rotations because they reduce the weed growth, diseases, insect problems and fixed nitrogen can be utilized by the succeeding crop.

India has a distinction of being world's largest producer of grain legumes. About a dozen pulse crops are grown over an area of 23.3 lack hectares adding 12.90 million tons of grains to the food basket of the country (FAO, 1990). The urd or black gram (Vigna mungo (L.)) is an errect spreading annual of Indian and central Asian origin. In India black gram is grown as a dry crop in region with less than 25 inches rain fall.

The area under black gram in Maharashtra state in 1990 was 3,82,400 ha with production of 1,47,300 tons. Considerable work on the germplasm screening and identification of promising material has been carried out in chickpea and pigeonpea.However, such information on other commonly grown legumes like black gram, mung bean, moth bean and horse gram is meagre.

Food legumes are known to contain a variety of substances, which may be considered toxic (growth inhibiting) to animals (Liener,1962). These include phytate, polyphenols and enzymes inhibitors. The occurrence and distribution of these

substances varies in different legumes. However, the information on the presence and nutritional significance of these factors in black gram is inadequate. Keeping in view the above facts, the present investigation on "Screening of promising black gram (*Vigna mungo* (L.) cultivars for chemical composition" was undertaken with the following objectives :

1) To screen 15 promising black gram cultivars for their proximate and chemical composition.

2) To study the limiting amino acids and antinutritional factors (polyphenols) in promising black gram cultivars.

3) To examine the protein quality of 15 promising black gram cultivars.

Chapter Opener Page

Review of Literature

.2. REVIEW OF LITERATURE

In this chapter, an attempt has been made to review the research work related to the proximate composition, minerals, limiting amino acids, polyphenols and protein fractionation of food legumes.

2.1 Proximate composition

The chemical composition of the grain is influenced by many factors such as cultivars, geographic origin, agronomical practices, soil fertility, application of fertilizers and use of chemicals like growth regulators (Gupta and Das, 1955; Dhage et al; 1984).

2.1.1. Moisture

Several workers have reported the moisture content (%) in black gram grains to be 11.2 (Pant and Tulsiani, 1969), 9.7 (Smartt, 1976), 10.9 (Gopalan et al; 1977), 10.6 (Doughty and Walker; 1982), 10.2 (Dhage, 1983) and 11.0 (Chaudhary and Singh, 1991)

2.1.2. Ash

The ash content is important from the nutritional point of view as it indicates the level of mineral matter in the seed.
Ash content (%) in black gram seed has been reported by several workers to be 3.4 (Pant and Tulsiani, 1969), 3.2-3.5 (Shobhana et al; 1976) 4.8 (Smartt, 1976) 3.4 (Gopalan et at; 1977), 3.37-3.4

(Dhage, 1983) and 3.41 (Chaudhary and Singh, 1991).

2.1.3. Protein content

The protein content in black gram has been found to be significantly influenced by genetic strain and environmental conditions (Chatterjee and Abrol, 1975, Mahadevappa and Raina, 1978 and Yadav and Tomer, 1985). Gopalan et al. (1977) reported that considerable genetic variation existed among the cultivars in their protein and amino acid content. These reports clearly indicate the possibility of obtaining high protein lines in black gram through germplasm screening.

The protein content in black gram has been studied by several investigators. The protein content upto 24% was reported by (Venkatrao et al; 1964, and Gopalan et al; 1977). Swaminathan and Jain (1972) reported that the protein content ranged from 21.2 to 31. % in 91 black gram cultivars. Shobhana et al. (1976) have studied some improved varieties of black gram for protein content which ranged from 23.2 to 27.4%. Smarrt (1976) reported 23.4% protein content while Doughty and Walker (1982) reported 21% in black gram. Salunkhe et al. (1985) observed a range of 21.2 to 31.3% protein Mehta and Singh (1989) found a range of 20.6 to 31.1% ,while Chaudhary and Singh (1991) found 24% Protein in black gram cultivars.

2.1.4 Lipids

Several workers have reported lipid content (%) in black gram to be 1.4 (Anonymous, 1970; Gopalan et al, 1977; Chaudhary and Singh 1991,) 1.69 (Pant and Tulsiani, 1969), 1.0 (Smartt 1978) and 1.6 (Doughty and Walker 1982). The total lipid content of thirteen genotypes of black gram ranged from 0.57 to 1.4% (Gupta, 1990), while Shobhana et al. (1976) observed that black gram seed contained 2.6 to 3.0% total lipids.

Legumes constitute 46-52% total lipids, 17-32% neutral lipids, 35-40% phospholipids and 10-12% glycolipids as fatty acid component. In all legumes, neutral lipids resembled phospholipids in fatty acid composition, but this was not so in black gram. Black gram carried high proportion (33-60%) of linolenic acid in all lipid classes while the other legume lipids were rich in linoleic acid (30-60%) (Mahadevappa and Raina, 1978)

2.1.5 Crude Fibre

The involvement of dietary fibre in lowering the blood cholesterol level has been reported. Cellulose is the least digestible component of dietary fibre and has a direct effect on the utilization of other nutrients (Ali et al, 1981).

Crude fibre content (%) of black gram seeds has been reported by several workers to be 3.6 to 4.4 (Shobhana et al, 1976), 3.8 (Smartt, 1976), 0.9 (Gopalan et al, 1977) 4.4; (Doughty and Walker, 1982). The crude fibre content of thirteen genotype of black gram ranged from 3.98 to 4.95% (Gupta, 1990)

2.1.6 Carbohydrates

Food legumes are good sources of dietary carbohydrates. Most legumes contain 50 to 60% carbohydrates, Black gram contains about 60% carbohydrates, including soluble sugars and oligosaccharides. Black gram seeds contained sucrose 0.7-1.5%, Raffinose 0-13% Stachyose 0.9-3.0% and Verbascose 3.4-3.5% (Reddy, et al.).

Carbohydrate content of black gram was 51.68% (Pant and Tulsiani 1969) 57.3%, (Smartt. 1976) 59.6%; (Gopalan et al. 1977) 63.4%, (Doughty and Walker, 1982) and 59.15% (Dhage 1983).

2.2 Minerals

Like most other legumes, black gram is a good source of dietary minerals such as calcium, phosphorus, iron, zinc and magnesium. The iron availability is highest in chickpea as compared to other legumes. (Cowan et al., 1967). The components such as phytic acid, oxalic acid, proteins, polyphenol and complex polysaccharides such as starch, fibre and lignin are known to react with minerals altering their bio availability. The information on the bio availability of minerals and their interactions with other nutrients in pulses is meagre (O' Dell, 1969).

The dietary importance of minerals is well recognised. Phosphorus is an essential constituent of every known tissue and cell in the body. Zinc was found to act as a cofactor in a variety of enzyme system. Zinc is also concerned with the

fundamental process of RNA and protein synthesis, while iron containing compounds, haemoglobin (heam protein) and myoglobin play vital role in oxygen transportation (Encyclopaedia of Food Science, Food Technology and Nutrition, 1993)

Gopalan et al, (1977) showed that in black gram cultivar Ca, P, Fe, Mg, Cu and Mo content was 154.4, 9.1, 185, 0.72 and 39.8 mg/ 100 g dry matter, respectively. The total mineral content of black gram is about 3.4% (Gopalan et al; 1977 and Doughty and Walker, 1982.). The mineral composition of black gram as reported by different workers is calcium, 131 mg; phosphorus, 395 mg, Iron, 7.1 mg/100g (Pant and Tulsiani, 1969). calcium, 161 to 230 mg, phosphorus, 332 to 397 mg, iron, 6.61-6.87 mg/100g (Shobhana et al, 1976); calcium, 76-154 mg, phosphorus, 385-560 mg, iron, 6.6-10.7 mg, Zn, 3.5 mg/100g (Reddy and Salunkhe 1980), calcium, 153.7 mg, phosphorus, 210 mg, iron, 12 mg and zinc, 5.4 mg/100g (Dhage, 1983), Holland, B et al; (1991) observed 11.1 mg/100 g iron in lentil.

2.3 Limiting amino acids

The total and limiting amino acid composition of black gram has been studied by several investigators. The values available in the literature for methionine content range from 0.97 to 1.57 g/16 gN (Vijayaraghavan and Srinivasan, 1953, Venkatrao and Leela, 1964, Hanumantrao and Subramanium, 1970; Gopalan et al, 1977, Kapoor et al; 1972; Shobhana et al; 1976; Chavan and Djurtoft, 1982, Dhage, 1983;) The value for tryptophan content ranged from 0.4 to 1.31 g/16 g N. (Vijayaraghavan and Srinivasan, 1953. Gopalan et al; 1971, Chatterjje and Abrol, 1975, Shobhana

et al, 1976 Chavan and Djurtoft, 1982, Dhage, 1983) These reports indicate that black gram proteins are deficient in sulfur containing amino acid and tryptophan (Hanumantrao and Subramanium, 1969). Black gram and green gram were found to be relatively better balanced in their amino acid composition. The black gram proteins are rich in lysine which is usually found deficit in most cereals.

Application of sulfur to black gram has been found to increase the methionine content significantly (Arora and Luthra, 1971). The correlation of both tryptophan and methionine in legume proteins is generally negative (Salunkhe, et al, 1985.), Hence, for increasing the protein content in most legumes by genetic or other manipulations, attention must be given to maintain the concentration of sulfur amino acids. Attempts are needed to increse methionine and tryptophan contents in legume proteins through proper breeding or biochemical manipulations.

2.4 Polyphenols

Polyphenols or tannins are generally defined as soluble, astringent complex phenolic substances of plant origin. The phenolic compounds are widely distributed in plants contributing to flavour and colour associated with legumes, cereal grains and oil seeds (Salunkhe et al; 1985). In recent years, polyphenols, have received increasing attention because of their influence on nutritional and aesthetic quality of food and biochemical physiological and pharmacological functions (Salunkhe et al; 1982). Polyphenols have been known to interact and form com-

plexes with proteins, minerals and vitamins and thereby reduce their bioavailability (Schaffert et al, 1974; Suscheft, 1975; Fishman and Neucere, 1980). Suscheft (1975) found a reduction in liver vitamin 'A' as a result of inclusion of 31.2% tannic acid in the diet.

The protein binding nature of polyphenols is known to inhibit the activities of several enzymes such as cellulase (Smart et al; 1961), beta-galactosidase (Goldstein and Swain, 1965) Trypsin (De lumen and Salamat, 1980). Inhibition of digestive enzymes like trypsin, lipase and amylase by polyphenols leads to the decreased digestibility of dietary proteins and carbohydrates (Salunkhe et al; 1982).

Polyphenols have been reported to depress the growth rate of rats and chicks and lower the protein and dry matter digestibility (Schaffert et al; 1974; Bressani and Elias, 1979) High level of tannic acid in diet has been reported to cause fatty liver in chicks (Change and Fuller, 1964). decrease blood haemoglobin in rats (Lease and Mitchell, 1940). Prolonged consumption of such compounds has been implicated in occurrence of oesophegeal cancer in many areas of the world (Morton, 1970). Dhage (1983) reported 1.22% polyphenols in black gram wheeas Rao and Deosthale (1986) found 0.54 to 1.19% polyphenols in black gram.

2.5 Protein fractonation

The special contribution of grain legumes to the human diet lies in the quantity and quality of the protein. Storage protein in legumes (globulin) account for about 80% of the seed nitrogen, and are usually classified as legumins or vicilins. Legumins are larger, less soluble in salt solution and more heat stable than vicilins. (Encyclopaedia of Food Science,Food Technology and Nutrition,1993).Black gram seed proteins have been fractionated into various solubility fractions viz, albumin (water soluble), globulin, (salt soluble), prolamin (alcobol soluble) and glutelin (alkali/acid soluble).

Padhay and Salunkhe (1977) fractionated black gram proteins into four fractions (albumin, globulin, prolamin and glutelin) Globulin amounted to about 81%, whereas albumins were 13%. Two minor fractions viz prolamins and glutelins, accounted for about 4 & 2% respectively. Venugopal and Rao (1978) reported the following proportion of solubility classes of black gram proteins: albumins, 24% globulins, 46.2%; prolamin, 0.9%; glutelin, 4.3% and 24.6% residual proteins.Shinde (1993) reported the following proportion of solubility classes of fieldpea: albumin, 12.97%; globulin, 56.24%; prolamin, 3.39%; glutelin, 17.57% at dry mature stage.

Chapter Opener Page

Material and Methods

3. MATERIAL AND METHODS

3.1 Materials

3.1.1 Seeds

Seeds of following fifteen promising cultivars of black gram were obtained from the Sr Pulse Breeder, Pulse Improvement Project Mahatma Phule Krishi Vidyapeeth, Rahuri.

Sr.No.	Cultivars
1.	Phule U-3
2.	TPU 3
3.	TPU 4
4.	TAU 2
5.	TAU 5
6.	VB-3
7.	UL-338
8.	DPU-88-1
9.	DPU-88-9
10.	DPU-88-31
11.	DPU-89-6-7
12.	Pant U-19
13.	Pant U-30
14.	T-9
15.	Sindakheda 1-1

These seeds were cleaned, ground to 60 mesh and stored at 4°C until used for analysis.

3.1.2 Chemicals

Most of the chemicals used in this investigation were of analytical grade. They were obtained from M/s Glaxo Laboratories, Bombay., Sarabhai M. Chemicals, Baroda, BDH, Qualigens Fine chemical and E. Merk (India), Bombay. DL-methionine was obtained from Sisco Research Laboratory, Bombay, while L-tryptophan was obtained from Loba-chemicals, Bombay.

3.2 Methods

3.2.1 Proximate composition

Moisture, ash, crude fat (ether extract) and total nitrogen (Kjeldhal's N) were determined accourding to the standard procedures of A.O.A.C. (1980). The % protein content was calculated by multiplying % N by 6.25.

3.2.2 Minerals

3.2.2.1 Phosphorus

Phosphorus in the meal was determined by the colorimetric method as described by Chapman and Pratt (1961).

Reagents :

1. Ammonium molybdovanadate solution

-: 22.5g ammonium molybdate in 400 ml distilled water, 1.25g ammonium vanadate in 300ml boiling distilled water and 250ml conc nitric acid were mixed together and volume was made to 1 liter with distilled water.

2. Standard Orthophosphate solution

-: 0.2194g potassium dihydrogen phosphate were dissolved in distilled water and diluted to 1 litre. This solution contained 50 µg p/ml.

Standard curve:

Standard working solution of 0,5,10,15,20 and 25 ml were taken in 50 ml volumetric flask and 10 ml ammonium molybdovanadate reagent was added. The contents were diluted to 50ml with distilled water and mixed well. The absorbance was read after 30 min at 470nm on a spectrophotometer (Spectronic 20).

*** Phosphorus in sample**

The digested sample (10ml) was taken in 50ml volumetric flask and 10ml ammonium molybdovanadate reagent was added. The contents were diluted to 50 ml and mixed well. The absorbance was read after 30 min at 470 nm on a spectrophotometer (Spectronic 20).

3.2.2.2 Iron and Zinc

The iron and zinc were estimated by atomic absorption method as described by Parkinson and Allen (1975).

3.2.3 Limiting amino acids.

3.2.3.1 Methionine

This amino acid was estimated by the chemical method based on McCarthy and Paille (1959).

Reagents :

1. 2N Hydrochloric acid.
2. 10% Sodium hydroxide.
3. 10% Sodium nitroprusside.
4. 10N Sodium hydroxide.
5. 3% glycine.
6. Concentrated phosphoric acid.
7. Standard methionine solution 100 mg DL. methionine was dissolved in 0.5 ml of 20% hydrochloric acid and diluted to 100 ml with distilled water. This solution contained 1 mg DL-Methionine/ml.

Extraction :

Methionine was extracted accourding to the method described by Gupta and Das (1955). Two grams of defatted sample was autoclaved with 25 ml of 2N HCl at 15 lb pressure for one hour. The hydrolysate was treated with a pinch of cultivated charcoal to get rid of colour, it was then heated to boiling and filtered. The charcoal was washed 3-4 times with hot water quickly and the washing were collected. The colour free extract was neutralised with 10 N NaoH to bring the pH to about 6.5. The volume was made to 200 ml.

Colour Development :

The colour was developed according to the method described by McCarthy and Paille (1959). Fifty ml of extract were taken in 250 ml conical flask and 6 ml of 10% NaoH were added. This was followed by addition of 0.3 ml of sodium nitro-

prusside and content were kept for 10 min with occasional shaking. After 10 min, 2 ml of glycine were added, shaken well and allowed to stand for 10 min. Four ml of concentrated orthophosphoric acid were added, shaken vigorously and colour intensities were measured after 10 min on Spectronic 20 at 540 nm.

Standard Curve :

For a standard curve different concentration of methionine (2,4,6 and 8 mg) were taken in duplicate, water was added to make up the volume to 50 ml. The colour was developed in the same way as described for samples. The methionine content in sample was calculated from standard curve and expressed as per cent on basis in moisture free meal and g/16g N.

3.2.3.2 Tryptophan

Tryptophan in the sample was determined by the colorimetric method of Spice and Chambers (1949).

Reagents :

1. 19 N Sulphuric acid.
2. 20.4 N Sulphuric acid.
3. 0.045% Sodium nitrate.
4. Para-dimethyl amino benzaldehyde.
5. Standard tryptophan solution.

L-tryptophan (20 mg) were dissolved in 100 ml distilled water (2 drops of 10% NaoH were added to solubilize tryptophan). This solution contained 200 µg L-tryptophan/ml.

Tryptophan in sample :

The ground defatted samples (40 mg) were taken in 50 ml conical flasks in triplicate and 30mg of para-dimethyl amino benzaldehyde were added to each flask. After keeping the flask in dark for 20 hrs at 30°C, 0.1 ml of $NaNO_2$ was added to each flask except in blanks, shaken gently and kept in dark for 30 min for colour development. The content were filtered through glass wool and the colour intensities were read at 600 nm on Spectronic-20.

Standard Curve :

The standard tryptophan solution (0-120 ug) was taken in 50 ml conical flasks in triplicate. Water was added to make up volume to 0.5 ml in each flask, followed by 9.4 ml of 20.4 NH_2SO_4 to each flask in order to make final strength of the acid 19 N. The p-dimethyl amino benzaldehyde (30 mg) were then added to each flask and flasks were kept in dark for 20 hrs at 30°C. Rest of the procedure was the same as described for samples. The tryptophan content was determined from the reference standard curve after making the necessary correction for the blank values of each sample. Tryptophan was expressed as per cent in meal and g/16g N.

3.2.4 Antinutritional factors

3.2.4.1 Polyphenols

The polyphenols in sample were determined by the method of A.O.A.C. (1980).

Reagents :

1. Folin-Denis reagent

One hundred g of sodium tungstate, 20 g of phosphomolybdic acid and 50 ml of concentrated phosphoric acid were added to 50 ml distilled water. The mixture was allowed to reflux for two hours on hot water bath and volume made to one litre with distilled water.

2. Alkaline reagent

Three hundred fifty g of sodium carbonate was dissolved in 1 litre of distiled water at 80°C and allow to stand overnigh at room temperature. It was then filtered throught glas wool.

3. Standard tannic acid solution (0.1%)

One hundred mg of tannic acid were dissolved in 100 ml distilled water.

Procedure :

One gram of 60 mesh ground sample was weighed into four 500 ml conical flasks and 150 ml water were added to each flask. Internal standards were prepared by adding 10, 15, 20 ml standard tannic acid in first three flasks and fourth flask was left as control. The contents in conical flask were gently heated and boiled for 30 min and centrifused at 5000xg for 20 min. The supernatent was collected in 250 ml volumetric flask and volume was adjusted with distilled water.

Colour development :

An aliquot of supernatant (10 ml) was taken in 100 ml flask to which 75 ml water, 2.5 ml Folin-Denis reagent and 5 ml standard sodium carbonate were added and volume was made upto 100 ml and allowed to stand for 30 min. The optical density was read at 740 nm. A standard curve was prepared plotting optical density versus tannic acid concentrations. Optical density of the control whenever it cuts Y axis was taken as new origin. The difference in optical density in first and second origin was taken as measure of tannic acid content in the sample, and the results expressed as tannic acid equivalent.

Chapter Opener Page

Results and Discussion

3.2.5 Protein fractionation

The proteins were fractioned by the Osborne method as modified by Nagy et al. (1941). The defatted flour was further ground in a glass morter and pestle so as to pass through 100 mesh sieve. The defatted sample was weighed into 50 ml capped centrifused tube and successively extracted (four shaking, each for one hour) with 100 ml of the following solvents : Distilled water for albumin+ globulin A, 5 percent w/v sodium chloride (for globulin), 70 percent ethanol v/v for prolamin and 2% sodium hydroxide for glutelin were used as solvents. To separate globulin a from Albumin, 20 ml of water soluble fraction was dialysed against distiled water. (4 changes, 5 lit each) at 4°C for 30 hours and centrifused. The albumins were obtained in the supernatant while globulin A in the precipitate (Pant and Tulsiani, 1969) The nitrogen in all the fractions was determined by microkjeldahl method of A.O.A.C. (1980) and protein per cent was calculated by N% x 6.25.

3.2.6 Statistical analysis

The data obtained for proximate composition, minerals, limiting amino acids and antinutritional factors in sample were statistically analysed by using the completely randomised design (CRD), as described by Panse and Sukhatme (1967).

4. RESULT AND DISCUSSION

Fifteen promising cultivars of black gram were analysed for their proximate composition, mineral contents, two limiting amino acids viz; methionine and tryptophan, polyphenols and major protein fractions. At dry maturity stage of black gram, the protein content of the grain was separated into Osborne solubility fractions. The results of these studies are presented and discussed under the following main headings :

4.1 Proximate composition

4.2 Mineral content

4.3 Limiting amino acids

4.4 Polyphenols

4.5 Protein fractionation

4.1 Proximate composition :

Fifteen promising black gram cultivars were analysed for the proximate composition of the whole seed at the dry maturity stage. The results obtained are presented in Table 1.

4.1.1 Moisture :

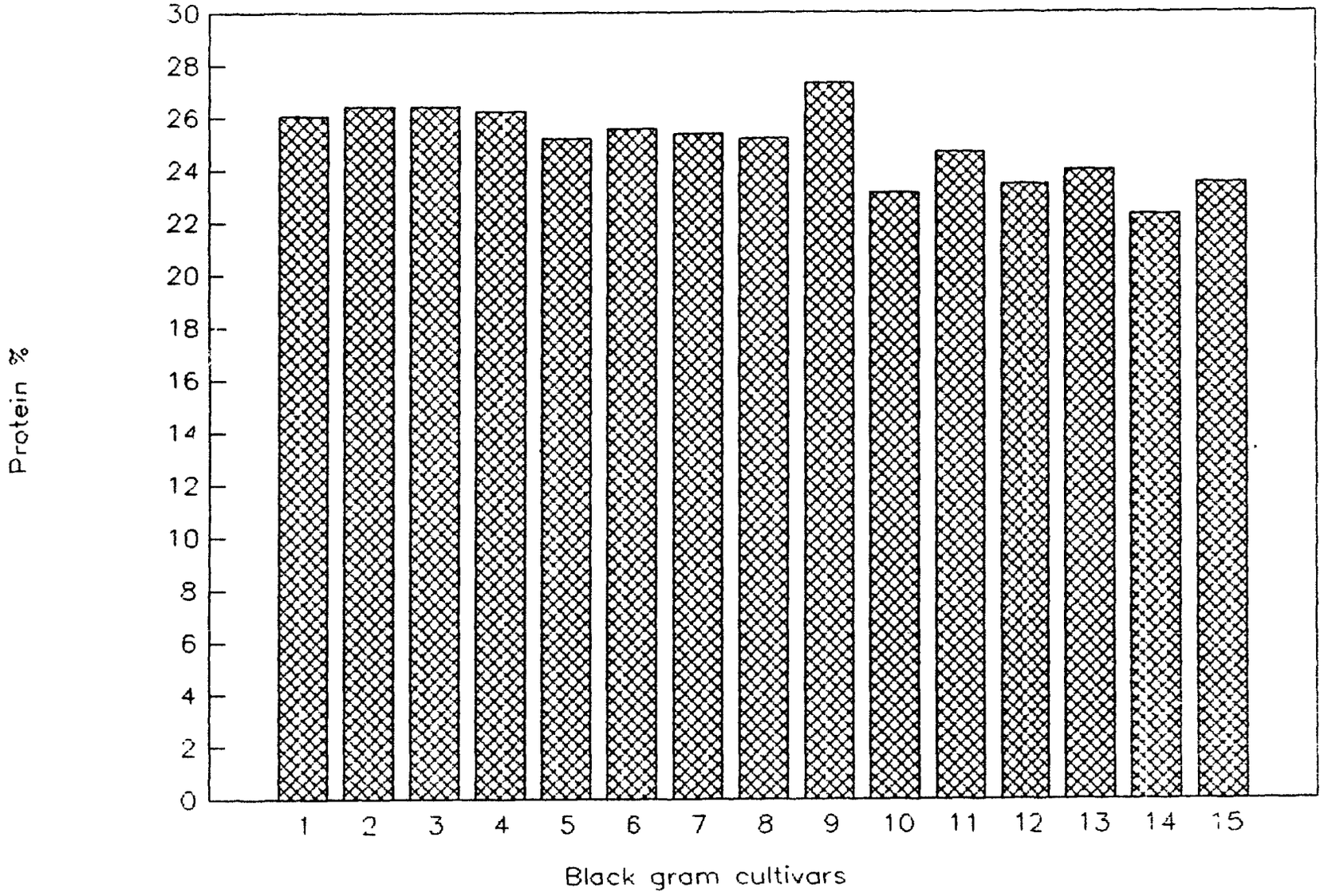
Moisture content of 15 black gram cultivars studied, ranged from 9.0 to 10.4% with a mean of 9.64 percent. The cultivars Pant-U-30 had the highest moisture (10.4 %), followed by

TABLE - 1

Proximate composition of black gram cultivars

Sr. No.	Cultivars	Moisture %	Ash %	Crude fat %	Crude protein %	Crude fibre %	Carbohydrates %
1.	DPU 88-1	10.0	3.2	1.6	26.07	2.95	56.18
2.	DPU 88-9	9.2	3.86	1.43	26.42	3.25	55.93
3.	DPU 88-31	10.1	3.23	1.40	26.42	3.05	55.83
4.	DPU 89-6-7	10.1	3.6	1.60	26.25	3.05	55.04
5.	Pant U-19	9.4	3.8	1.20	25.20	2.6	57.80
6.	Pant U-30	10.4	4.0	1.26	25.55	2.9	55.95
7.	Phule U-3	9.2	3.0	1.20	25.37	3.1	58.13
8.	S-1-1	9.3	3.6	1.23	25.20	2.7	58.0
9.	T9	9.1	4.0	1.8	27.30	2.9	54.09
10.	TAU-2	9.0	3.86	1.0	23.10	3.15	59.95
11.	TAU-5	9.4	3.8	1.8	24.67	3.1	57.23
12.	TPU-3	10.2	4.2	1.41	23.45	3.0	57.75
13.	TPU-4	10.2	3.0	1.60	23.97	2.8	55.98
14.	UL-338	9.6	2.8	1.40	22.29	2.9	58.43
15.	VB-3	9.4	3.2	1.46	23.5	3.15	59.35
MEAN		9.64	3.54	1.43	24.98	2.97	57.06
SE		0.58	0.246	0.075	0.427	0.1348	0.114
CD at 5%		NS	0.714	0.218	1.23	0.390	0.332

fig-1 Protein content of black gram cultivars



Cultivars.

1. *DPU 88-1*
2. *DPU 88-9*
3. *DPU 88-31*
4. *DPU 89-6-7*
5. *Pant U-19*
6. *Pant U-30*
7. *Phule U-3*
8. *S 1-1*
9. *T - 9*
10. *TAU - 2*
11. *TAU - 3*
12. *TPU - 3*
13. *TPU 4*
14. *UL - 338*
15. *VB - 3*

TPU-3 and TPU-4 (10.2%). Whereas the lowest moisture was found in TAU-2 (9.0%). However, there was no significant difference in the moisture content of different cultivars (Table 1).

The reported values of moisture content were 10.9% (Gopalan et al, 1977), 10.6% (Doughty and Walker, 1982), 10.2% (Dhage, 1983). The data obtained in this study conform with these reports.

4.1.2 Ash :

The total ash content of black gram cultivars at the dry maturity stage ranged from 2.8% to 4.2%, with a mean value of 3.53%. The cultivar TPU-3 had the highest ash content (4.2%), followed by Pant-U-30 and T9 (4.0%). The lowest ash content was found in UL-338 (2.8%).

Total ash content in black gram seed have been reported by several workers which ranged from 3.0 to 4.8 percent (Shobhana et al; 1976, Smartt, 1976, Dhage; 1983, Chaudhary and Singh 1991).

4.1.3 Crude protein :

The crude protein content of 15 black gram cultivars at the dry mature stage ranged from 22.29 to 27.30 percent with a mean value of 24.98 percent. The cultivar T9 had the highest protein content (27.3 percent), followed by DPU 88-9 and DPU 88-31 (26.42 percent); The lowest protein content was in UL-338 (22.29 percent). The varietal difference in protein content of

black gram seed was significant.

Yadav and Tomer (1985) found that protein content in black gram was significantly influenced by the genetic strain and environmental conditions. The reported values for the protein content (%) in black gram are : 24 (Gopalan et al. 1977), 21.2 to 31.1 (Swaminathan and Jain, 1972), 23.2 to 27.4 (Shobhana et al, 1976) 21.2 to 31.3 (Salunkhe et al, 1985) 20.6 to 31.1 (Mehta and Singh, 1989). The values obtained in the present study conform to these reports.

4.1.4 Crude fat :

The crude fat content of the grain of 15 black gram cultivars at the dry maturity stage ranged from 1.0 to 1.8 percent with a mean value of 1.41 percent. The cultivars T9 and TAU-5 had the highest fat content (1.8 per cent) followed by TPU-4 and DPU 88-1 (1.6 per cent). The lowest value was in cultivar TAU-2 (1.0 per cent). The reported values of crude fat in black gram are : 1.4 per cent (Gopalan et al 1977, Chaudhary and Singh 1991). 1.69 per cent (Pant and Tulsiani, 1969), 1.0 per cent (Smartt 1976). Gupta (1990) reported that total lipid content of thirteen genotypes of black gram ranged from 0.57 to 1.4 percent. The values obtained in this study appear to be slightly higher than the reported values.

4.1.5 Crude fibre :

The crude fibre content of black gram cultivars at the dry mature stage ranged from 2.6 to 3.25 per cent, with a mean value of 2.97 per cent. The cultivars DPU 88-9, TAU-2, VB-3 had the highest crude fibre content (3.25 and 3.15 respectively), followed by Phule-U-3, TAU-5, DPU 88-31 and DPU 89-6-7, (3.1, 3.1, 3.05 per cent respectively). Cultivar Pant-U-19 had the lowest crude fibre content of 2.6 per cent.

Crude fibre content of black gram seeds has been reported by several workers.(%) 3.8 (Smartt, 1976); 0.9 (Gopalan et al. 1977); 4.4 (Doughty and Walker, 1982) and 3.98 to 4.95 (Gupta, 1990). The values for crude fibre content observed in the present study are within the ranges reported earlier

4.1.6 Total carbohydrates :

The total carbohydrate content of 15 black gram cultivars at the dry mature stage ranged from 54.09 to 59.95 per cent with a mean value of 57.06 per cent. The cultivars TAU-2 and VB-3 had the highest content of total carbohydrates. (59.95 and 59.35 per cent, respectively), followed by UL-338 and phule U-3 (58.43 and 58.13 % respectively). The cultivar T9 had the lowest carbohydrates(54.09 %). There was significant varietal difference in carbohydrate content of the black gram cultivars studied.

Carbohydrate content (%) in blackgram reported by earlier workers (%) are: 51.68 (Pant and Tulsiani, 1969) 57.3 (Smartt,1976) 59.6 (Gopalan et al , 1977) 63.4 (Doughty and Walkar

, 1983) 60 (Chaudhary and Singh, 1991). The values of carbohydrates content obtained in the present study conform to these reports.

4.2. Minerals :

Fifteen black gram cultivars were also analysed for their important mineral content (P, Fe, Zn) of the whole seed at the dry mature stage. The results obtained are presented in Table 2.

4.2.1 Phosphorus :

The phosphorus content of black gram cultivars ranged from 215.5 to 329.2 mg/100g, with a mean value of 264.8 mg/100 g. Among 15 cultivars tested, T9 had the highest phosphorus content (329.2 mg/100g), followed by Pant U-19, TPU-4, (323.3 mg/100 g). The lowest being found in TAU-5 (215.5 mg/100 g).

The reported values for the phosphorus content (mg/100g) in black gram are as followes: 385 (Gopalan et al; 1977) 385 to 560 (Reddy and salunkhe, 1980): 210 (Dhage, 1983) and 400 (Chaudhary and Singh, 1991) The values of phosphorus content for 15 black gram cultivars obtained in this study are relatively lower than those reported earlier.

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ABSTRACT DIGITIZED

Table.2 Phosphorus,iron,zinc content of black gram cultivars

Sr. No.	Cultivars	Minerals (mg/100g)		
		Phosphorus	Iron	Zinc
1.	DPU 88-1	274.29	10.7	4.5
2.	DPU 88-9	286.05	7.90	4.59
3.	DPU 88-31	274.29	7.01	4.47
4.	DPU 89-6-7	280.17	6.61	4.37
5.	Pant U-19	323.28	8.87	5.2
6.	Pant U-30	309.56	8.1	4.5
7.	Phule U-3	309.56	12.0	5.15
8.	S-1-1	286.05	10.7	4.27
9.	T9	329.15	11.6	5.4
10.	TAU 2	221.39	7.01	4.47
11.	TAU 5	213.51	6.61	4.70
12.	TPU-3	244.91	9.1	4.35
13.	TPU-4	323.28	6.87	4.7
14.	UL-338	291.93	7.71	4.47
15.	VB-3	250.78	9.1	4.12
	MEAN	264.89	8.66	4.61
	SE	18.33	1.41	0.169
	CD at 5%	53.13	4.09	0.491

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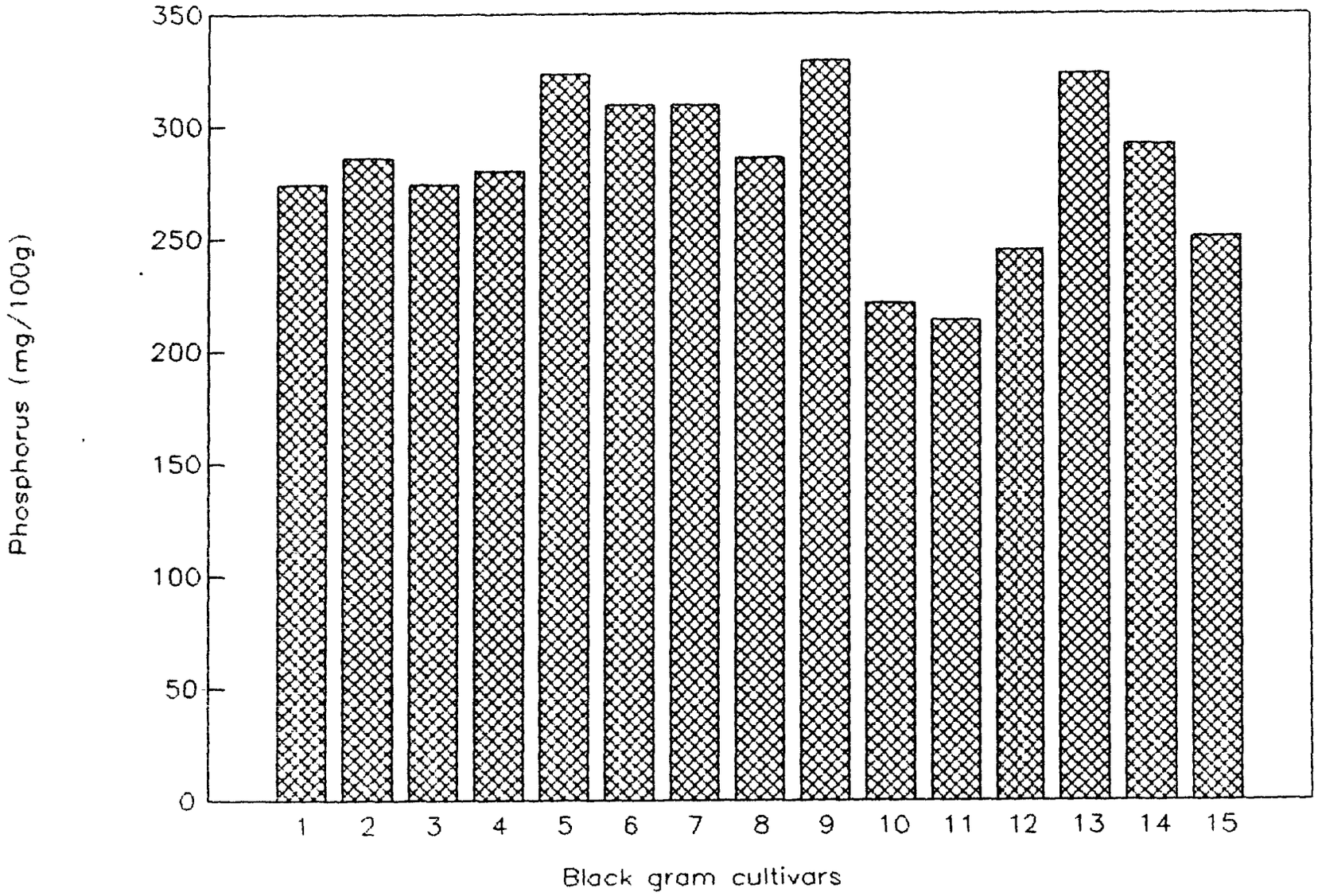
fig-2 Phosphorus content of black gram cultivars



Cultivars.

1. *DPU 88-1*
2. *DPU 88-9*
3. *DPU 88-31*
4. *DPU 89-6-7*
5. *Pant U-19*
6. *Pant U-30*
7. *Phule U-3*
8. *S 1-1*
9. *T - 9*
10. *TAU - 2*
11. *TAU - 3*
12. *TPU - 3*
13. *TPU 4*
14. *UL - 338*
15. *VB - 3*

4.2.2 Iron :

The iron content of black gram cultivars ranged from 6.61 to 12 mg/100 g, with a mean value of 8.66 mg/100 g. Among fifteen cultivars tested, Phule U-3 had the highest iron content (12 mg/100g) followed by Tg, S-1-1, and DPU 88-1 (11.6, 10.7, 10.7, respectively). The lowest value was found in DPU 89-6-7 and TAU-5 (6.61 mg/100 g).

The reported values for iron content (mg/100g) in black gram are as followes, 9.1 (Gopalan et al;) 6.6 to 10.7 (Reddy and Salunkhe, 1980) 12 to 13.37 (Dhage, 1983) The value of iron content obtained in the present study conform to these reports.

4.2.3 Zinc :

The zinc content of black gram cultivars ranged from 4.12 to 5.4 mg/100 g with a mean value of 4.61 mg/100g. The cultivar T9 had the highest zinc content (5.4 mg/100g), followed by Pant U-19 and Phule U-3 (5.2, 5.15 mg /100g, respectively). The lowest value was found in VB-3(4.12 mg/100g).

Reported values for zinc content in black gram (mg/100g) are as followes. 3.5 (Reddy and Salunkhe, 1980) and 5.4 (Dhage, 1983). The values for zinc content observed in the present study are within the ranges reported earlier.

4.3 Limiting amino acids :

Methionine is the first limiting amino acid and tryptophan is the second one in most of the grain legumes including black gram. The contents of these two amino acids were therefore determined in the different black gram cultivars and the results are presented in Table 3.

4.3.1 Methionine :

The methionine content in protein ranged from 0.69-0.95 g/16 gN, with a mean value of 0.83 g/16 gN. Amoung 15 cultivars tested, Pant U-19 had the highest methionine content of 0.95g/16gN followed by TPU-3 and VB-3 (o.94g/16g N). The lowest value was found in DPU 88-1 (0.69 g/16 gN).

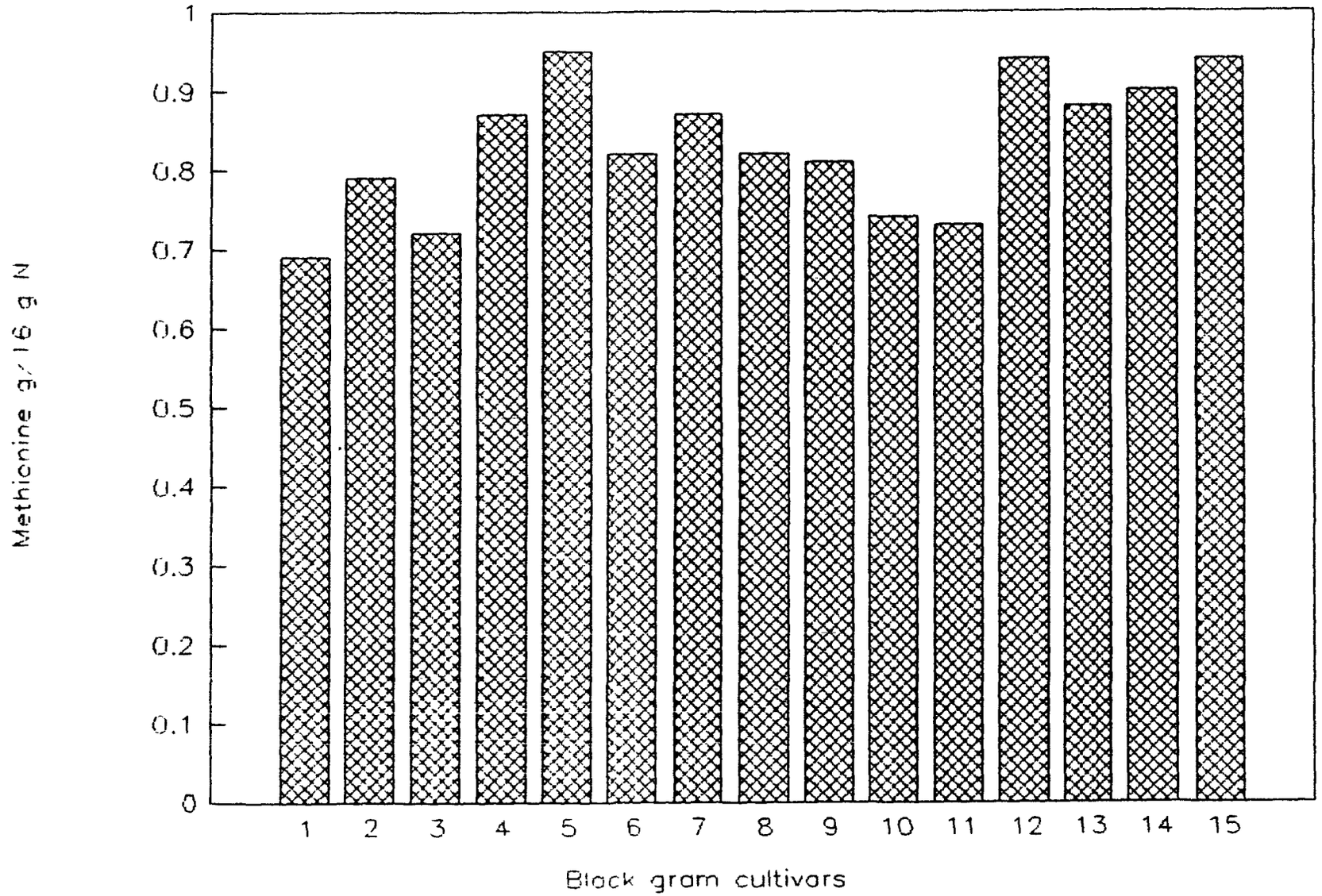
The reported values of methionine content (g/16 gN) in black gram are as followes: 1.1 (Vijayaraghavan and Srinivasan, 1953 and Venkatrao et al, 1964), 1.44 (Gopalan et al, 1971); 0.97-1.1 (Shobhana et al, 1976) and 1.41-1.52 (Dhage, 1983). The values of methionine content recorded in the present study are fairly in agreement with those reported earlier though they are slightly towards lowerside.

Table.3

Methionine and tryptophan content of black gram cultivars

Sr. No.	Cultivars	Tryptophan g/16 g N	Methionine g/16 g N
1.	DPU 88-1	0.49	0.69
2.	DPU 88-9	0.56	0.79
3.	DPU 88-31	0.53	0.72
4.	DPU 89-6-7	0.49	0.87
5.	Pant U-19	0.51	0.95
6.	Pant U-30	0.47	0.82
7.	Phule U-3	0.75	0.87
8.	S-1-1	0.51	0.83
9.	T9	0.62	0.81
10.	TAU-2	0.56	0.74
11.	TAU-5	0.73	0.73
12.	TPU-3	0.59	0.94
13.	TPU-4	0.75	0.88
14.	UL-338	0.85	0.90
15.	VB-3	0.64	0.94
	MEAN	0.60	0.83
	SE	0.054	0.055
	CD at 5%	0.156	0.158

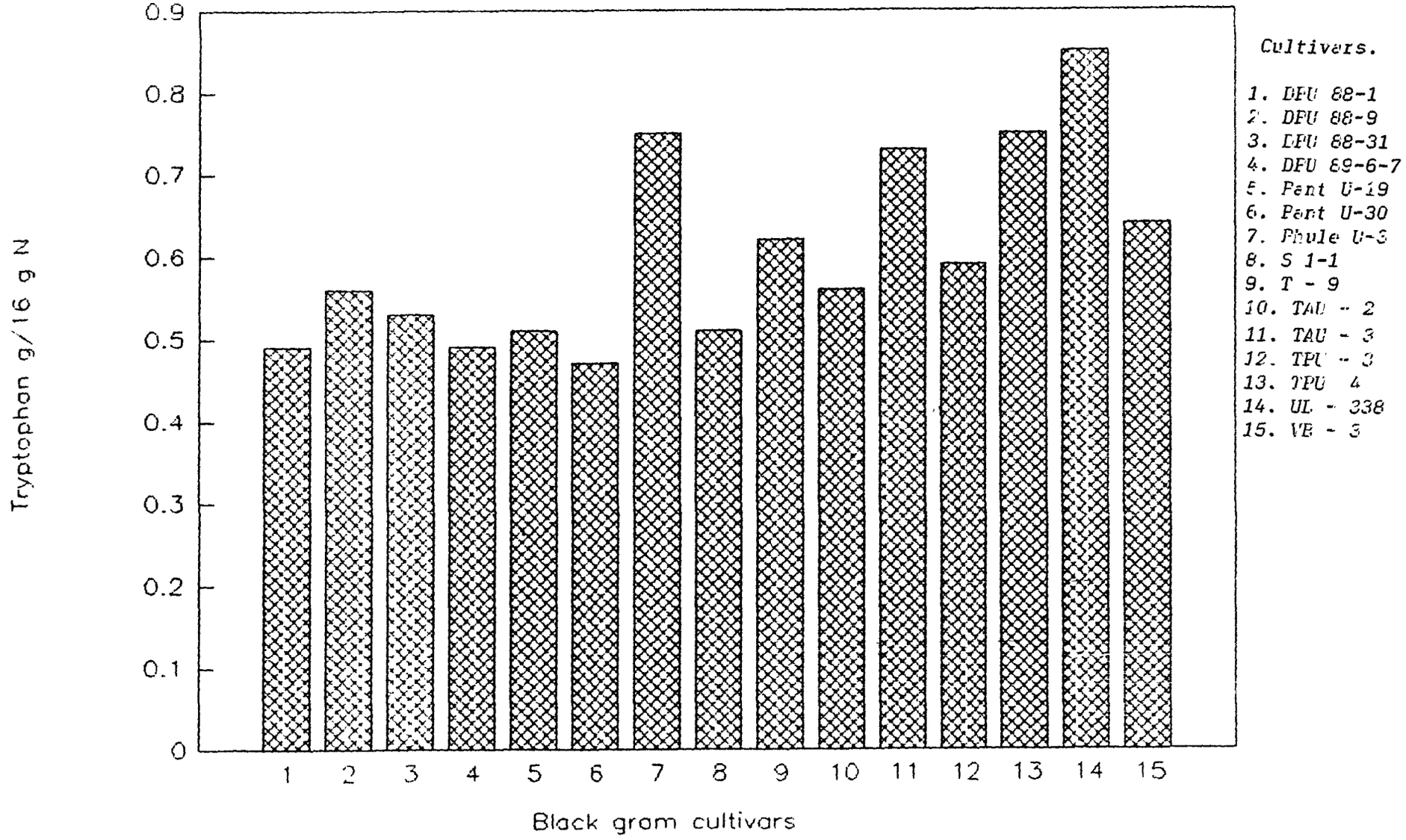
fig-3 Methionine content of black gram cultivars



Cultivars.

1. *DPU 88-1*
2. *DPU 88-9*
3. *DPU 88-31*
4. *DPU 89-6-7*
5. *Pant U-19*
6. *Pant U-30*
7. *Phule U-3*
8. *S 1-1*
9. *T - 9*
10. *TAU - 2*
11. *TAU - 3*
12. *TPU - 3*
13. *TPU 4*
14. *UL - 338*
15. *VB - 3*

fig-4 Tryptophan content of black gram cultivars



4.3.2 Tryptophan :

The tryptophan content in protein ranged from 0.49 to 0.85 g/16 gN, with a mean value of 0.60 g/16 gN. Amoung 15 cultivars tested, UL-338 had the highest tryptophan content of 0.85 g/16 gN, followed by Phule-U-3 and TPU-4 (0.75 g/16gN), the lowest being found in DPU-88-1 and DPU 89-6-7 (0.49 g/16 gN)

The reported values for tryptophan content (g/16gN) in black gram are as followes.0.5 (Vijayaraghavan and Srinivasan 1953); 1.12 (Gopalan et al, 1971); 0.62-0.79 (Shobhana et al 1976) and 1.23-1.31 (Dhage, 1983). The values for tryptophan content observed in the present study are within the ranges reported earlier.

4.4. Polyphenols :

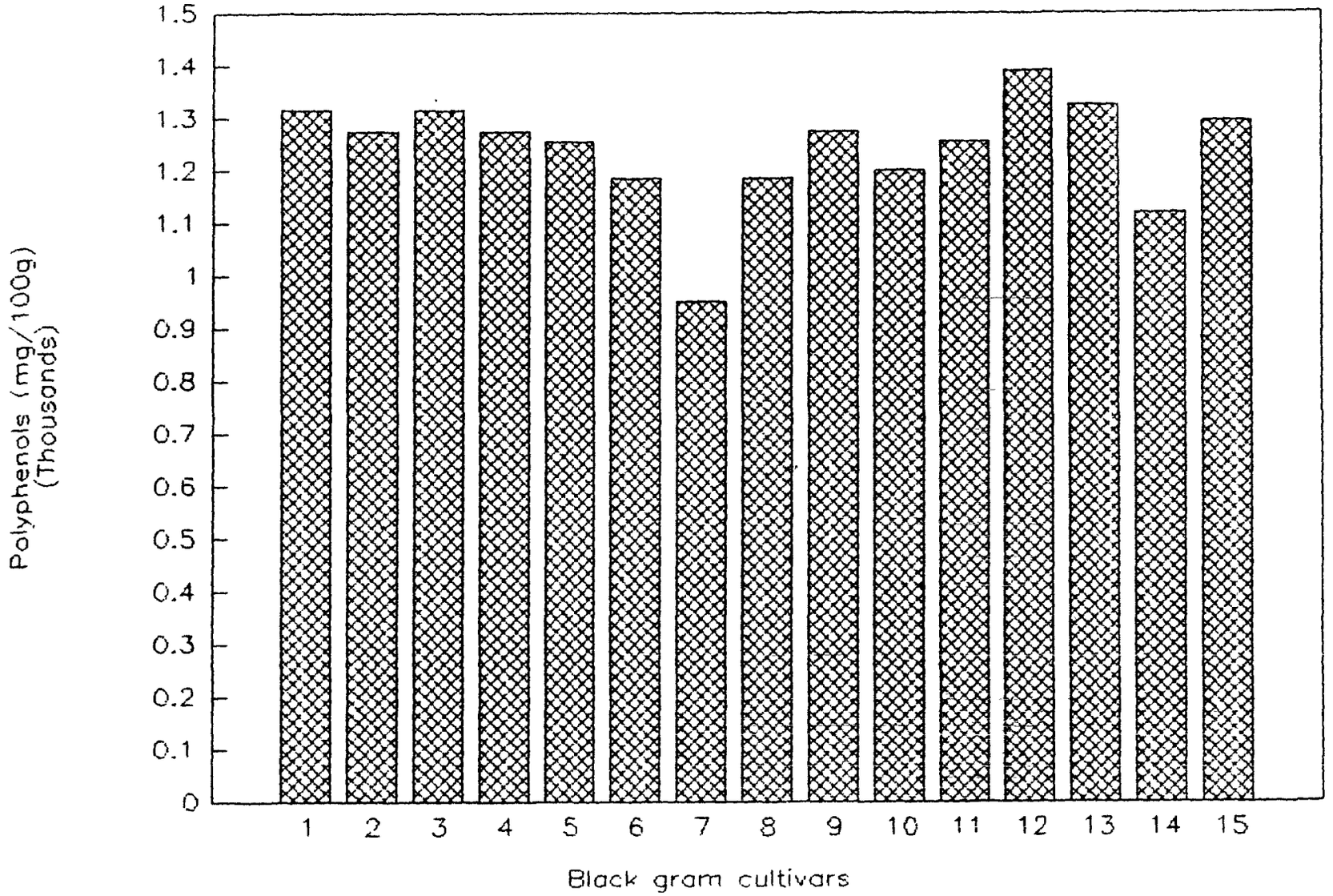
The content of polyphenols in the whole seed of 15 black gram cultivars at the dry mature stage are presented in Table 4. The total polyphenol content in whole seeds of 15 black gram cultivars ranged from 950-1390 mg/100g. The cultivars TAU-3 and TPU-4 had the highest polyphenol content (1390 and 1325 mg/100 g, respectively), followed by DPU-88-1 and DPU 88-31 (1315 mg/100g). The lowest polyphenol content was found in Phule-U-3 (950 mg/100 g). The differences between the varieties with respect to polyphenols were significant.

Rao and Deosthale (1982) reported that the total polyphenols in the black gram seed ranged from 540-1197 mg/100g while Dhage (1983) reported 1110 to 1550 mg/100g polyphenols in black gram, which are in agreement with the data recorded in this

Table.4 Polyphenols contents of black gram cultivars

Sr. No.	Cultivars	Polyphenols mg/100g
1.	DPU 88-1	1315
2.	DPU 88-9	1275
3.	DPU 88-31	1315
4.	DPU 89-6-7	1275
5.	Pant U-19	1255
6.	Pant U-30	1185
7.	Phule U-3	950
8.	S-1-1	1185
9.	T9	1275
10.	TAU-2	1200
11.	TAU-5	1255
12.	TPU-3	1390
13.	TPU-4	1325
14.	UL-338	1120
15.	VB-3	1295
	MEAN	1241
	SE	31.41
	CD AT 5%	91.04

fig-5 Polyphenol content of black gram cultivars



Cultivars.

1. *DPU 88-1*
2. *DPU 88-9*
3. *DPU 88-31*
4. *DPU 89-6-7*
5. *Pant U-19*
6. *Pant U-30*
7. *Phule U-3*
8. *S 1-1*
9. *T - 9*
10. *TAU - 2*
11. *TAU - 3*
12. *TPU - 3*
13. *TPU 4*
14. *UL - 338*
15. *VB - 3*

study.

4.5 Protein fractionation :

The black gram cultivar T9, which had the highest protein content, was fractionated into the major solubility fractions viz., albumin, globulin, prolamin and glutelin (Table 5). The cultivar T 9 had the following protein fractions. viz., albumin, 19.04%; globulin, 57.21; prolamin, 2.14; glutelin, 3.37% residual proteins (NPN) 17.82%; and total protein (detatted) 27.3% with a recovery of protein to the extent of 81.76%. The black gram cultivar UL-338 with the lowest protein content was aslo fractionated, which had the following fractions viz; albumin, 16.50%; globulin, 59.49%; Prolamin, 2.31%; glutelin, 4.25% and residual protein (NPN), 16.24% and total protein (detatted) 22.29% with a recovery of protein to the extent of 82.50%

Padhye and Salunkhe, (1977) reported the following proportion of solubility fractions of black gram proteins: albumin, 13%; globulin, 81%; prolamin, 4% and glutelin, 2%. Venugopal and Rao, (1978) reported that black gram proteins contained following solubility fractions : albumin, 24%; globulin, 46.2%; prolamin, 0.9%, glutelin, 4.3% and residual protein 24.6%. The values of protein solubility fractions reported here fairly conform to those reported in literature.

TABLE .5

Distribution of protein in various solubility fractions of black gram (T9).(at dry mature stage)

Sr. No.	Type of protein Fraction	Per cent
1.	**ALBUMIN**	
	(a) % in whole grain	5.20
	(b) % in extracted protein	19.04
2.	**GLOBULIN**	
	(a) % in whole grain	15.62
	(b) % in extracted protein	57.21
3.	**PROLAMIN**	
	(a) % in whole grain	0.58
	(b) % in extracted protein	2.14
4.	**GLUTELIN**	
	(a) % in whole grain	0.92
	(b) % in extracted protein	3.37
	Protein recovered	22.32
	Protein % (N % x 6.25)	27.3
	Percent recovery of protein	81.76

Table .6

Distribution of protein in various solubility fractions of black gram (UL 338) (at dry mature stage)

Sr. No.	Type of protein fraction	Per cent
1.	**ALBUMIN**	
	(a) % in whole grain	3.67
	(b) % in extracted protein	16.50
2.	**GLOBULIN**	
	(a) % in whole grain	13.26
	(b) % in extracted protein	59.49
3.	**PROLAMIN**	
	(a) % in whole grain	0.51
	(b) % in extracted protein	2.31
4.	**GLUTELIN**	
	(a) % in whole grain	0.95
	(b) % in extracted protein	4.25
	Protein recovered	18.39
	Protein % (N% x 6.25)	22.29
	Percent recovery of protein	82.50

Vita

Chapter Opener Page

Summary and Conclusions

5. SUMMARY AND CONCLUSION

Black gram is one of the most important food legumes of dry land agriculture. This legume give dependable yeild on low fertility soil. It is a rich source of dietary protein, calories, minerals and certain B-group vitamins to the vegetarian population. Besides, this grain legume is known to contain lower levels of commonly observed antinutritional factors in most other grain legumes. However, like other legumes, black gram is known to be deficient in certain essential amino acids like methionine and tryptophan.Considering, its agronomical and nutritional advantage over other legumes, it is essntial to develop cultivars which are rich in these limiting amino acids, low in antinutritional factors like polyphenols and phytate phosphorus. With this purpose in view, the available germplasm was screened to identify nutritionally superior lines, if any. Such information will be useful for the selection of propermaterial for genetic improvement of this important food legume. The results obtained are briefly summarised as follows:

1. The nutritional composition of 15 black gram cultivars at the dry mature stage varied markedly : crude protein, 22.3 to 27.3%; crude fat, 1.0 to 1.8%, crude fibre 2.6 to 3.25%; and ash, 2.8 to 4.2%. T 9, DPU 88-9 and DPU 88-31 were found to be the highprotein cultivars (27.3% and 26.42%, respectively).

2. The mineral composition of 15 black gram cultivars at the dry mature stage (mg/100g) was : phosphorus 215.51 to 329.15, iron,

Chapter Opener Page

Literature Cited

6. Literature Cited

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Chapter Opener Page

Vita

7. VITA

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