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BIOCHEMICAL PROFILING OF LENTIL (*Lens culinaris* Medik) GERMPLASM AT DIFFERENT GROWTH STAGES

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ABSTRACT

The lentil (*Lens culinaris* MediK) is an annual, bushy herb, erect or sub-erect with many branches. The genus name Lens is derived from the typical shape of the lentil seed. It is one of the most nutritious of all pulses that are traditionally grown in Central and Southwest Asia. Lentil is a self-pollinating diploid (2n = 14 chromosomes) legume crop that is highly valued as a high protein food. For this biochemical profiling analysis study seventy six lentil germplasm were collected from different sources to characterize the germplasm based on biomolecules concentration. Biochemical parameters viz. total sugar content estimation and protein content estimations were studied in this study at different growth intervals.

Keywords: Biochemical analysis, *Lens culinaris*, Germplasm, Sugar estimation, Protein estimation.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important cool-season crop of North Africa, West Asia, the Middle East, the Indian Subcontinent and North America [1]. It is a highly valued annual food legume crop that coevolved with wheat, barley and other cool season pulses in the Near East arc about 8000 years ago [2,3]. It is an important source of dietary protein (25 percent) in both human and animal diets, second only to soybeans as a source of usable protein (CGIAR). Lentil ranks seventh among grain legumes and is grown on over 3.5 million hectares in over 48 countries with a total production of over 3 million metric tons. The major lentil producing regions are Asia (58 percent of the area) and the West Asia-North Africa region (37 percent of the acreage of developing countries). Lentils contain high levels of proteins, including the essential amino acids isoleucine and lysine, and are an essential source of inexpensive protein in many parts of the world. Lentils are deficient in two essential amino acids, methionine and cystine. However, sprouted lentils contain sufficient levels of all essential amino acids, including methionine and cystine. Apart from a high level of proteins, lentils also contain dietary fiber, Folate, vitamin B1, and minerals. Red (or pink) lentils contain a lower concentration of fiber than green lentils (11% rather than 31%). Lentils are one of the best vegetable sources of iron. This makes them an important part of a vegetarian diet, and useful for preventing iron deficiency.

Protein concentration of lentils reportedly range from 22-34.6% and 100 g of dried seeds contain 340-346 g calories, 12% moisture, 20.2 g protein, 0.6 g fat, 65.0 g total carbohydrate, about 4 g fiber, 2.1 g ash, 68 mg Ca, 325 mg P, 7.0

mg Fe, 29 mg Na, 780 mg K, 0.46 mg thiamine, 0.33 mg riboflavin, 1.3 mg niacin [4,5]. Among the cool season legume crops, lentil is the richest in the important amino acids (lysine, arginine, leucine, and sulpher containing amino acids) [6]. The starch content ranges from 35-53% in the seed and 42% in dry matter while amylose varies from 20.7 to 38.5% of the seed starch [7,8]. "One hundred grams of decorticated lentil seed contain 344 calories, 9.9 % moisture, 25.8 g protein, 1.8 g fat, 58.8 g total carbohydrate, 0.9 g fiber, 3.7 g ash, 24 mg Ca, 271 mg P, 10.6 mg Fe, 0.47 mg thiamine, 0.21 mg riboflavin, and 1.5 mg niacin. Lentils are a good source of B vitamins, containing per 100 g: 0.26 mg thiamine, 0.21 mg riboflavin, 1.7 mg nicotinic acid, 223 mg choline, 107 mg folic acid, 130 mg inositol, 1.6 mg Pantothenic acid, 13.2 mg biotin, and 0.49 mg pyridoxine. Vitamins, except folic and Pantothenic acids, increase markedly during sprouting. Dry lentil husks contain 11.1% protein (1.3% digestible), 0.7% fat, 47.5% carbohydrate, 25.6% fiber, and 3.1% ash" [9]. About 90% of lentil protein is found in the cotyledons with albumins and globulins being the major fractions. Digestibility coefficients for lentil are relatively high and range from 78-93%, while biological values range from 32-58%. Oleic, palmitic and linoleic are the dominant fatty acids [8].

MATERIAL AND METHODS

Collection of Germplasm

A core collection of lentil germplasm was collected from CSA Kanpur, GBPUAT Pantnagar and NBPGR New Delhi (Table 1). This germplasm were sown during *rabi* season of 2011-12 at Crop Research Centre of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, (India). The fresh green leaves of each germplasm were collected from field and used for biochemical analysis.

Table 1: Germplasm of lentil (*Lens culinaris* Medik.) collected from different sources and were used for analysis.

SN	Germplasm	Source	SN	Germplasm	Source
1	IPL-110	CSA, Kanpur	39	P-722	GBPUAT, Pant Nagar
2	IPL-114	CSA, Kanpur	40	P-768	GBPUAT, Pant Nagar
3	IPL-401	CSA, Kanpur	41	P-867	GBPUAT, Pant Nagar
4	IPL-139	CSA, Kanpur	42	P-869	GBPUAT, Pant Nagar
5	IPL-202	CSA, Kanpur	43	P-870	GBPUAT, Pant Nagar
6	IPL-203	CSA, Kanpur	44	VL-515	GBPUAT, Pant Nagar
7	IPL-204	CSA, Kanpur	45	VL-133	GBPUAT, Pant Nagar
8	IPL-128	CSA, Kanpur	46	L-4603	GBPUAT, Pant Nagar
9	IPL-404	CSA, Kanpur	47	P-888	GBPUAT, Pant Nagar
10	IPL-406	CSA, Kanpur	48	VL-125	GBPUAT, Pant Nagar
11	IPL-2016	CSA, Kanpur	49	VLM-4	GBPUAT, Pant Nagar
12	VK5-1771	CSA, Kanpur	50	VL-134	GBPUAT, Pant Nagar
13	ILL-7616	CSA, Kanpur	51	VL-135	GBPUAT, Pant Nagar
14	ILL-639	CSA, Kanpur	52	PL-2	GBPUAT, Pant Nagar
15	ILL-6002	CSA, Kanpur	53	VL-516	GBPUAT, Pant Nagar
16	ILL-7723	CSA, Kanpur	54	Pl-406	GBPUAT, Pant Nagar
17	ILL-8114	CSA, Kanpur	55	VL-507	GBPUAT, Pant Nagar
18	VR5-16/11	CSA, Kanpur	56	IC-248966	NBPGR, New Delhi
19	KLB-8611	CSA, Kanpur	57	EC299676	NBPGR, New Delhi
20	KLB-8617	CSA, Kanpur	58	IC243364	NBPGR, New Delhi
21	K-75	CSA, Kanpur	59	IC208337	NBPGR, New Delhi
22	KL5-218	CSA, Kanpur	60	IC201683	NBPGR, New Delhi
23	LL-492	CSA, Kanpur	61	IC201743	NBPGR, New Delhi
24	P-319	CSA, Kanpur	62	IC208327	NBPGR, New Delhi
25	P-334	CSA, Kanpur	63	IC201655	NBPGR, New Delhi
26	P-364	CSA, Kanpur	64	IC201777	NBPGR, New Delhi
27	P-390	CSA, Kanpur	65	IC248956	NBPGR, New Delhi
28	PL-639	CSA, Kanpur	66	IC201793	NBPGR, New Delhi
29	P-434	CSA, Kanpur	67	IC218359	NBPGR, New Delhi
30	KL-133	GBPUAT, Pant Nagar	68	IC248964	NBPGR, New Delhi
31	P-508	GBPUAT, Pant Nagar	69	EC299646	NBPGR, New Delhi
32	P-509	GBPUAT, Pant Nagar	70	IC208331	NBPGR, New Delhi
33	KL5-137	GBPUAT, Pant Nagar	71	IC201699	NBPGR, New Delhi
34	L-4076	GBPUAT, Pant Nagar	72	IC248963	NBPGR, New Delhi
35	P-582	GBPUAT, Pant Nagar	73	IC201798	NBPGR, New Delhi
36	P-589	GBPUAT, Pant Nagar	74	IC201786	NBPGR, New Delhi
37	P-635	GBPUAT, Pant Nagar	75	IC248959	NBPGR, New Delhi
38	P-7101	GBPUAT, Pant Nagar	76	IC212688	NBPGR, New Delhi

Biochemical Profiling Analysis of Lentil Germplasm

Estimation of Total Sugar Content

The biochemical characterization was done on the basis of total sugar content and protein content**.** The phenol sulphuric acid method [10] is used to estimate total sugar content in lentil at 30, 60, 90 Days after sowing (DAS) and at the time of harvesting. The principle involved in estimation of total sugar is, phenol in the presence of sulphuric acid reacts with carbohydrate to produce orange yellow colour. This can be measured by taking absorbance at 490 nm using a spectrophotometer (Microprocessor spectrophotometer Model 1305).

Estimation of Protein content

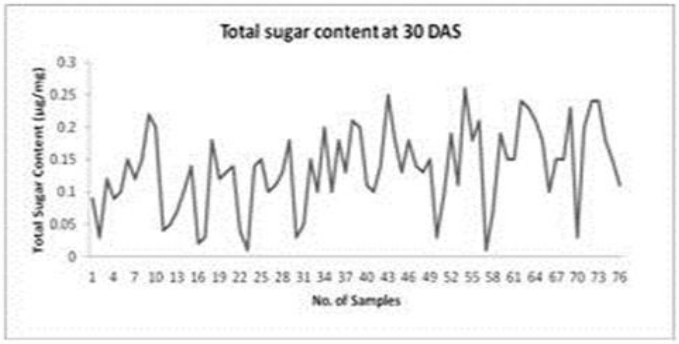
The protein content was estimated by Lowry method [11] in lentil at 30, 60, 90 Days after sowing (DAS) and at the time of harvesting. The blue colour developed by the reduction of Phosphomolybdic-phosphotungentic components in the Folin-Ciocalteu reagent by the amino acid tyrosine and tryptophan present in the protein plus colour developed by the biuret reaction of protein with the alkaline cupric tartarate is measured in the Lowry's method. This can be measured by taking absorbance at 660 nm using a spectrophotometer (Microprocessor spectrophotometer Model 1305).

RESULTS AND DISCUSSION

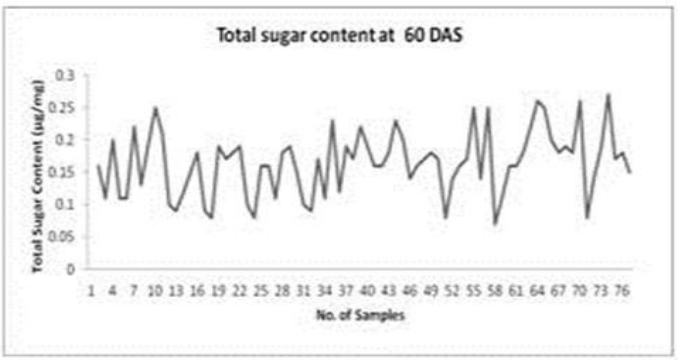
Biochemical Profiling Analysis of Lentil Germplasm

Total Sugar Content

Total carbohydrates represent the major component of lentil seeds [12] with starches occupying most of the carbohydrate mass. The total sugar content was estimated in all the seventy six lentil leaves extract using phenol sulphuric acid method at 30, 60, 90 DAS and at the time of harvest to characterize the biochemical properties of lentil germplasm and to standardization the carbohydrate content in lentil. A reference curve was prepared using different concentrations of glucose ranging between 0-1000 µl/ml and calibration curve was determined experimentally. With the help of this reference curve total sugars from all the germplasm were estimated. This was measured by taking absorbance at 490 nm using a spectrophotometer. The total sugar content of seventy six germplasm was range 0.01µg/mg to 0.26µg/mg with the mean value of 0.13µg/mg at 30 DAS, the germplasm LL-492 showed lowest value of 0.01µg/mg and PI-406 showed highest value of 0.26µg/mg at 30 DAS (Figure 1A). The total sugar content at 60 DAS ranged between 0.07µg/mg to 0.27µg/ml with the mean value of 0.16µg/mg. The EC-299676 showed lowest value of 0.07µg/mg and IC-201798 showed highest value of 0.27µg/mg at 60 DAS (Figure 1B). The total sugar content at 90 DAS ranged between 0.12µg/mg to 0.48µg/mg with the mean value of 0.28µg/mg, the P-768 showed lowest value of 0.12µg/mg and IPL-406 showed highest value of 0.48µg/mg at 90 DAS (Figure 1C). The total sugar content of seventy six germplasm was ranged between 0.15µg/mg to 0.38µg/mg with the mean value of 0.24µg/mg at harvest, IC-248964 showed lowest value i.e. 0.15µg/mg and IPL-406 showed highest value i.e. 0.38µg/mg of total sugar content at harvest (Figure 1D).

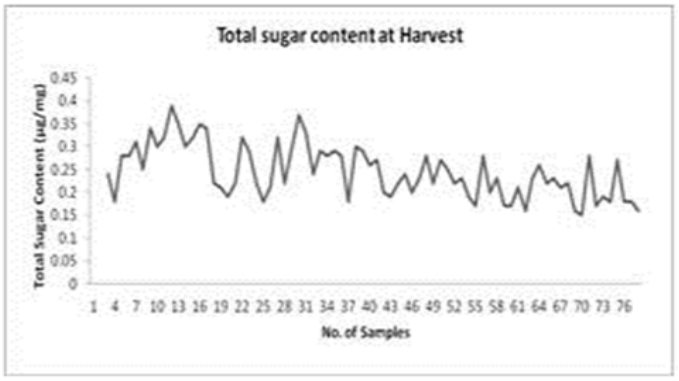


(A)



(B)

(C)



(D)

Figure 1: Total Sugar content (µg mg^{-1} fresh weight) in leaves of Lentil at 30, 60, 90 DAS and at Harvest

Among seventy six germplasm LL-492 shows lowest total sugar content i.e. 0.11µg/mg and IPL-406 showed highest value i.e. 0.32µg/mg of total sugar content. At different growth stages none of data available regards to carbohydrate content in lentil however Singh *et al.* [13] reported carbohydrate content results in mango.

Protein Estimation

Lentils are considered to be a good source of proteins. This high protein content in lentils and other pulses makes them a significant food source for developing countries and low-income people [14]. Most of the pulse proteins are storage proteins, which are usually consumed by the germ during seed germination. In this view the protein content was estimated in all the seventy six lentil leaves extract using Lowry's method at 30, 60, 90 DAS and at the time of harvest to characterize the biochemical properties of lentil germplasm and also to standardization the protein content in lentil. A reference curve was prepared using different concentrations of bovine serum albumin ranging between 0-1000 µl/ml and calibration curve was determined experimentally. With the help of this reference curve proteins from all the varieties were estimated. This was measured by taking absorbance at 660 nm using a spectrophotometer. The protein content of seventy six germplasm was range 0.08µg/ml to 0.28µg/mg with the mean value of 0.15µg/mg at 30 DAS, the germplasm KLB-8611 showed lowest value of 0.08µg/mg and P-768 showed highest value of 0.28µg/mg at 30 DAS (Figure 2A). The protein content at 60 DAS ranged between 0.19µg/mg to 0.39µg/mg with the mean value of 0.27µg/mg, the PL-2 showed lowest value of 0.19µg/mg and L-4076 showed highest value of 0.39µg/mg at 60 DAS (Figure 2B). The protein content at 90 DAS ranged between 0.12µg/mg to 0.67µg/mg with the mean value of 0.43µg/mg, the P-722 showed lowest value of 0.12µg/mg and P-7101 showed highest value of 0.67µg/mg at 90 DAS (Figure 2C). The protein content of seventy six germplasm was ranged between 0.20 µg/mg to 0.43µg/mg with the mean value of 0.30µg/mg at harvest, IPL-404 showed lowest value i.e. 0.20µg/mg and P-867 showed highest value (0.43µg/mg) of protein content at harvest (Figure 2D). Among seventy six germplasm IPL-404 shows lowest protein content i.e. 0.21µg/mg and P-7101 showed highest value i.e. 0.39µg/mg of protein contents at all the growth stages of lentil germplasm. At different growth stages none of data available regards to protein content in lentil. The protein yields among lentil genotypes were reported by Tomar *et al.* [15] at harvest strength the results and protein content was also reported at maturity by Iqbal and Khan [16] in wheat.

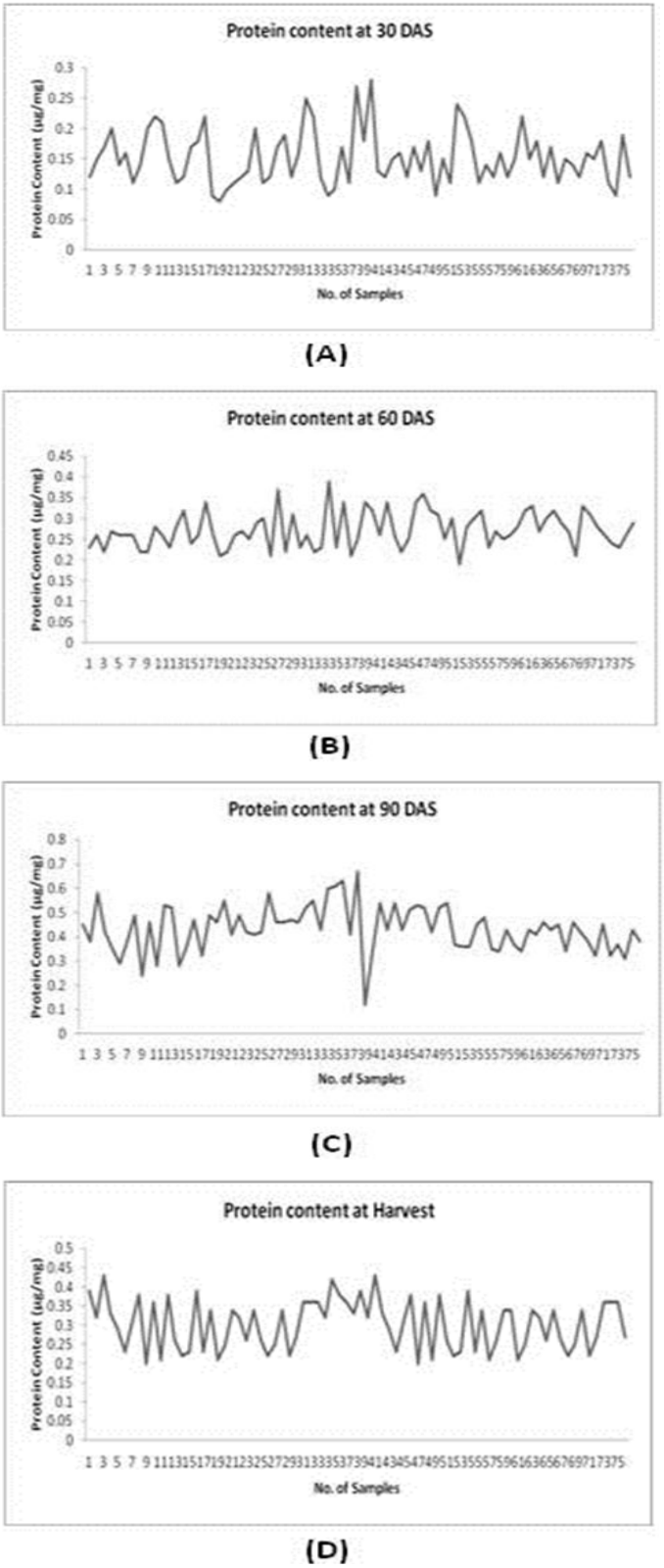


Figure 2: Total Protein content (µg mg^{-1} fresh weight) in leaves of Lentil at 30, 60, 90 DAS and at Harvest

CONCLUSION

Dry leguminous seeds, also known as pulses, represent important sources of plant protein and carbohydrates in the human diet. Lentil, dry pea, chickpea and dry bean are widely cultivated and consumed worldwide, and are considered foods with great nutritional value. The carbohydrate and protein content at different growth stages are discussed and reported in this paper. The overall results revealed that significant variation was observed in seventy six germplasm of lentil at different growth stages. These biochemical markers could be useful in identifying lentil cultivars, contributing to prepare maps and also contribute useful data in phylogenetic analyses in the genus *Lens*.

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Mr. Amit Kumar Singh received his B. Tech. and M. Tech. in Biotechnology from Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. Then he worked as a Junior Research Fellow in CST funded project, entitled Molecular, biochemical and morphological characterization of lentil germplasm/ cultivars for yield improvement under limited water condition at College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. He has 3 years of experience in research. Presently he is Pursuing Ph.D. in Biotechnology from Sam Higginbottom Institute of Agriculture, Technology and Science, Allahabad.

