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Research Article

Comparitive Analysis of Total Flavonoid Content in Various Wheat (*Triticum aestivum* L.) Landraces in Rainfed Conditions of Rawalakot

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ABSTRACT

Wheat and its products are considered as chief source of essential nutrients like antioxidants and flavonoids. Because health promoting effects of wheat grain are result of these bioactive compounds. These phytochemicals such as polyphenols and flavonoids are linked with reduced risk of many fatal diseases. The aim of research was to quantify the total amount of variability present in the various genotypes for total flavonoid contents. Study was conducted in the experimental area and laboratory of Plant Breeding and Molecular Genetics, University of Poonch Rawalakot. Study was comprised of twenty five landraces with one check variety BARS-2009 (total 26 genotypes). Total flavonoid contents were estimated by following method by keeping Quercetin as a standard . TFC mean values ranged from (3.99-8.13 mg/g). Least value for TFC for noted in LR-12 (3.79). Whereas Check variety BARS-2009 added (4.92) for TFC. Maximum TFC was contributed by LR-34 (8.13) followed by LR-13 (7.95) so LR-34 and LR- 13. Thses could be our desired Landraces for future wheat improvement programs especially in rainfed conditions.

Key words: *Flavonoid, Rainfed, Landraces, Phytochemical, Quercetin*

INTRODUCTION

Wheat is one of most important cereal crop that is consumed by majority of world population's (approx 36 %) as a staple food. Wheat along with other cereals is consumed worldwide on large scale due to their nutritional importance such as cereal grains are contributing significantly to antioxidant intake with beneficial health effects[1-,3]. Studies proved that cereals like wheat contains different types of bioactive compounds that can be serve as antioxidant, enzymes and other health-beneficial compounds in human nutrition plus reducing the risk of non-communicable diseases [4- 6].

In addition many bioactive compounds such as carotenoids, tocopherols, phenolic acids and flavonoids have antioxidant activity [7, 8]. Flavonoids or bioflavonoid are also known as Vitamin P because of effect that flavonoids had on permeability of vascular capillaries [9] .The word flavonoid is from Latin word flavus meaning yellow, are a class of plant secondary metabolites [10]. Flavonoids are polyphenolic compounds usually categorized into various groups such as into flavonols, flavanones, flavones and isoflavones according to chemical structure [11]. Approximately 4,000 different flavonoids have been identified [12]. Many reports suggested that these bioactive compounds are vital for human[13].

Flavonoids have been reported to have anti-allergic, antitumor, and anti-inflammatory and antioxidant activities (free radical scavenging) and antioxidants are substances that protect living cells from destructive effects of reactive oxygen species such as peroxyl, superoxide and hydroxyl radicals [14, 15].

Position of hydroxyl groups in chemical structure of flavonoids is very important for free radical scavenging activities [16,- 18].

Imbalance between these reactive oxygen species and antioxidants results in oxidative stress that may leads to cellular damage [19]. Furthermore oxidative stress is responsible to cause cancer, inflammation, aging and many neurodegenerative diseases like Alzheimer's. Some other compounds also have antioxidant activity; these compounds include Lycopene, Vitamin A, Beta-carotene, and Vitamin C. They may involve in cellular metabolism [20]. Quercetin is most abundant dietary flavonol that is a potent antioxidant because it has all the right structural features to act as a free radical scavenger [21]. Flavonoids and phenolic acids are present both in free and conjugated forms in various cereals such as in wheat. Concentration of flavonoids is high in aleurone layer of wheat but these compounds may also found in seed coat and embryos of kernels[22-24]. In case of plants, flavonoids are widely distributed performing many functions[25]. For plants, flavonoids are actually pigments producing flower coloration, such as red or yellow pigmentation in petals [26]. In many other higher plants, these may act as a chemical messenger, physiological regulators; they are involved in UV filtration and protect plants against many diseases[27]. But the concentration of all these phytochemicals like flavonoid and phenolic in crop plant may influenced by soil type, rainfall, location and plant genetics etc [28- 30].

METHODOLOGY

Sample description:

Total flavonoids contents were determined in winter wheat samples grown during 2013-2014. Seed of accessions was acquired from Department of Plant Breeding and Genetics, PMAS-University of Arid Agriculture, Rawalpindi-Pakistan.

Twenty five different land races of wheat with one check variety BARS-2009 were used for study (table 1). The research study was conducted in the experimental field and laboratory of Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture, University of Poonch, Rawalakot. Genotypes were sown in the experimental field of University of the Poonch Rawalakot Azad Kashmir Pakistan on 3-11-13 and harvested on 21-6-14. Seeds of selected genotypes were sown in well prepared soil with row to row distance of 30cm. Fertilizer containing nitrogen was applied twice. All other suggested cultural practices were exercised uniformly. Biochemical analysis (TFC) was carried out from seed.

Table 1: List of wheat genotypes

Sr. No.	Genotypes	Sr. No.	Genotypes
01	BARS-2009	14	LR-27
02	LR-03	15	LR-30
03	LR-05	16	LR-33
04	LR-06	17	LR-34
05	LR-07	18	LR-35
06	LR-10	19	LR-36
07	LR-11	20	LR-37
08	LR-12	21	LR-38
09	LR-13	22	LR-41
10	LR-15	23	LR-42
11	LR-16	24	LR-43
12	LR-20	25	LR-44
13	LR-26	26	LR-45

Reagents and Chemicals:

Aluminium chloride, potassium acetate, seed extract, distil water.

Preparation of wheat extracts:

Seeds of all desired genotypes were taken, grinded and then finally taken 0.1 gram of fine powder of sample and dissolved in 10 ml of hot water then kept all samples at room temperature overnight. Next day 0.1 ml of aluminium chloride, 0.1 ml of extract, 0.1 ml of potassium acetate and 1.2 ml of water was taken for test (sample).

Preparation of Aluminium chloride:

Ten grams of aluminium chloride was taken and it was dissolved in 100 ml of distilled water.

Preparation of Potassium acetate:

For preparation of potassium acetate, 10 grams of potassium acetate was dissolved in 100 ml of distilled water.

Estimation of total flavonoid contents:

Total flavonoid content was determined by method [1]. Flavonoid content was measured using 0.1 g of seed sample dissolved in 10 ml of hot water, 0.1 ml of aluminium chloride, 0.1 ml of potassium acetate and 2.8 ml of distilled water. After kept the

mixture at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm on spectrophotometer.

STATISTICAL ANALYSIS

All data are expressed as Mean. Cluster analysis, simple statistics was analyzed by help of computer software PAST [31]. The cluster analysis was done on the basis of standard distance of k-means and in each cluster the genotypes were then analyzed for the basic statistics.

RESULTS AND DISCUSSION

Divergence in means:

Mean values for TFC ranged from (3.99-8.13 mg/g) in table 2. Figure 1, 2 and 3 indicated the divergence for TFC among all genotypes studied. For TFC the maximum mean value was noted in LR-34 of 8.13 followed by the LR-13 (7.95), LR-16 (7.91), LR-26 (7.40) where as two landraces LR-05 and LR-42 shared the same means (5.72) for TFC. Minimum mean value for TFC was recorded in LR-12 (3.79). Whereas the Check variety BARS-2009 contributed to (4.92) to the TFC. Quercetin was used as a standard. Quercetin is the most common flavonoid that present in most of the food and extensively distributed in nature. It is well known for having anti-inflammatory and antiviral characteristics. Most importantly it has the antioxidant ability by acting as a scavenger of various free radicals. Aim of study was to identify the wheat landraces that were rich in TFC, as the flavonoids have great health promoting factors such as flavonoids have antioxidant, anti inflammatory, antiallergic and antiviral abilities. In another study conducted by ''van'' in 2009, they estimated the total flavonoid contents in wheat grain, they found that flavonoid content were higher in outer layer of grain[32]. Our results were not matched with that previous study as TFC vary from genotype to genotype due to difference in genetic makeup. LR-34, LR-13 and LR-16 showed maximum TPC and could be our target accessions for wheat improvement program regarding breeding for improved nutritional value.

Cluster Analysis

Hierarchical Clustering:

Twenty six genotypes including one check variety (BARS-2009) for TFC were classified into two main clusters. Cluster analysis estimated diversity that was based on Euclidean distance using Ward's method. There was two key clusters namely as I, II at linkage distance of '6'showed in figure 4. Cluster 'I' was comprised of two sub clusters 'A' and 'B'. Sub cluster 'A' was again divided into two sub-sub groups 'a1' and 'a2'. Sub-sub cluster 'a1' contained only two genotypes LR-33 and LR-36. Sub-sub cluster 'a2' was comprised of four genotypes namely LR-30, LR-38, LR-7 and LR-35. No outlier was seen in whole sub cluster 'A' of main cluster 'I'. Sub cluster 'B' included the following genotypes LR-26, LR-34, LR-13 and LR-16. In this sub cluster LR-26 and LR-34 was outliers by showing maximum divergence. Main cluster 'II' was also contained two sub groups 'C' and 'D'. Whereas sub cluster 'C' of main cluster II was again distributed into two new sub-sub clusters 'c1' and 'c2'. Sub-sub cluster 'c1' was a small cluster had two genotypes LR-10 and LR-37 due to similarities in mean values for TFC. Whereas sub-sub cluster 'c2' was large sub-sub group of sub cluster 'C' that had five different genotypes such as LR-44, LR-5, LR-42, 15 and LR-43. LR-44, LR-43 were declared as the outliers because they showed the variation. In addition sub cluster 'B' of key cluster 'II' was categorized into two new sub-sub groups 'd1' and 'd2' where 'd1' included BARS-2009, LR-11, LR-27 and LR-41. LR-11 and BARS-2009 declared as outliers. While 'd2' of main cluster 'D' had LR-6, LR-20, LR-12 and LR-45. No such outlier was noted in this sub-sub group. Table 3 showed that over all cluster 'I' had 10 members and cluster 'II" had 16 members.

Table 2: Depicting TFC mean values in different genotypes

Genotypes	TFC mg/g	Genotypes	TFC mg/g
BARS-2009	4.92	LR-27	4.70
LR-03	5.58	LR-30	5.98
LR-05	5.72	LR-33	6.45
LR-06	4.19	LR-34	8.13
LR-07	6.31	LR-35	6.27
LR-10	5.29	LR-36	6.71
LR-11	4.63	LR-37	5.25
LR-12	3.79	LR-38	6.09
LR-13	7.95	LR-41	4.67
LR-15	5.65	LR-42	5.72
LR-16	7.91	LR-43	5.47
LR-20	4.37	LR-44	5.83
LR-26	7.40	LR-45	3.94

Table 3: Members of cluster based on hierarchical clustering

Cluster No.	Members
Cluster I	LR-33, LR-36, LR-30, LR-38, LR-7, LR-35, LR-26, LR-34, LR-13, LR-16
Cluster II	LR-10, LR-37, LR-44, LR-5, LR-42, LR-15, LR-43, BARS-2009, LR-3, LR-11, LR-27, LR-41, LR-6, LR-20, LR-12, LR-45

Graphical Representation

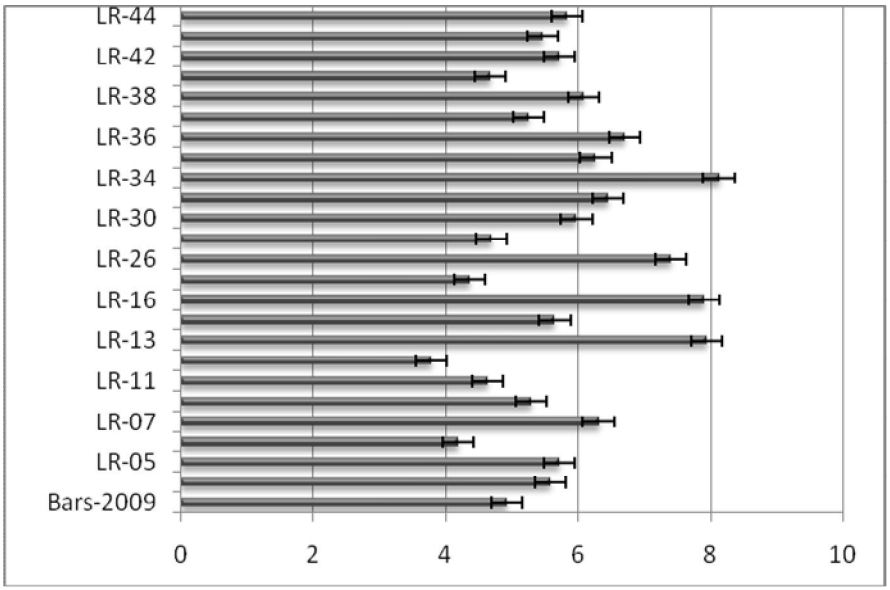

Figure.1: Diversity indicted by graphical representation

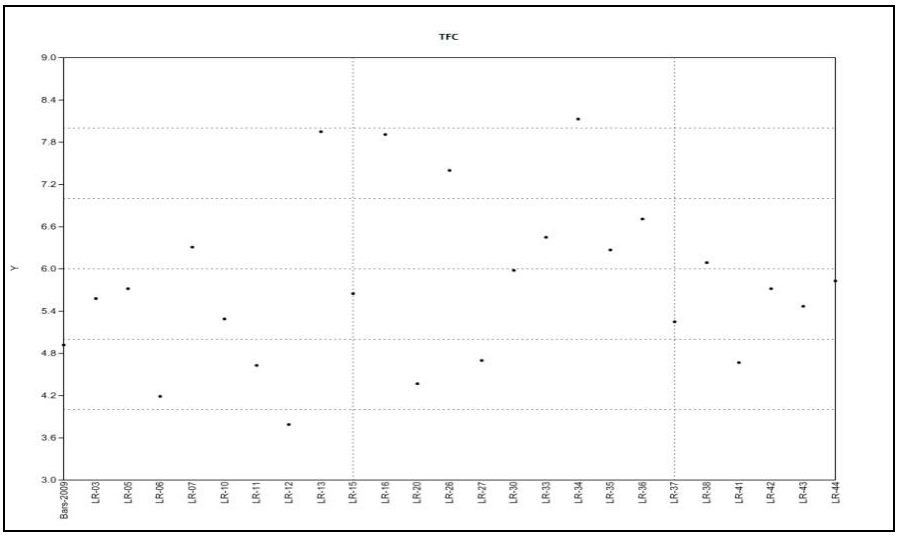

Figure.2: Point diagram showing accessions diversity for TFC

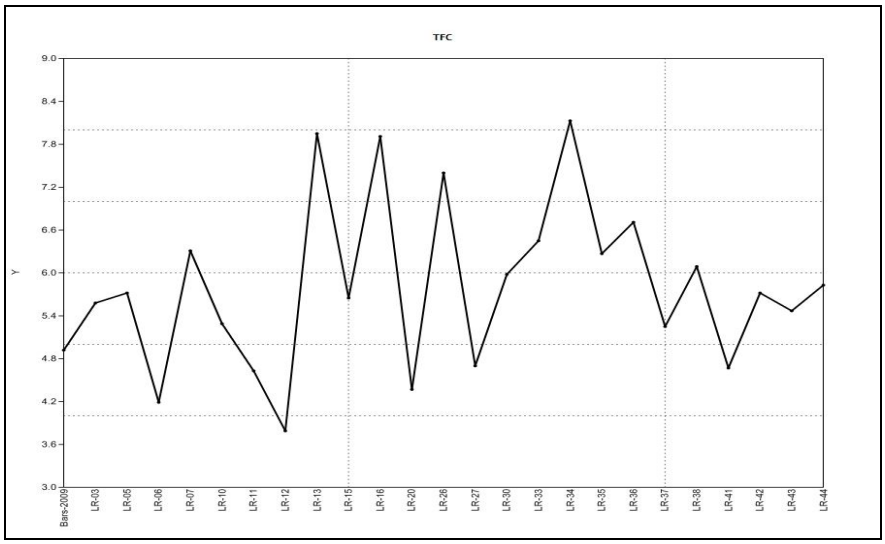

Figure.3: Line diagram showing TFC divergence among wheat accessions

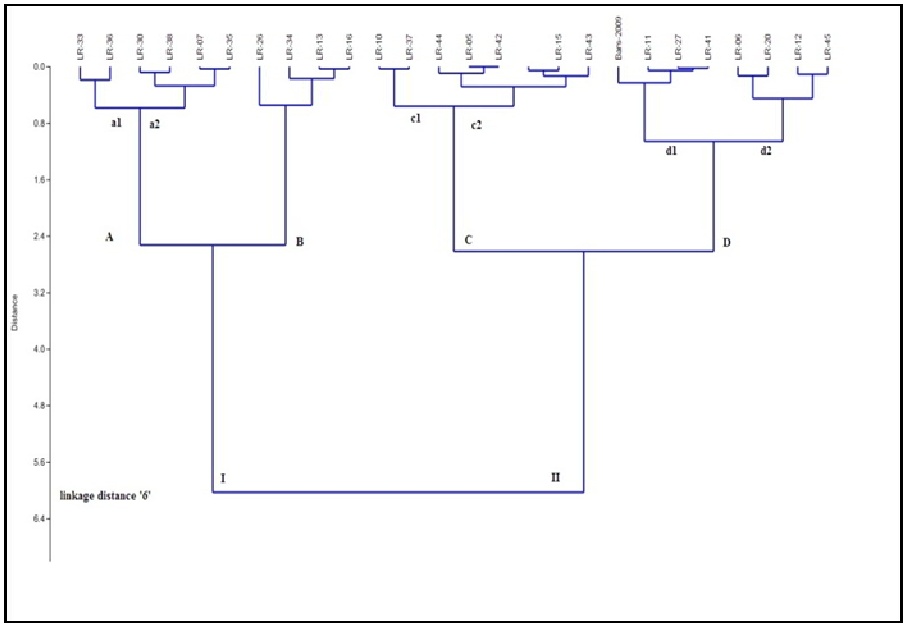

Figure.4: Dendrogram based on average linkage distance in 26 genotypes

Flavonoids are the plant secondary metabolites that protect plants against various environmental stresses, including biotic and abiotic. Among flavonoids, Quercetin is most abundant dietary flavonol, plus it is potent antioxidant because that has all right structural features for free radical scavenging activity. Epidemiological studies have proved that flavonoid intake in our diet is inversely related to mortality from coronary heart disease.

Flavonoids along with phenolic acids are abundant in the aleurone layer of wheat and other cereal, but these can be also found in embryos and seed coat of kernels. Being a staple crop assessment of diversity for biochemical traits in wheat along with quantitative and qualitative traits is of acute important to be incorporated into breeding stocks and in plant breeding program, genetic diversity is assessed by using various multivariate techniques such as cluster analysis, that is a efficient method for determining the family relationships.

In our study we used cluster analysis, graphical representation, line and point diagrams to reveal diversity among landraces for highest flavonoid..

Cluster diagram showed the most diverse or outliers depending upon the genetic distance between different landraces, while line, point and graphical studies showed most divergent landraces on the basis of their mean values. LR-26, LR-34, LR 43, LR-44, LR-11 and BARS-2009 were outliers in cluster analysis. Whereas graphical study and other diagrams showed that TFC mean values ranged from (3.99-8.13 mg/g). For TFC the maximum mean value was noted in LR-34 of 8.13 followed by the LR-13 (7.95), LR-16 (7.91) so these could be our desired landraces having highest amount of flavonoid content.

CONCLUSION

Flavonoids are one of the most vital phytochemicals mostly present in outer layer of wheat grain. They are responsible for biofunctionality of whole wheat kernel. Our research study demonstrated the importance of flavonoid and flavonoid rich wheat landraces. Our work emphasizes the need to explore flavonoid rich staple food because flavonoids are associated with many health promoting effects such as they are antioxidant, anti-viral and involve in anti-tumors activities so reduce the development of several diseases. Current study showed that LR-34, LR-13 and LR-16 had utmost TFC (8.13), (7.95) and (7.91 mg/g) respectively. Therefore these three landraces could be our desired genotypes for future studies in various rainfed areas. It is recommended that characterization of these flavonoid rich landraces should be done in future along with comparison of TFC in various commercial cultivars.

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AUTHOR'S CONTRIBUTION

Main project was of Huma Tariq. All other authors of this paper contributed equally, all helped in its discussion and involved in overall planning of work.

ABBREVIATIONS: TFC: Total flavonoid content, LR: Landrace, mg/g: Milligram per gram

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