Comparative Analysis of Pakistani Wheat Germplasm for Primary and Seconday Metabolites

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Abstract

Wheat is a major source of energy and essential components of daily diet. However, modern wheat varieties are deficient of minerals, primary and secondary metabolites. There is a need to conduct metabolic profiling of Pakistani wheat germplasm locked in germplasm banks. This study was designed to evaluate Pakistani wheat germplasm for primary and secondary metabolites. Ten Pakistani wheat landraces, collected from diverse geographic region of Pakistan, along ten wheat varieties and ten advanced CIMMYT durum wheat lines were sown in Randomized Complete Block Design (RCBD) with three replications at Gomal University D.I. Khan. Primary metabolites (starch, protein and gluten) were determined through Near Infrared Spectroscopy (NIR) while secondary metabolite anthocyanin contents were measured through Gas Chromatography- Mass spectrometry (GC-MS). Results showed that highest starch contents (60.52±0.09g/100g) were possessed by wheat variety Shahkar, while highest grain protein (14.46±0.16g/100g) and gluten contents (21.12±0.09g/100g) were possessed by landrace 011179. Similarly, highest anthocyanin contents (39.46±0.13mg/kg) were also possessed by landrace 011179. Compared to modern wheat varieties and durum wheat lines, wheat landraces exhibited better metabolic profile and significant variations in primary and secondary metabolites, especially grain protein, gluten and anthocyanin contents ($p = 0.05$). Similarly, significant variation was observed for anthocyanin expression at coleoptile, auricle and anthesis stages ($p = 0.05$). The expression of anthocyanin in F1 progenies developed from the cross of anthocyanin-rich landrace 011179 and anthocyanin-deficient Khaista variety provides novel source for use in breeding program aimed at developing nutritional superior and high yielding wheat, particularly anthocyanin-rich wheat lines.

Keywords: Wheat landrace, Durum wheat, Metabolites, Gluten, Anthocyanin.

DOI:	https://zenodo.org/records/14589204
Journal Link:	https://jai.bwo-researches.com/index.php/jwr/index
Paper Link:	https://jai.bwo-researches.com/index.php/jwr/article/view/86
Publication Process	Received: 8 Jan 2024/ Revised: 15 Mar 2024/ Accepted: 23 Mar 2024/ Published: 31 March 2024
ISSN:	Online [3007-0929], Print [3007-0910]
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Indexing:	
Publisher:	BWO Research International (15162394 Canada Inc.) https://www.bwo-researches.com

Introduction

Bread wheat (*Triticium aestivum* L) is the third largest cereal grain crop after rice and maize. The era of green revolution and post green revolution has witnessed a tremendous boost in wheat yield. Modern breeding technologies contributed to develop semi-dwarf, disease resistant and high yielding wheat cultivars with improved end use quality. However, in the pursuit of such huge wheat yield gain, the nutritional quality of wheat has been greatly compromised (Poudel *et al*., 2021). Wheat wild relatives, wheat progenitor's species and wheat landraces possess nutritional superiority in terms of primary and secondary metabolites over modern day high yielding wheat cultivars (Migliorini *et al*., 2016). Consequently, more than three billion people around the globe are suffering from hidden hunger; a situation refers to the lack of required amount of proteins, iron, zinc, minerals, vitamins and anthocyanin in daily diet. The hidden hunger is responsible for different types of disorders such as impaired growth, mental retardation, anemia and compromised immune system especially in new born and lactating women (Ikram *et al*., 2024). Thus, consumers are showing a growing demand for wheat meals enriched in nutrients, especially primary and secondary metabolites.

Metabolic profiling is a key strategy that enable us to unravel the complex interaction between living system and environmental factors, especially biotic and abiotic stresses. It also facilitates genetic, genomics, molecular and biochemical analysis to understand the complex mechanisms/pathways governing the accumulation of primary and secondary metabolites in wheat developing grain (Hawkesford *et al*., 2018). Wheat grain is a repository of various types of primary metabolites such as starch, proteins and glutens, and secondary metabolites such as anthocyanin, iron, zinc, vitamins and minerals. The combination of these metabolites in a developing wheat grain not only determines the end use quality of wheat but also the nutritional status of a particular wheat genotype (Yan et al., 2024).

Among primary metabolites, starch constitutes a large bulk of a wheat grain, estimating up to 90% of the wheat kernel endosperm. Modern breeding technologies have focused on developing wheat varieties with enhanced starch contents to provide food and energy to constantly increasing human population (Boukid *et al*., 2018). In wheat grain, starch is present mainly in the endosperm as a complex mixture of amylose and amylopectin. The distinct proportion of amylose and amylopectin is crucial to ensure special physiochemical properties of wheat flour (Sofi *et al*., 2013). Similarly, grain protein content is a key influencing factor of wheat end use quality. The bread making quality of any wheat variety is greatly influenced by the varying concentration of protein contents in wheat grain. Grain protein contents, such as gluten and non-gluten contents, are in turn influenced by genotype, ecological dynamics and their exchange (Mujeeb-Kazi 2013; Arzani and Ashraf, 2016). Among all cereals, wheat kernels possess highest amount of gluten protein that enable wheat flour to make flexible paste to cook lighten bread. This distinct flexible bread making quality has made wheat to be cultivated on the large cultivated land from east to west and made it a staple food for than half of the global human population (Wrigley, 2009).

Among secondary metabolites, iron, zinc and anthocyanin play a crucial role to defend human body against several disorders such as anemia, proper growth and development, and confer immunity against viral, bacterial and fungal pathogens. Anthocyanin belong colored phenolic compounds mainly present in colored vegetables, fruits and cereals. They are abundantly present as red, blue, purple and black pigments in colored fruits, vegetables and cereals (Haverlentova *et al*., 2014). Anthocyanin acts antioxidants and have been found to defend against metabolic disorders, inflammation, diabetes, cardiac disease, cancer, aging and obesity (Chen et al., 2013). The current study was designed to conduct comparative metabolic profiling for primary and secondary metabolites among wheat landraces, durum wheat lines and modern high yielding Pakistani wheat varieties.

Material and Method

Sampling site description

The current study was conducted at the research field of the Department of Plant Breeding and Genetic, Faculty of Agriculture, Gomal University Dera Ismail Khan. The weather of D.I. Khan is desert subtropical, with very hard summers, quite moderate winters (though they are frequently cold at night), and quite dry winters. The lowest temperature is 6°C in winter and the highest is 50°C in summer. The current study was carried out during the November 2022–June 2023 wheat growing season in the Gomal University Dera Ismail Khan agriculture department.

Materials

The germplasm used in this study comprised of total thirty genotypes of which ten were durum wheat lines, ten modern varieties and ten wheat landraces (Table 1). **Table 1** Wheat germplasm used in this study.

S. No.	Type of wheat	Acc#/ Name	S. No.	Type of wheat	Acc#/Name	S. No.	Type of wheat	Acc#/Name
1	Durum wheat	IDN-P701	11	Variety	Hashim-08	21	Landrace	011145
2	Durum wheat	IDN-718	12	Variety	AZRC Dera	22	Landrace	011154
3	Durum wheat	IDN-720	13	Variety	Khaista	23	Landrace	011166
4	Durum wheat	IDN-727	14	Variety	Wadan	24	Landrace	011169
5	Durum wheat	IDN-733	15	Variety	Akbar-19	25	Landrace	011174
6	Durum wheat	IDN-P736	16	Variety	Akbar-Punjab	26	Landrace	011175
7	Durum wheat	IDN-738	17	Variety	Shahkar	27	Landrace	011179
8	Durum wheat	IDN-P744	18	Variety	Gulzar-19	28	Landrace	011180
9	Durum wheat	IDN-P745	19	Variety	Shahid-17	29	Landrace	011181
10	Durum wheat	IDN-P750	20	Variety	Peer Sabaq-13	30	Landrace	011183

Research design

The wheat germplasm was grown in Randomized Complete Block Design (RCBD) with three replications. The length of each line was kept at 4 meter, plant to plant distance was maintained at 15 cm, and row to row distance was standardized at 30cm. The sowing was done in lines with 10–15 seeds per row being sown using a funnel.

Practices

Land was prepared using 2-3 deep ploughings accompanied by rotavator operation. There was application of regular necessary agricultural practices such as fertilizer application of recommended doses of nitrogen, phosphorous, potassium, humic acid, herbicide application and irrigation *etc*.

Primary and secondary metabolites determination

Near infrared spectroscopy (NIR)

NIR was used to determine the concentration of primary metabolites such as starch, protein and gluten according to the method devised by Vergara-Diaz *et al*., (2020). Wheat seeds were crushed into fine powder using a Retsch ZM 200 laboratory mill (Retsch, Haan, Germany), powder kept at +5°C for fourier transform infrared spectroscopy study. FT-NIR spectroscopic investigation of ground macaroni sample was (about 30 g) conducted by using all in one material workstation (Thermo Fisher Scientific Inc., Madison, WI, USA). Spectrum was obtained by utilizing 32 x spectrograph with a designation of 4 cm-1 in the 10,000-4000 cm-1 range. FT-NIR spectral data was normalized. Multivariate Statistical analysis was performed by using (The Un Scrambler) program.

Gas Chromatography- Mass Spectrometry (GC-MS)

For anthocyanin contents determination in wheat kernel, GC-MS technique was used (Prinsen *et al*., 2014), with a 0.5 mm sieve, 100 g of wheat kernels were grinded in a Teflon plastic-coated grinder, and mash in a mortar to a powder. In a Fast Prep Instrument, the metabolomes were obtained from ten to twenty mg of meal using 1 mL of eighty percent CH_3OH with 10 M five pentose de-oxy ribose alcohol as an internal reference utilizing zirconia beads of 1 mm.

Statistical analysis

Data was subjected to R-package (https://www.r-project.org/) for Analysis of Variance (ANOVA) and to calculate standard mean and stander error.

Results

Grain starch contents

All 30 wheat genotypes exhibited a wide range of variations in the mean values of kernel starch concentrations. Among durum wheat advanced lines, the highest starch contents (56.45±0.16g/100g) were possessed by IDN-P750 while lowest concentration (51.53±0.08g/100g) was possessed by IDN-738. Similarly, among wheat varieties the highest starch contents (60.52±0.09g/100g) were possessed by variety Shahkar and the lowest concentration (53.40±0.11g/100g) was possessed by variety Gulzar-19. Among landraces, the highest starch contents (56.68±0.12g/100g) were possessed by variety landrace 011174 and the lowest concentration (50.43±0.09g/100g) was possessed by landrace 011179. As a whole, modern wheat varieties used in this study exhibited better starch contents as compared to landraces and durum lines (Table 2).

Table 2 Primary and secondary metabolites contents in wheat genotypes.

S.No.	**Type of wheat**	**Genotype name**	**Mean starch contents** (g/100g)		**Mean protein contents** (g/100g)		**Mean gluten contents** (g/100g)		**Mean anthocyanin contents** (mg/kg)	
			Mean	Std. Error	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
1	Durum wheat	IDN-P701	53.46	0.10	11.65	0.13	17.33	0.17	23.38	0.10
2	Durum wheat	IDN-718	53.55	0.10	11.58	0.12	16.35	0.20	22.43	0.09
3	Durum wheat	IDN-720	52.57	0.13	11.42	0.15	18.26	0.184	22.60	0.14
4	Durum wheat	IDN-727	52.38	0.10	10.64	0.10	16.29	0.17	21.59	0.13
5	Durum wheat	IDN-733	53.63	0.13	11.54	0.14	14.29	0.21	24.36	0.10
6	Durum wheat	IDN-P736	52.56	0.12	11.69	0.09	17.19	0.12	24.69	0.05
7	Durum wheat	IDN-738	51.53	0.08	11.58	0.11	18.15	0.20	23.36	0.10
8	Durum wheat	IDN-P744	54.83	0.04	10.75	0.09	15.24	0.19	25.53	0.07
9	Durum wheat	IDN-P745	55.61	0.11	11.56	0.12	17.11	0.12	23.51	0.16
10	Durum wheat	IDN-P750	56.45	0.16	10.50	0.16	19.20	0.15	25.67	0.07
11	Variety	Hashim-08	56.76	0.10	12.73	0.08	19.17	0.20	22.42	0.11
12	Variety	AZRC Dera	57.41	0.16	12.66	0.12	18.32	0.21	25.64	0.14
13	Variety	Khaista	59.48	0.12	12.59	0.15	17.69	0.21	19.48	0.10
14	Variety	Wadan	58.66	0.12	11.81	0.04	19.26	0.23	24.47	0.14
15	Variety	Akbar-19	59.51	0.14	12.46	0.11	17.37	0.11	25.42	0.12
16	Variety	Akbar-Punjab	58.56	0.12	11.66	0.07	18.24	0.19	20.45	0.15
17	Variety	Shahkar	60.52	0.09	13.54	0.14	19.12	0.11	21.64	0.12
18	Variety	Gulzar-19	53.40	0.11	11.76	0.11	19.06	0.17	24.39	0.11
19	Variety	Shahid-17	56.69	0.12	11.52	0.04	17.33	0.27	22.67	0.13
20	Variety	Peer Sabaq-13	53.54	0.14	11.64	0.15	19.20	0.25	26.38	0.09
21	Landrace	011145	55.48	0.11	11.68	0.11	19.21	0.20	27.70	0.11
22	Landrace	011154	56.55	0.13	11.49	0.08	17.04	0.06	25.66	0.14
23	Landrace	011166	53.37	0.10	11.64	0.13	15.16	0.05	34.57	0.12
24	Landrace	011169	56.52	0.14	12.61	0.11	19.21	0.18	24.64	0.09
25	Landrace	011174	56.68	0.12	11.69	0.11	18.10	0.13	31.52	0.16
26	Landrace	011175	56.47	0.15	12.50	0.08	19.16	0.08	29.83	0.07
27	Landrace	011179	50.43	0.09	14.46	0.16	21.12	0.09	39.46	0.13
28	Landrace	011180	53.61	0.15	11.72	0.12	17.07	0.10	33.57	0.16
29	Landrace	011181	54.66	0.09	11.53	0.12	19.08	0.10	29.65	0.13
30	Landrace	011183	56.36	0.11	12.40	0.13	16.21	0.14	32.55	0.11

Grain protein contents

All 30 wheat genotypes exhibited a wide range of variations in the mean values of grain protein contents. Among durum wheat advanced lines, the highest grain protein contents (11.65±0.13g/100g) were possessed by IDN-P701 while lowest concentration (10.50±0.16g/100g) was possessed by IDN-P750. Similarly, among wheat varieties the highest grain protein contents (13.54±0.14g/100g) were possessed by variety Shahkar and the lowest concentration (11.52±0.12g/100g) was possessed by variety Shahid-17. Among landraces, the highest grain protein contents (14.46±0.16g/100g) were possessed by landrace 011179 and the lowest concentration (11.49±0.08g/100g) was possessed by landrace 011154. As a whole, wheat landraces used in this study exhibited better grain protein contents as compared to modern wheat varieties and durum lines (Table 2).

Grain gluten contents

All 30 wheat genotypes exhibited a wide range of variations in the mean values of grain gluten contents. Among durum wheat advanced lines, the highest grain protein contents (19.20±0.15g/100g) were possessed by IDN-P750 while lowest concentration (15.24±0.19g/100g) was possessed by IDN-P744. Similarly, among wheat varieties the highest grain gluten contents (19.26±0.23g/100g) were possessed by variety Wadan and the lowest concentration (17.33±0.27g/100g) was possessed by variety Shahid-17. Among landraces, the highest grain protein contents (21.12±0.09g/100g) were possessed by landrace 011179 and the lowest concentration (15.16±0.05g/100g) was possessed by landrace 011166. As a whole, wheat landraces used in this study exhibited better grain gluten contents as compared to modern wheat varieties and durum lines (Table 2).

Grain anthocyanin contents

All 30 wheat genotypes exhibited a wide range of variations in the mean values of grain anthocyanin contents. Among durum wheat advanced lines, the highest grain anthocyanin contents (25.67±0.07mg/kg) were possessed by IDN-P750 while lowest concentration (21.59±0.13mg/kg) was possessed by IDN-727. Similarly, among wheat varieties the highest grain anthocyanin contents (26.38±0.09mg/kg) were possessed by variety Peer Sabaq-13 and the lowest concentration (19.48±0.10mg/kg) was possessed by variety Khaista. Among landraces, the highest grain anthocyanin contents (39.46±0.13g/100g) were possessed by landrace 011179 and the lowest concentration (24.64±0.09mg/kg) was possessed by landrace 011169. As a whole, wheat landraces used in this study exhibited better grain anthocyanin contents as compared to modern wheat varieties and durum lines (Table 2).

Anthocyanin accumulation in seed, coleoptile and auricle

Anthocyanin expression and accumulation were observed in mature seeds and coleoptile. Majority of the wheat varieties and durum lines appeared white possessing trace amount of anthocyanin while majority of the landraces possessed high anthocyanin contents and turned red, dark red and light purple. The same anthocyanin accumulation trend was also observed in coleoptile (grown in pots) in which the coleoptile of anthocyanin rich genotypes appeared dark purple and those deficient in anthocyanin appeared green (Fig. 1).

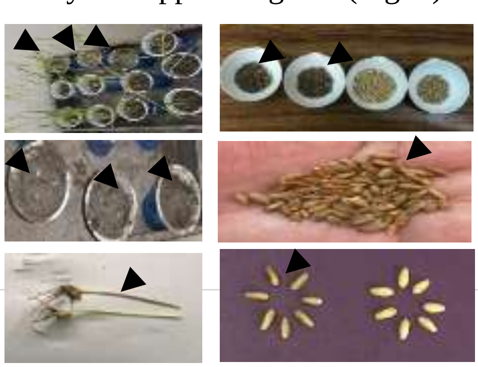

Fig. 1 Anthocyanin expression and accumulation in seeds and coleoptile. Black arrows represent anthocyanin color in seeds and coleoptile of anthocyanin-rich genotypes.

Similarly, the auricle of anthocyanin-rich genotypes appeared dark purple and those of anthocyanin-deficient genotypes turned green. Moreover, majority of the wheat varieties and durum lines used in this study exhibited green auricle (89%), showing trace amounts of anthocyanin while majority of the wheat landraces showed either moderately purple auricle (7%) or strong purple (4%), representing high anthocyanin accumulation in auricles (Fig. 2).

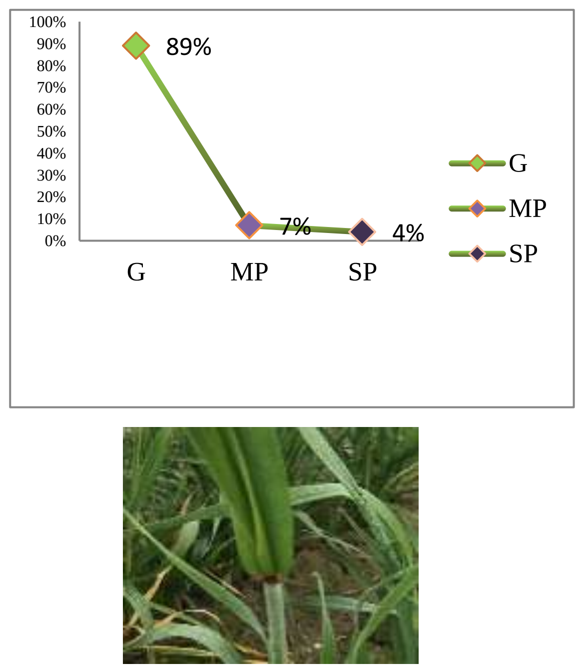

Fig. 2 Anthocyanin expression and accumulation in auricle. **G**: green, **MP**: moderately purple, **SP**: strong purple.

Anthocyanin expression in F_1 progenies seeds and auricle

The cross of anthocyanin-deficient variety Khaista (white colored seeds and green auricle) with anthocyanin-rich landrace 011179 (dark red colored seeds and dark purple colored auricle) produced F_1 progenies having dark purple colored seeds showing successful transfer of anthocyanin from landrace donor parent to recipient variety Khaista. The F_1 seeds were grown into F_1 plants during wheat season 2023-2024 which exhibited dark purple auricle, showing a stable transfer of the trait into next generation (Fig. 3).

Fig. 3 Expression of anthocyanin in the seeds and auricle of F_1 progenies.

Discussion

Wheat is the staple food of more than half of the global human population. Wheat is an important cereal crop offering a series of primary and secondary metabolites such as starch, proteins, glutens, fibers, minerals, vitamins, Fe, Zn and anthocyanin. Primary metabolites act as primary reservoirs of energy and food for human. On the other hand, secondary metabolites such as Fe, Zn, vitamins are important components of wheat kernel that ensure proper growth and development, nourish blood with Fe contents and safeguard against different bacterial, viral and fungal pathogens (Balk *et al*., 2019; Gupta *et al*., 2021; Ikram *et al*., 2024). To feed the constantly growing human population, wheat breeders have developed semi-dwarf, disease resistant and high yielding wheat varieties. Selecting breeding for high yield potential caused a major breakthrough in wheat yield what is commonly known as "Green revolution" during 1960-1970. However, the breeders completely neglected the nutritional quality in breeding programs, especially Fe, Zn, vitamins, minerals and anthocyanin. Thus, majority of the wheat varieties developed during and post-green revolution lack or possess trace amounts of these secondary metabolites. In our study, the highest amount primary metabolites such as grain protein and gluten, and secondary metabolite anthocyanin were possessed by wheat landraces as compared to wheat varieties and durum wheat lines (Table 2). Our results are in accordance with the findings of Balk *et al*., 2019; Gupta *et al*., 2021 and Ikram *et al*., 2024).

Anthocyanin are phenolic derivatives and expressed as secondary metabolites in different parts of wheat such as coleoptile, auricle and mature kernels. They accumulate in aleurone and pericarp layers of colored wheat kernels, conferring red, blue, purple and black colors. Previously, high anthocyanin accumulation has been found wheat progenitor species, tetraploid wheat and landraces while modern wheat varieties lack anthocyanin contents (Abdel-Aal *et al*., 2008; Knievel *et al*., 2011). Our findings also confirmed highest accumulation of anthocyanin in wheat landraces, especially landrace 011179 being the most anthocyanin-rich genotype containing (39.46±0.13mg/kg) of anthocyanin. Breeding efforts have been made to successfully transfer anthocyanin from low yielding colored wheat lines into high yielding but low anthocyanin lines. The resultant hybrid progenies experienced high accumulation of anthocyanin (Varga *et al*., 2013; Monika *et al*., 2016; Gordeeva *et al*., 2019). In our study, the cross of anthocyanin rich landrace 011179 with anthocyanin-deficient variety Khaista produced F_1 progeny with enhanced anthocyanin level (Fig. 3), thereby supporting the previous findings of Varga *et al*., 2013; Monika *et al*., 2016 and Gordeeva *et al*., 2019.

Conclusion

Among 30 genotypes investigated for primary and secondary metabolites in this study, the highest starch contents (60.52±0.09g/100g) were possessed by wheat variety Shahkar, while highest grain protein (14.46±0.16g/100g) and gluten contents (21.12±0.09g/100g) were possessed by landrace 011179. Similarly, highest anthocyanin contents (39.46±0.13mg/kg) were also possessed by landrace 011179. Compared to modern wheat varieties and durum wheat lines, wheat landraces exhibited better metabolic profile and significant variations in primary and secondary metabolites, especially grain protein, gluten and anthocyanin contents ($p = 0.05$). Similarly, significant variation was observed for anthocyanin expression at coleoptile, auricle and anthesis stages ($p = 0.05$). The expression of anthocyanin in F_1 progenies developed from the cross of anthocyanin-rich landrace 011179 and anthocyanin-deficient Khaista variety provides novel source for use in breeding program aimed at developing nutritional superior and high yielding wheat, particularly anthocyanin-rich wheat lines.

Conflicts of interest

The authors declare no conflicts of interest.

Authors contribution

Nasr Ullah Khan conceived the idea, designed the study and drafted the manuscript. Maham Jamshed and Saeed Ur Rehman conducted the experiments and collected the data. Muhammad Anas Khan and Abu Baker Sidiq helped in data collection and data analysis.

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