5th International Conference on Recent Development in Engineering Science, Humanities and Management
National Institute of Technical Teachers Training & Research, Chandigarh, India (ESHM-17)
16th April 2017, www.conferenceworld.in ISBN: 978-93-86171-39-9

STUDIES ON SOME PRIMARY METABOLITE'S QUANTIFICATION OF WHEAT SPECIES (TRITICUM AESTIVUM L AND TRITICUM DURUM DESF)

Praveen Kumar Chachaiya[1],Gargi Mehta[2]

[1]*Research Scholar, Department of Sciences, Career Point University, Kota, Rajasthan (India)*

[2]*Department of Botany, Jiwaji University, Gwalior, Madhya Pradesh (India)*

ABSTRACT

During the present investigation, comparative study of primary metabolites i.e. Total Soluble Sugar, Starch, Protein, Lipids and Phenol of genus Triticum were determined. The two wheat species Triticum aestivum L. and Triticum durum desf. were undertaken for study and powdered seed material was analyzed for their chemical composition. It was found that the overall higher amount of Total soluble Sugar, starch, protein, lipids was observed in Triticum aestivum L. genotype GW 366 (15.8; 15.6; 22.2; 60.0 mg/gdw) respectively and phenol content was higher in another Triticum aestivum L. genotype GW 322 (8.5 mg/gdw). However, minimum value of Total soluble Sugar and Phenol was found in Triticum durum desf. genotype HI 8737 (10.0 and 2.8 mg/gdw) respectively, equal lower amount of Starch was found in Triticum aestivum L. and Triticum durum desf. genotypes Raj 4238 and MPO 1215 (6.2 mg/gdw) respectivly. The lowest content of protein and lipids was found in Triticum aestivum L. genotypes Raj 4238 (10.2 mg/gdw) and GW 322 (7.5 mg/gdw) respectively. The study provided the basis for further investigations into the adaptability of the wheat genotypes for the selection of valuable nutrient and nutraceutical quality.

Keywords: Genotype, Primary metabolite, Triticum aestivum L., Triticum durum desf., Wheat

I. INTRODUCTION

Nature is a rich store house of herbal remedies. They are known more and more for their crucial benefits in many areas. The medicinal properties of wheat attributed to their chemical compositions. Many of the crop species that bestow medicinal herbs have been scientifically assess for their possible medicinal prosperities. Plants play a significant role for the development of new remedies for human being. Around 20,000 medicinal plant species have been recorded (Dev, 1997). In India, massive coffers of medicinal plants that are used in traditional medical treatments. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti *et al*. 2008).

ISBN: 978-93-86171-39-9

Human beings have been utilizing wheat crop for basic preventive and curative health care since ancient time. Primary metabolites are synthesis during photosynthesis and these compounds is essential for crop life, growth and development. Some primary metabolites *viz*, Total Soluble Sugar, Starch, protein, lipid, Phenol plays a unique role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. Carbohydrates can be divided into two main types: available and unavailable. Available carbohydrates are those who digested and absorbed by mankind, which include (non-resistant) starch and soluble sugars. Starch is used to store energy. It is the last product of photosynthesis and can be stored for later use in seeds. Chemically, starch is a polysaccharide comprised of glucose molecules linked together in long chains (Barthakur *et al*., 1995). In wheat, starch is the most abundant component present in the grain endosperm (Lineback and Rasper, 1988). It consists of the glucose polymers, amylose and amylopectin. Proteins are also derived by carbohydrates through the formation of amino acids. Wheat is an important source of proteins in vegetarian diet. Grain based foods like wheat which provide complex carbohydrates, is the best fuel for our bodies. Wheat is high in fiber, low in fat, full of vitamins (i.e. Thiamin, Riboflavin, Niacin), Folic acid and meager amount of iron too. The main characteristic of proteins is their high nitrogen content (Fairbairn and Shrestha, 1967). Essential fatty acids (palmitic and linoleic acids), fat-soluble vitamins and phytosterols are important components of wheat grain lipids (Ruibal-Mendieta *et al*., 2004). Flour lipids have a positive effect on formation of dough during the process of bread-making. The volume and softness of steamed bread and morphology of short-dough biscuits is also influenced by lipids (Pomeranz *et al*., 1991; Papantoniou *et al*., 2004). Phenols are the most important antioxidant compounds of the wheat grain. They are mainly made up of phenolic acids and flavonoids (Dinelli *et al.*, 2009). In wheat kernel, polyphenols can be found in the free soluble and the bound insoluble forms, both possessing strong antiradicalic power as well as anticancer and anti-inflammatory properties (Thompson, 1994)

II STUDY AREA and METHODOLOGY

In the present study basic primary metabolites of *Triticum* species seed material of 10 wheat genotypes from Agriculture Research Station, Ummedganj, Kota viz. five genotypes of *Triticum aestivum L.* (Raj 4037, Raj 4238, GW 322, GW 366, HI 1544) and five genotypes of *Triticum durum desf*. (Raj 6560, MPO 1215, HI 8498, HI 8737, HD 4728) and used for estimation of primary metabolites (Total soluble sugar, starch, protein, Lipids and Phenol). Different protocols were used for the quantitative estimation of primary metabolites. The seed powder of various wheat species was used for analysis of Total Soluble Sugar and Starch (Dubois *et al*, 1951), Protein (Lowry *et al*, 1951), Lipids (Jayaraman J., 1980) and Phenol (Bray & Thorpe, 1951). The investigation carried out at the Seminal Applied Bioscience Laboratory, Jaipur (Raj.) and Agriculture Research Station, Ummedganj, Kota during 2016-17. All experiments were repeated in triplicate and means were calculated.

III ISOLATION AND QUANTIFICATION OF PRIMARY METABOLITES

Wheat grain were dried and powdered for evaluation of various primary metabolites.

ISBN: 978-93-86171-39-9

3.1 Carbohydrates

3.1.1 Total Soluble Sugars Extraction

The dried wheat seed (50 mg each) was homogenized in pestle and mortar with 20 mL of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected separately and concentrated on a water bath using the method of Loomis and Shull (1973). Distilled water was added to make up the volume up to 50 mL and processed further for quantitative analysis.

3.1.2 Starch Extraction

The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5 mL of 52% perchloric acid (Mc Cready *et al* , 1950). Later, 6.5 mL of water was added to each sample and the mixture was shaken vigorously for 5 minutes.

3.1.3 Quantitative Estimation

1mL of aliquot of each sample was used for the estimation of carbohydrates using the phenol sulphuric acid method of Dubois *et al* (1951). A standard regression curve of standard sugar (glucose) was prepared. A stock solution of glucose (100µg mL^{-1}) was prepared in distill water. From this solution, 0.1 to 0.8 mL was pipette out into eight separate test tubes and volume was made up to 1 mL with distilled water. These tubes were kept on ice; 1 mL of 5% phenol was added in each tube and shaken gently. 5 mL of conc. sulphuric acid added was rapidly poured so that the steam hits the liquid and tubes were gently shaken during the addition of the acid. Finally the mixture was allowed to stand on water bath at 26-30^{0}C for 20 minutes. The characteristics yellow orange colour was developed. The optical density was measured at 490 nm using spectrophotometer (Carl Zeiss, Jena DDR, VSU 2 P), after setting for 100% transmission against a blank (distilled water). Standard regression curve was computed between the known concentration of glucose and their respective optical density, which followed Lambert Beer's Law. All samples were analyzed in the same way as described above and contents of total soluble sugars and starch were calculated by computing optical density of each of the samples with standard curve.

3.2 Proteins Extraction

The test sample (50mg each) were separately homogenized in 10 mL of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4^{0}C for 24 hours. These mixtures were centrifuged separately and supernatants were discarded. Each of the residues was again suspended in 10 mL of 5% TCA and heated at 80^0 on a water bath for 30 minutes. The samples were cooled, centrifuged and supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight at room temperature (Osborne, 1962).

3.2.1 Quantitative Estimation

Each of the above samples (1 mL) was taken and the total protein content was estimated using the spectrophotometer through method of Lowry et al (1951). A regression curve was prepared. A stock solution of BSA (Sigma Chem. Co., St. Louis, USA) was prepared in 1N NaOH (1mgL^{-1}). Eight concentrations (ranging from 0.1 to 0.8 mgL^{-1}) were separately measured in test tube and volume of each sample was made to1 mL by adding

ISBN: 978-93-86171-39-9

distilled water. To each, 5 mL of freshly prepared alkaline solution (Prepared by mixing 50 mL of 2% Na_2CO_3 in 0.1 N NaOH and 1 mL of 0.5 % $CuSO_4$.$5H_2O$ in 1% Sodium potassium tartarate) was added and kept at room temperature for 10 minutes. In each sample 0.5mL of Folin-Ciocalteau reagent (commercially available reagent was diluted with equal volume of distilled water just before use) was added rapidly with immediate mixing and optical density of each sample was measured after 30 minutes at 750 nm using spectrophotometer against blank. Five replicates of each concentration were taken and average value was plotted against their respective concentrations to compute regression curve.

All samples were processed in the same manner and the concentration of the total protein content in each sample was calculated by referring the optical density of each sample with standard curve. Five replicates of each concentration were taken and their mean value was calculated.

3.3 Lipids Extraction and Quantification

The test sample were dried, powdered and 100mg was macerated with 10 mL distilled water, transferred to a conical flask containing 30 mL of chloroform and methanol (2/1:v/v; Jayaram, 1981) . The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 mL of chloroform mixed with 2 mL of water were added and centrifuged. Two layers were separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the preweighed glass vials and the colored aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers dried *in vacuo* and weighed. Each treatment was repeated thrice and their mean values were calculated.

3.4 Phenol Extraction

The deproteinized test materials (200mg each) were macerated with 10 mL of 80% ethanol for 2 hours, and left overnight at room temperature. The mixtures were centrifuged and the supernatants were collected separately and maintained up to 40 mL by adding 80% ethanol .

3.4.1 Quantitative Estimation

Total phenol content in each sample was estimated by spectrophotometer method of Bray and Thorpe (1954). It includes the preparation of a regression curve of standard phenol (Tannic acid). A stock solution of tannic acid was prepared by mixing 40 mg of standard phenol in 1 mL of 80% ethanol. Eight concentrations ranging from 0.1 to 0.8 mL were prepared in the test tube and volume was raised to 1mL by addition of 80% ethanol. To each test tube , 1mL of Folin-Ciocalteau reagent (commercially available reagent was diluted by distilled water in 1:2 ratio just before use) and 2 mL of 20% sodium carbonate solution was added and then mixture was shaken thoroughly. The samples were placed in boiling water for 1 min and cooled under running water. These reaction mixtures were diluted to 25 mL by adding distilled water and optical density was read at 750 nm against a blank. The optical density of each sample was plotted against the respective concentration of total phenols to compute regression curve. The concentrations in the test samples were calculated by referring the respective optical density of test sample against standard curve of tannic acid.

ISBN: 978-93-86171-39-9

IV RESULT AND DISCUSSION

Biologically active compounds contain a remarkably diverse assay of organic compounds and the carbohydrates are not only the first formed organic compounds in the plants as a result of photosynthesis but, also a major source of energy. Not only this, all the biochemical compounds are directly/indirectly derived from them for the important structure or they also modify the physico-chemical characters of other groups of compounds by combining with them.

4.1 Total Soluble Sugar

Higher concentration of soluble sugars was observed equal in both wheat species *Triticum aestivum L.* and *Triticum durum desf.* genotypes (GW 366; 15.8 mg/gdw) and (MPO 1215; 15.8 mg/gdw) respectively and lower concentration was observed in *Triticum aestivum L.* genotype (HI 1544; 12.0 mg/gdw) and *Triticum durum desf.* genotype (HI 8737; 10.0 mg/gdw). Sugar can be used as artificial sweetener and they can even help in diabetes by supporting the body in its rebuilding (Freeze, 1998).

4.2 Starch

The highest concentration level of starch was observed in *Triticum aestivum L.* genotype (GW 366; 15.6 mg/gdw) and *Triticum durum desf.* genotype (HI 8737; 13.6mg/gdw) and lowest concentration level was measured equal in both species of *Triticum aestivum L.* genotype (Raj 4238; 6.2 mg/gdw) and *Triticum durum desf.* genotype (MPO 1215; 6.2mg/gdw) respectively. The major sources of starch are wheat, potato and cassava mostly used as food (Tester and Karkalas, 2001). Although, starch is also used in cosmetic formulation like face powder and in dusting preparations that use aerosol dispensing systems (Griffin and Wang, 1983), Starch may also be used as a substitute for petroleum based plastics (Schwach and Averous, 2004).

4.3 Protein

The maximum content of protein was observed in *Triticum aestivum L.* genotype (GW 366; 22.2 mg/gdw) and *Triticum durum desf.* genotype (MPO 1215; 21.0 mg/gdw), while minimum content was in *Triticum aestivum L.* genotype (Raj 4238; 10.2 mg/g.dw) and *Triticum durum desf.* genotype (HI 8737; 12.8 mg/gdw). The presence of higher protein level in the plant parts towards their possible increase in food value or that a protein based bioactive compounds can also be utilized in future for enhancing food quality (Thomsen *et. al.*, 1991).

4.4 Lipids

The amount of lipids was maximum in *Triticum aestivum L.* genotype (GW 366; 60.0 mg/gdw) and equal amount in two *Triticum durum desf.* genotypes (HI 8737 and HD 4728; 20 mg/gdw), minimum amount of lipids was in *Triticum aestivum L.* genotype (GW 322; 7.5 mg/gdw) and *Triticum durum desf.* genotype (Raj 6560; 8.0 mg/gdw). Lipid is a diverse group of primary metabolites, include reserve plant material such as fats, essential oils, waxes terpenoids and oleoresin. Plant lipids have developed products that work with diverse requirements, be it culinary, medicinal or cosmetic (Yadav and Tyagi, 2006).

ISBN: 978-93-86171-39-9

4.5 Phenol

The highest content of phenols was observed in *Triticum aestivum L.* genotype (GW 322; 8.5 mg/gdw) and *Triticum durum desf.* genotype (HI 8498; 5.6 mg/gdw), while lowest content of phenol was in *Triticum aestivum L.* genotype (HI 1544; 4.4 mg/g.dw) and *Triticum durum desf.* genotype (HI 8737; 2.8 mg/gdw). Phenolic compounds are receiving increasing attention, because of their health promoting effects, attributed to their antioxidant activity. Indeed, these compounds may have beneficial effects on human health (Meot-Duros and Magne, 2009; Chahal *et.al.*, 2011). Phenols possess a number of biological activities such as antioxidant, antiseptic, disinfectant fungicide and pesticides. The higher amount of phenols is important in the regulation of plant growth, development and diseases resistance. Plant phenols may interfere with all stages of cancer process, potentially resulting in a reduction of cancer risk (Hollman, 2001).

Table1 Estimation of primary metabolites from genotypes of *Triticum aestivum L.* (mg/gdw)

S. No.	Genotypes	Primary Metabolite (mg/gdw)				
		TSS	Starch	Protein	Lipids	Phenol
1	Raj 4037	14.2	7.2	16.0	10.0	8.0
2	Raj 4238	13.0	6.2	10.2	10.0	6.7
3	GW 322	14.4	11.8	19.4	7.5	8.5
4	GW 366	15.8	15.6	22.2	60.0	6.9
5	HI 1544	12.0	9.2	14.6	8.0	4.4

Table 2 Estimation of primary metabolites from genotypes of *Triticum durum desf*. (mg/gdw)

S. No.	Genotypes	Primary Metabolite (mg/gdw)				
		TSS	Starch	Protein	Lipids	Phenol
1	Raj 6560	12.0	11.6	20.2	8.0	4.3
2	MPO 1215	15.8	6.2	21.0	10.0	4.9
3	HI 8498	13.2	12.8	14.7	15.0	5.6
4	HI 8737	10.0	13.6	12.8	20.0	2.8
5	HD 4728	13.6	9.0	17.0	20.0	3.7

Abbreviations : mg/gdw = milli gram dry weight, TSS = Total Soluble Sugar

ISBN: 978-93-86171-39-9

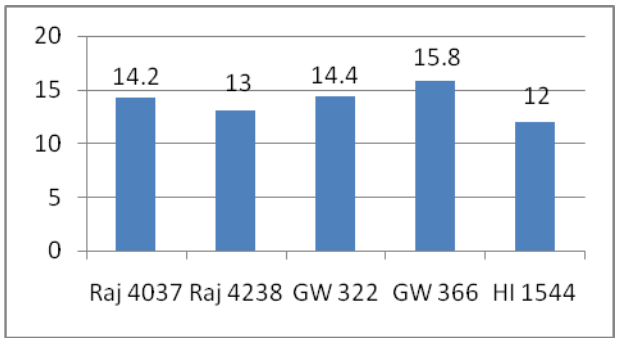

Fig. 1 Total Soluble Sugar of *Triticum aestivum L.* (mg/gdw)

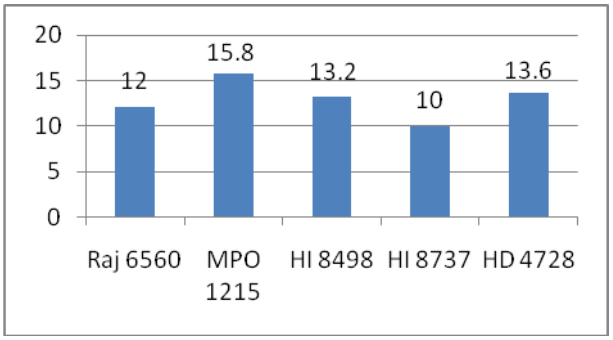

Fig. 2 Total Soluble Sugar of *Triticum durum desf.* (mg/gdw)

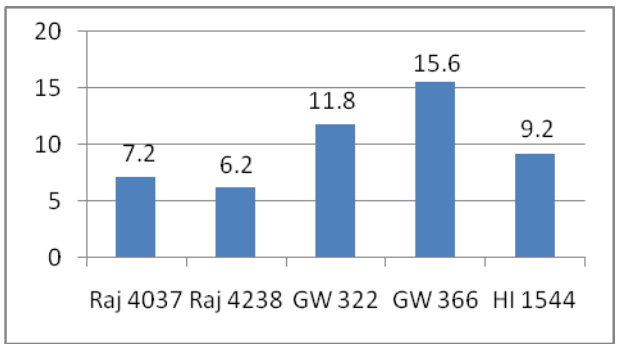

Fig. 3 Starch of *Triticum aestivum L.* (mg/gdw)

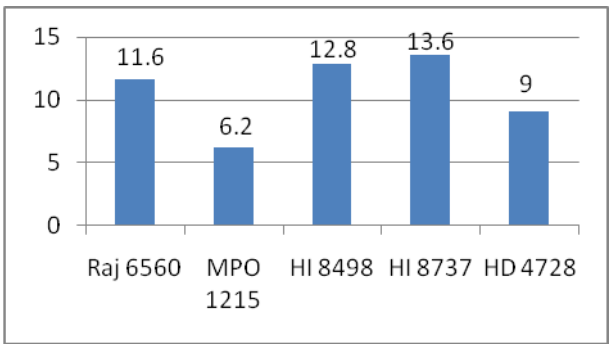

Fig. 4 Starch of *Triticum durum desf.* (mg/gdw)

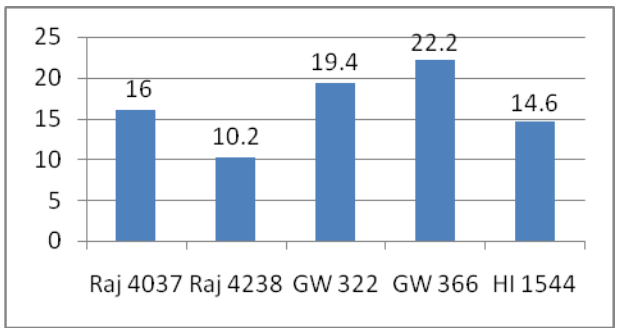

Fig. 5 Protein of *Triticum aestivum L.* (mg/gdw)

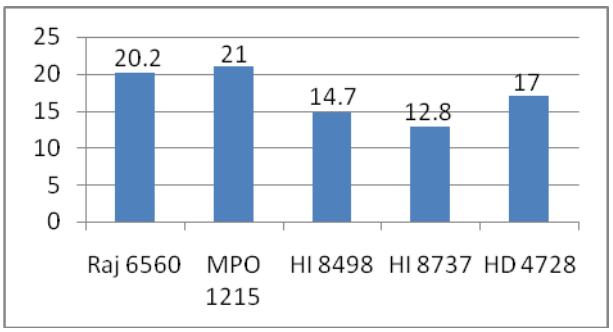

Fig. 6 Protein of *Triticum durum desf.* (mg/gdw)

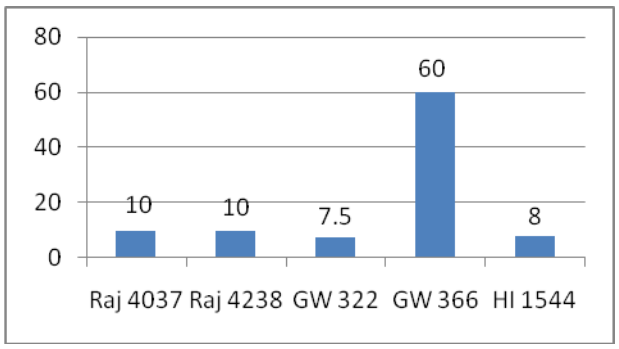

Fig.7 Lipids of *Triticum aestivum L.* (mg/gdw)

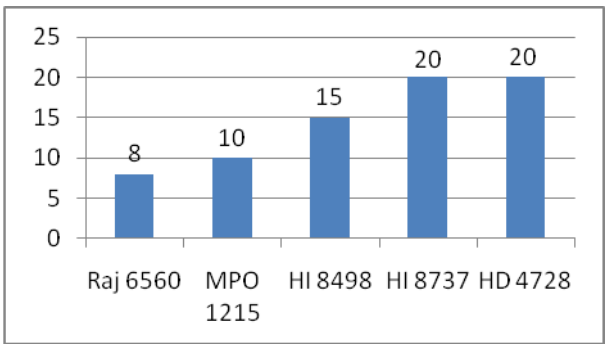

Fig. 8 Lipids of *Triticum durum desf.* (mg/gdw)

ISBN: 978-93-86171-39-9

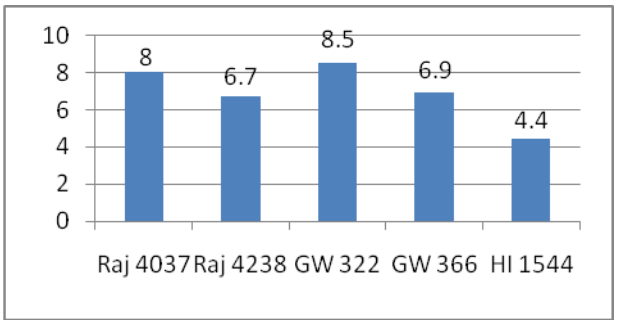

Fig. 9 Phenol of *Triticum aestivum L.* (mg/gdw)

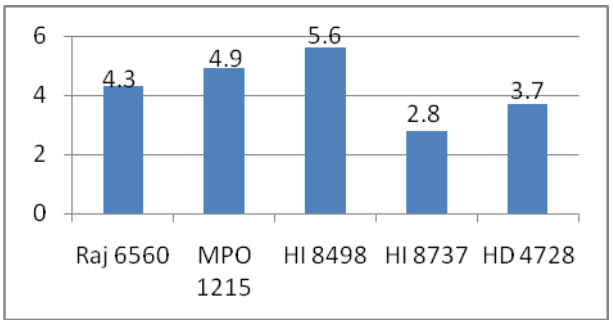

Fig.10 Phenol of *Triticum durum desf.* (mg/gdw)

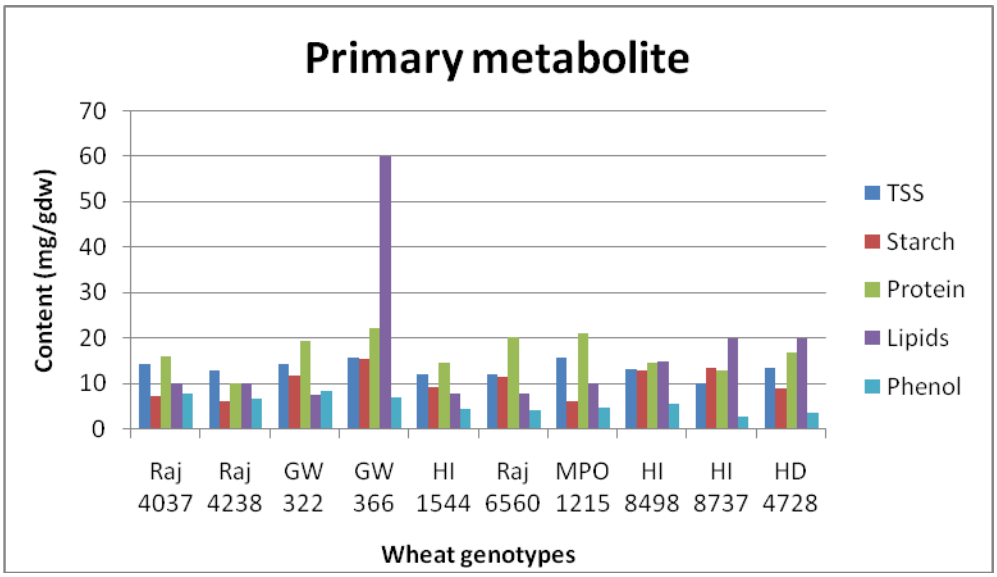

Fig. 11 Comparative performance of primary metabolite's in Wheat Species

V. CONCLUSION

During the present research work, It was found that the overall higher level of Total Soluble Sugar, starch, protein, lipids was observed in *Triticum aestivum L.* genotype GW 366 and phenol content was also higher in *Triticum aestivum L.* genotype GW 322. Similarly, minimum value of Total Soluble Sugar and Phenol was found in *Triticum durum desf. genotype* HI 8737, equal lower amount of starch found in *Triticum aestivum L.* and *Triticum durum desf. genotypes* Raj 4238 and MPO 1215 respectively. The lowest content of protein and Lipids was in *Triticum aestivum L.* genotypes Raj 4238 and GW 322 respectively. The results revealed that the wheat species are rich in primary metabolites and the quantities of these primary metabolites are differs in specific genotypes (Fig. 11). These results are indication of primary bioactive compound of commercially importance and may result in great interest in pharmaceuticals. Primary metabolites analysis is necessary for knowing the nutritional potential of plants and also the precursors for the synthesis of secondary metabolites (Vijayvergia and Kumar, 2007).

ISBN: 978-93-86171-39-9

VI. ACKNOWLEDGEMENT

Authors are thankful to research scientists of Seminal Applied Bioscience Laboratory, Jaipur for providing laboratory facilities and sincere efforts in writing this research paper and gratitude to Dr. Krishnendra Singh Nama and also grateful to scientists of Agricultural Research Station, Ummedganj, Kota (Agriculture University, Kota) for their regular helps.

REFERENCES

[1] Dev S., Ethnotherapeutic and modern drug development: The potential of Ayurveda, Curr. Sci. 73 (11), 1997, 909- 928.

[2] Prusti A., Mishra S. R., Sahoo S. and Mishra S. K., Antibacterial activity of some Indian medicinal Plants, Ethnobotanical Leaflets 12, 2008, 227-230.

[3] Barthakur N. N, N. P. Arnold and I. Alli. The Indian Labernum (Cassia fistula L.) fruit: an analysis of its chemical constituents, Plant Foods Human Nutr. 47, 1995,55-62.

[4] Lineback, D. R. and Rasper, V. F. , Wheat, Chemistry and Technology St Paul, MN: AACC, 1988.

[5] Fairbairn J. W. and A. B. Shrestha. The distribution of anthraquinone glycosides in Cassia senna L. Phytochemistry, 6, 1967, 1203-1207.

[6] Ruibal-Mendieta, N. L., Rozenberg, R., Delacroix, D. L., Petitjean, G., Dekeyser, A., Baccelli, C., Marques, C., Delzenne, N. M., Meurens, M., Habib-Jiwan, J.-L. et al., Spelt (Triticum spelta L.) and Winter Wheat (Triticum aestivum L.) Wholemeals Have Similar Sterol Profiles, As Determined by Quantitative Liquid Chromatography and Mass Spectrometry Analysis. Journal of Agricultural and Food Chemistry 52, 2004, 4802-4807.

[7] Pomeranz, Y., Huang, M. and Rubenthaler, G. L., Steamed bread. III. Role of lipids. Cereal Chemistry 68, 1991, 353-356.

[8] Papantoniou, E., Hammond, E. W., Scriven, F., Gordon, M. H. and Schofield, J. D., Effects of endogenous flour lipids on the quality of short-dough biscuits. Journal of the Science of Food and Agriculture 84, 2004, 1371-1380.

[9] Dinelli G, Carretero AS, Di Silvestro R, Marotti I, Fu S, et al., Determination of phenolic compounds in modern and old varieties of durum wheat using liquid chromatography coupled with time-of-flight mass spectrometry. J Chromatogr A 1216(43), 2009, 7229-7240.

[10] LU Thompson, Antioxidant and hormone-mediated health benefits of whole grains. Crit. Rev. Food Sci. 34, 1994, 473-97.

[11] Dubois, M., Gills, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F.. Colorimetric method for determination of sugar and related substances. Anal. Chem. 28, 1956, 350-356.

[12] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the phenol. J. Biol. Chem. 193, 1951, 265-275.

ISBN: 978-93-86171-39-9

[13] Jayaraman, J., Laboratory Manual in Biochemistry. New Delhi: wiley Eastern Limited. 1981

[14] Bray, H. G. and W. V. Thorpe. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal.,1, 1954, 27-52.

[15] Loomls, W.E. & Shull CA, Methods in plant physiology. McGraw Hill, New York,1973.

[16] Mccready R. M., J. Guggoiz, V. Silviera and H. S. Owens. Determination of starch and amylase in vegetables. Anal. Chem., 22, 1950, 1156-1158.

[17] Osborne, D.J, Effect of Kinetin on protein and nuclic acid metabolism in Xanthium leaves during senescence. Plant Physoil. 37, 1962, 595-602.

[18] Freeze, H., Disorder in protein glycosylation and protein therapy. The Journal of Pediatrics. 133 (5), 1998, 553-600.

[19] Tester, R. F. and Karkalas, J., The effects of environmental conditions on the structural features and physico-chemical properties of starches. Starch. 53, 2001, 513–519.

[20] Griffin, G. J. L. and Wang, J. K., In Taro. JK Wang (Ed) University of Hawaii press, Honolulu, USA. p., 1983, 400.

[21] Schwach, E. and Averous, L., Starch based biodegeadable blends: Morphology and interface properties. Polymer International. 53, 2004, 2115- 2124.

[22] Thomsen, S., Handen, H. S. and Nymn, V., Ribosome inhibiting proteins from in vitro cutures of Phytolacea decandra. Planta Medica. 57, 1991, 232-236.

[23] Yadav, P. R. and Tyagi, R., Lipid Biotechnology. 1 Discovery Publishing House -New Delhi. p., 2006, 89.

[24] Meot, D.L. and Magne, C., Antioxidant activity and phenol content of Crithmumm aritimum L. leaves. Plant Physiol. Biochem., 47, 2009, 37-41.

[25] Chahal, J.K., Sarin, R. and Malwal, M., Biochemical estimation of selective metabolites of two plants of Verbenaceae family. J. Ind. Bot. Soc. 90 (3&4), 2011, 303-306.

[26] Hollman, P. C., Evidence for health benefits of plant phenol. J. Sci. of Food and Agr. 89, 2001, 842-852.

[27] Vijayvergia R. and Kumar J., Quantificaton of primary metabolites of Nerium indicum Mill. Asian J. Exp. Sci. 21(1), 2007, 123-128.