Journal of Cereal Research
Volume 14 (3): 308-320
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR
Research Article

Identification of wheat genotypes with higher levels of antioxidant properties across environments in India

Sunil Kumar*, Priti, Sewa Ram, Om Parkash Gupta, Sendhil Ramdas, Vanita Pandey and Gyanendra Pratap Singh

Division of Quality & Basic Sciences, PO Box-158, Agrasain Marg, ICAR-Indian Institute of Wheat & Barley Research, Karnal-132001 (India)

Article history:
Received: 06 Sept., 2022
Revised: 13 Nov., 2022
Accepted: 11 Dec., 2022

Citation:
Kumar S, Priti, S Ram, OP Gupta, Sendhil R, V Pandey and GP Singh. 2022. Identification of wheat genotypes with higher levels of antioxidant properties across environments in India. *Journal of Cereal Research* ***14 (3):*** *308-320.* http://doi.org/10.25174/2582-2675/2022/124827

****Corresponding author:***
E-mail: sunil.kumar212@icar.gov.in

Abstract

Wheat is an important source of energy and nutrition including antioxidants having health benefits to humans. In this investigation, 19 and 12 prominent wheat genotypes grown (during cropping season 2019-20) at three locations in two production environments representing North-Western Plains Zone (NWPZ) and Central Zone (CZ), respectively, were used for evaluating their antioxidant potential. There was a significant positive correlation between total soluble phenol and ABTS activity ($p<0.01$) while non-significant with DPPH scavenging activity in NWPZ genotype. However, there was a significant positive correlation of total soluble phenol with DPPH ($p<0.01$) and ABTS ($p<0.05$) radical scavenging activities in CZ genotypes. The genotypes HI 1628 and PBW 771 of NWPZ and HI 1634, CG 1029 and GW 322 of CZ exhibited higher mean phenolic content and antioxidant potential and were found comparatively stable across their environments with respect to the parameters tested. Overall, the Karnal location (NWPZ) was shown to have higher phenolic content and ABTS activity compared to genotypes grown at different locations of both the zones. The additive main effects and multiplicative interaction-based ANOVA indicated highly significant effect of environment, genotype and genotype(x) environment interactions on soluble phenolics and antioxidants among genotypes in both the zones. The identified genotypes can be used for cultivation and improving nutritional value.

Key words: Whole meal flour, Soluble phenol, Trolox equivalent antioxidant capacity, GGE biplot, AMMI, Stability, Correlation studies

1. Introduction

Recently there is surge in demand of food products high in bioactive compounds because of their role in thwarting various chronic diseases (Moore *et al.,* 2006; Sadeer *et al.,* 2020; Tan *et al.,* 2018). Bioactive compounds play an active role in reducing reactive oxygen species (ROS) generated during normal metabolic processes. The free radicals are the by-products of inescapable seepage of electrons on to molecular oxygen during the electron transport activities in the cellular arena *viz.* plasma membrane, chloroplast and mitochondria (Kumar and Malhotra, 2008; Kumar *et al.,* 2011). The reduction of molecular oxygen to water requires four electrons, but ironically these electrons are taken up one by one and not as a whole, thus leading to the generation of reactive oxygen species (ROS) during normal metabolism; notable among those are $^{\cdot}O_2^-$ (superoxide anion), H_2O_2 (hydrogen peroxide), $^{\cdot}OH^{\cdot}$ (hydroxyl radical) and $^{\cdot}O_2^{'}$ (singlet oxygen). The ROS accumulation in turn, opens a

pandora of deteriorative changes in several biomolecules, *viz.* DNA, protein, enzyme and lipids in the form of mutation, denaturation, inactivation and peroxidation, respectively (Regoli and Winston, 1999). Ultimately, this may lead to the augmented occurrences of a panoply of degenerative diseases like cancer, cardiovascular diseases, brain and neural dysfunction, and arthritis *etc.* (Sadeer *et al.,* 2020). Although, the human body comprehends several endogenous antioxidant systems, yet a better-quality diet can impart an imperative share of exogenous antioxidants to maintain the redox status of the entity in question (Narwal *et al.,* 2014). Among cereals' health-promoting phytochemicals, phenolic compounds have been considered among scientific research in full swing owing to their potent antioxidant behaviour (Gu *et al.,* 2019; Žilić, 2016).

Globally, wheat is one of the main sources of nutrients, contributing more than 20% of the required calories and proteins to most public across the world (Braun *et al.,* 2010). India is the second-largest producer of wheat in the world where a substantial part of human population consumes wheat grains as energy and nutrient source. Apart from being a stockpile of essential nutrients like carbohydrates and proteins, wheat contains significant amount of antioxidants activity (Narwal *et al.,* 2014; Sedej *et al.,* 2011). Phenolics, such as ferulic, vanillic, and caffeic acids *etc.*, are the major antioxidants in wheat and concentrated mainly in its outer bran layer and might play significant role in antioxidant mediated health benefits (Adom *et al.,* 2003; Beta *et al.,* 2005; Moore *et al.,* 2006). Generally, phenolic compounds encompass phenolic acids, flavonoids, stilbenes, coumarins, lignans and tannins; still, in cereals, the former duo represent the most abundant phenolic compounds (Žilić *et al.,* 2011). Phenolic acids' antioxidant properties are attributable to their reactive hydroxyl substituent on the aromatic ring of phenol moiety (Žilić, 2016). The concentration of cereal phenolic compounds is influenced by the types of the compound and also the cultivars and grain part where these are concentrated (Žilić *et al.,* 2011; Žilić *et al.,* 2012). Many of these phenolics scavenge or neutralize free radicals and obviate oxidative mediated damage to cellular proteins, DNA, and lipids. This, in turn, leads to the inhibition of diseases like cancer and cardiovascular ailments, which may be caused or exacerbated once the disease has progressed as a consequence of mounting cellular oxidative stress (Verma *et al.,* 2008).

Since wheat is an important source of energy and nutrition to large part of Indian population, the knowledge of antioxidant potential of different wheat varieties will help in improving wheat for health benefits. Traditionally, wheat cultivars have been developed taking into account of agronomic features such as yield and pathogen resistance and some quality traits for end product quality. However, phenolic content and antioxidant capacity have not been used as criteria for developing varieties. Therefore, understanding the phenolic and antioxidant characteristics of the diverse wheat genotypes will lead to enhanced health benefits for large population in the country. Furthermore, the levels of phenolic compounds as well as the antioxidant properties can appreciably vary due to environmental growing conditions (Moore *et al.,* 2006; Verma *et al.,* 2008). Therefore, in the present investigation, the soluble phenolic content and antioxidant potential of different wheat varieties of three different locations each of the North-western plain zone and Central zone in India, were evaluated.

2. Materials and methods

2.1 Materials

Nineteen and twelve released wheat cultivars were selected and grown in three production environment/ locations of North-Western Plains Zone (NWPZ) (*viz.* Karnal, Ludhiana and Pantnagar) and Central Zone (CZ) (viz. Junagarh, Powarkhera and Vijapur) of India, respectively, the details of which regarding location, growing condition *etc.* have been furnished in Table 1. The cultivars selected were some prominent genotypes of the last two decades having superior yield or other superior agronomic traits. The mentioned genotypes were grown during the cropping season 2019-20 at respective locations with the recommended package of practice. The conditions of sowing included: Irrigated timely sown (ITS), Restricted irrigation timely sown (RITS) and Irrigated late sown (ILS); the respective details of each genotype concerning sowing conditions have been elaborated in Table 1. The chemicals, ABTS, DPPH, potassium persulfate, Trolox, and gallic acid were purchased from Sigma-Aldrich company. All other chemicals and solvents were of the highest analytical grade.

Table 1: Varieties of bread wheat employed for experimentation in North-Western Plains Zone and Central Zone grown at three different locations

Zone (Centres)	Growing condition	Sample code	Genotype	Year of release (Pedigree)
North Western Plains Zone (Ludhiana, Karnal, Pantnagar)	ITS	S1	DBW 88	2014 (KAUZ//ALTAR84/AOS/3/MILAN/ KAUZ/4/ HUITES)
		S2	DBW 187	2019 (NAC/TH.AC//3*PVN/3/MIRLO/BUC/ 4/2*PASTOR/5/KACHU/6/KACHU (45th IBWSN-1316)
		S3	HD 2967	2011 (ALD/COC//URESH/HD2160M/HD2278)
		S4	WH 1105	2013 (MILAN/S87230//BABAX)
		S5	DBW 222	2020 (KACHU/SAUAL/8/ATTILA*2/PBW65 /6/ PVN//CAR422/ANA/5/BOW/CROW//BUC/PVN/3/ YR/4/TRAP#1/7/ATTILA/2*PASTOR)
		S6	HD 3086	2014 (DBW14/HD2733//HUW468)
		S7	PBW 550	2008 (WH 594/RAJ 3856//W 485)
	ILS	S8	HD 3059	2013 (KAUZ//ALTAR84/AOS/3/MILAN /KAUZ/4/ HUITES)
		S9	DBW 173	2018 (KAUZ/AA//KAUZ/PBW602)
		S10	WH 1021	2008 (NYOT95(GW 296)/SONAK)
		S11	PBW 771	2020 (BW 3246/2*DBW17)
		S12	HD 3298	2021 (CL1449/PBW343//CL882/HD2009)
		S13	WH 1124	2014 (MUNIA/CHTO/AMSEL)
	RITS	S14	HD 3043	2012 (PJN/BOW//OPATA*2/3/CROC_1/ *Ae.squarrosa*(224)//OPATA)
		S15	PBW 644	2012 (PBW175/HD2643)
		S16	HI 1628	2020 (FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/ TRAP// KAUZ/5/PFAU/WEAVER//BRAMBLING)
		S17	WH 1080	2011 (21STSAWSN151)
		S18	WH 1142	2015 (CHEN/AEGILOPS SQUARROSA (TAUS)// FCT/3/2*WEAVER)
		S19	NIAW 3170	2020 (SKOLL/ROLF07)
Central Zone (Junagarh, Powarkhera, Vijapur	ILS	S1	HI 1634	2021 (GW 322/PBW 498)
		S2	HD 2932	2008 (KAUZ/STAR//HD 2643)
		S3	MP 3336	2013 (HD 2402/GW 173)
		S4	HD 2864	2005 (DL 509-2/ DL 377-8)
		S5	CG 1029	2021 (HW 2004/ PHS 725)
	ITS	S6	GW 322	2002 (PBW 173/GW 196)
		S7	HI 1544	2008 (HD2402/HW 3007)

RITS	S8	MP 3288	2011 (DOVE/BUC/DL 788-2)
	S9	HI 8627 (D)	2007 (HD 4672 / PDW 233)
	S10	UAS 466 (D)	2020 (Amruth/Bijaga Yellow//AKDW 2997-16)
	S11	DBW 110	2015 (KIRITAT/4/2*SERI*2/3/KAUZ*2/ BOW// KAUZ)
	S12	DDW 47 (D)	2020 (PBW34/RAJ1555//PDW314)

ITS: Irrigated timely sown; RITS: Restricted irrigation timely sown; ILS: Irrigated late sown; D = Durum

2.2 Sample preparation and extraction

The whole-meal was prepared in the Cyclotec-1093 mill (Tecator) using a 0.5 mm screen. The antioxidant activity and soluble phenolics were analyzed in whole-meal flour. The extraction was done as per Beta *et al.* (2005) with minor modifications. Methanol extracts were prepared by adding 1.0 mL of 80% methanol to 0.1 g of each flour sample and mixing followed by constant shaking for 2 h in order to release the soluble phenolic. After shaking, the contents were centrifuged at 5000xg for 10 min (Hermle Z383K, India). The supernatants obtained were collected as methanolic extracts and used to estimate the phenols and total antioxidant activity. Unless stated otherwise, all the estimations were performed in four replicates.

2.3 Total soluble phenolic content

The total soluble phenolic content of 80% methanolic extracts was assessed as described by Singleton *et al., (*1999). Briefly, 50 µL of the extracts were mixed with 1.55 mL of water and oxidized with 100 µL of the Folin-Ciocalteu reagent (1 N). After 5 min, the reaction was stopped by adding 300 µL of 20% sodium carbonate solution followed by incubation at 40 °C in a water bath for 30 min. The absorbance was recorded at 765 nm (Systronics UV-Vis, 2202, India) and the concentration of the soluble phenolic compound was determined against the gallic acid standard and the content was expressed as micrograms of gallic acid equivalent (GAE) g^{-1} d. wt. (dry weight) of whole meal flour.

2.4 Determination of total antioxidant activity

2.4.1 ABTS assay

The radical cation $ABTS^{\cdot+}$ scavenging activity was determined by following Re *et al.* (1999). For the ABTS reagent, 7 mM ABTS was dissolved in water (stock solution), added 2.45 mM potassium persulphate and allowed the reaction to occur by keeping the mixture in the dark for nearly 16 h. The reagent was diluted with methanol just before using to an OD value of 0.7 at 734 nm against blank. The methanol extract (10 µL) was reacted with 1000 µL of ABTS reagent. The absorbance was recorded after 6 min using 80% methanol as blank at 734 nm. The ABTS radical scavenging activity was calculated as % discolouration of sample and expressed as Trolox equivalent antioxidant capacity (TEAC) and defined as nmols of TEAC g^{-1} d. wt. of whole meal flour.

2.4.2 DPPH assay

The free radical scavenging activity was measured using DPPH, a stable free radical (Beta *et al.,* 2005). The methanol extract (25 µL) was reacted with 975 µL of 6×10^{-5} mol L^{-1} of DPPH solution. The absorbance was recorded after 30 min using 80% methanol as blank at 515 nm. Antioxidant activity was calculated as % discolouration of the sample and radical scavenging activity was expressed as Trolox equivalent antioxidant capacity (TEAC) and defined as nmols of TEAC g^{-1} d. wt. of whole meal flour.

2.5 Statistical analysis

Data was reported as the mean ± standard error of the deviation for each sample. The ANOVA, two-tailed 't' test and Pearson's correlation were performed using the SPSS software (version 26.0) to identify differences between values, location *etc.* Least significant difference was calculated using Statistical Tools for Agricultural Research (STAR) software. Principal component analysis and GGE biplot analysis were performed using the GEA-R (G x E Analysis with R) software (version 4.1) to study variations in soluble phenolic and antioxidant activity between selected genotypes under investigation, interactions of genotype (G), environment (E) and G x E, and the stability of genotypes across various locations.The statistical significance was declared at $P<0.05$ (significant) and $P<0.01$ (highly significant).

3. Results & Discussion

There has always been a quest to evaluate the food commodity for its antioxidant potential and components, which can augment the deterrence to various human diseases prevalent in recent times including heart disease and cancer (Laddomada *et al.,* 2015; Sadeer *et al.,* 2020). India is the second largest producer of wheat in the world and a large number of varieties have been developed with different genotypic and phenotypic traits across the environments. Selected wheat varieties grown under different agro-climatic conditions at three different locations in each of NWPZ and CZ were evaluated for their total antioxidant activity and soluble phenol content. The antioxidant activity was measured by the employment of two radical systems *i.e.,* ABTS and DPPH radical scavenging antioxidant capacities in the present study thus,making it more informative (Narwal *et al.,* 2014; Opitz *et al.,* 2014; Sadeer *et al.,* 2020).

3.1 Effect of location on the content of soluble phenolics and antioxidant capacity of wheat genotypes representing NWPZ

The data for soluble phenolic content, ABTS and DPPH radical scavenging activity of whole meal flour has been elaborated in Table 2. Average phenolic content of whole meal flour ranged from 764±12 (DBW 222; S5) to 1086±20 (PBW 771; S11) µg GAE g^{-1}d. wt. basis (Table 2). Overall, Karnal location (1322±20) had nearly two times higher mean phenolic content compared to Ludhiana (664±20) and Pantnagar (664±40) locations in terms of GAE g^{-1} d. wt. basis. However, the DPPH trolox equivalent antioxidant capacity (TEAC) of phenolic extract ranged from 626±79 (WH 1080; S17) to 1150±43 (PBW 771; S11) n mols TEAC g^{-1} d. wt. basis (Table 2). Among NWPZ environment, Pantnagar location had the highest mean DPPH activity (998±68) followed by Karnal (932±31), and Ludhiana (823±52) locations expressed as nmols TEAC g^{-1} d. wt. basis. The ABTS TEAC of phenolic extract ranged from 4634±151 (PBW 771; S11) to 5716±146 (WH 1080; S17) nmols TEAC g^{-1} d. wt. basis (Table 2). The Karnal location had the highest mean ABTS activity (5328±113) followed by Ludhiana (5039±150), and Pantnagar (4966±142) locations expressed as nmols TEAC g^{-1} d. wt. basis for NWPZ genotypes. Pearson's correlation studies showed a significant correlation of soluble phenol (r= 0.278**) with ABTS activity while non-significant correlation (r= 0.117) with DPPH radical scavenging activity. However, the ABTS and DPPH activities showed a significant negative correlation (r= -0.213**). Principal component analysis was carried out to identify better performing genotypes using software-mediated simulations (GEA-R, version 4.1) taking stability, overall mean and ranking of genotypes into considerations (Verma and Singh, 2021).

The analysis of variance based on additive main effects and multiplicative interaction (AMMI) indicated highly significant environment, genotype and genotype-environment (GxE) interactions, with a total variation of 17.65, 47.35 and 35.01%, respectively for ABTS; the corresponding variations were 10.03, 37.29 and 52.68%, for DPPH radical scavenging activity (Table 3). However, in case of phenols, the environment had maximum contribution (81.71%) to variation followed by GxE (11.59%) and G (6.70%) (Table 3). The GGE biplots "Mean vs. stability" of total soluble phenol (1a), DPPH (1b) and ABTS (1c) for NWPZ explain the stability of various genotypes with respect to their mean performance (Yan and Tinker, 2006) (Fig. 1). The highest mean values were reported with PBW 771 (S11) for phenol and DPPH while WH 1080 (S17) for ABTS activity (Fig. 1; Table 2). On the basis of the performance of various genotypes with respect to their phenolic content and ABTS activity, HI 1628 (S16) was proved most stable while with respect to DPPH radical scavenging activity, WH 1124 (S13) was found most stable across environments.The genotype PBW 771 (S11) (with highest mean for phenols and DPPH) was found having comparable stability with respective stable genotypes for DPPH (3rd next) and phenol (4thnext). The genotype WH 1080 (S17) (with highest mean values for ABTS) was 4th most stable genotype(Fig. 1; Table 2).

Table 2: Soluble phenolic content and trolox equivalent antioxidant activity of various genotypes from three different locations of North-Western Plains Zone

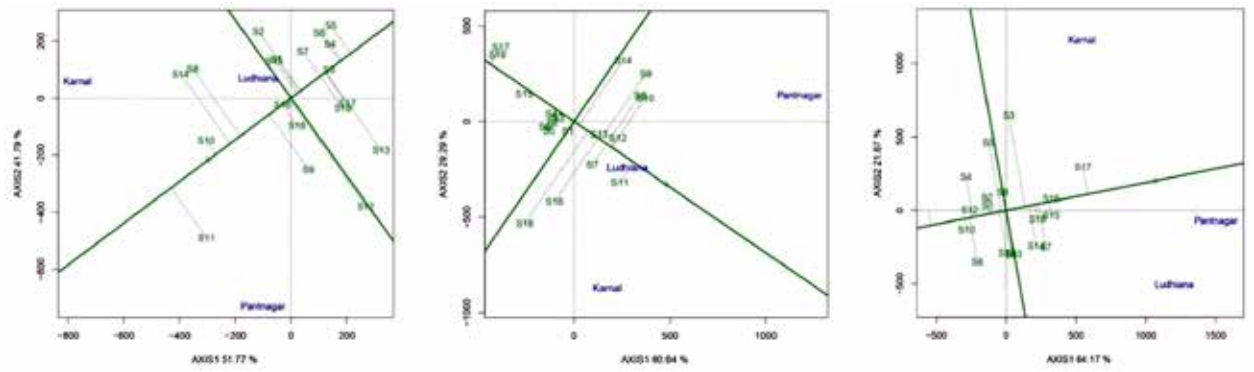
Genotype	Sample code	Soluble Phenolic content (µg GAE g^{-1} d. wt.)				DPPH radical scavenging activity (nmols TEAC g^{-1} d. wt.)				ABTS radical scavenging activity (nmols TEAC g^{-1} d. wt.)			
		Karnal	Ludhiana	Pantnagar	Mean	Karnal	Ludhiana	Pantnagar	Mean	Karnal	Ludhiana	Pantnagar	Mean
DBW 88	S1	(1377±25)^{e}	(629±27)^{f,g}	(555±31)^{h,I,j}	(854±28)	(920±33)^{f}	(982±27)^{a,b,c}	(942±17)^{f}	(948±26)	(5262±84)^{c,d,e,f}	(4795±177)^{g,h}	(4862±122)^{e,f,g,h}	(4973±128)
DBW 187	S2	(1442±17)^{d}	(588±42)^{h}	(477±26)^{j,k}	(836±28)	(900±20)^{f,g}	(885±30)^{d}	(860±54)^{f,g}	(882±35)	(5334±113)^{c,d,e}	(4819±172)^{f,g,h}	(4814±201)^{f,g,h}	(4989±162)
HD 2967	S3	(1213±11)^{i}	(588±11)^{h}	(579±38)^{h,i}	(793±20)	(854±15)^{g,h}	(978±16)^{b,c}	(884±43)^{f,g}	(905±24)	(5894±78)^{a}	(4658±37)^{h,i}	(5096±16)^{c,d}	(5216±44)
WH 1105	S4	(1210±35)^{i}		(507±24)^{I,j,k}	(773±22)	(923±58)^{f}	(699±91)^{f,g,h}	(870±115)^{f,g}	(831±88)	(5334±121)^{c,d,e}	(4449±119)^{i}	(4733±119)^{g,h,i}	(4839±120)
DBW 222	S5	(1206±16)^{i}	(628±10)^{f,g}	(457±11)^{k}	(764±12)§	(989±30)^{e}	(724±14)^{e,f,g}	(846±47)^{f,g}	(853±30)	(5758±66)^{a}	(4843±106)^{e,f,g,h}	(4685±156)^{h,i}	(5095±109)
HD 3086	S6	(1247±15)^{h}	(587±34)^{h}	(476±5)^{j,k}	(770±18)	(1003±15)^{e}	(619±120)^{h}	(829±41)^{g}	(817±59)	(4837±58)^{j}	(4808±422)^{g,h}	(4822±180)^{f,g,h}	(4822±220)
PBW 550	S7	(1285±9)^{g}	(697±9)^{c,d}	(534±12)^{h,I,j,k}	(839±10)	(1143±46)^{c}	(812±21)^{d,e}	(1100±144)^{e}	(1018±71)	(5286±120)^{c,d,e,f}	(5590±69)^{a,b}	(5226±150)^{b,c}	(5367±113)
HD 3059	S8	(1625±17)^{b}	(770±37)^{a}	(594±78)^{g,h}	(996±44)	(831±15)^{h}	(895±36)^{c,d}	(1412±43)^{a,b}	(1046±31)	(4957±170)^{I,j}	(4931±107)^{e,f,g}	(5234±237)^{b,c}	(5041±171)
DBW 173	S9		(593±8)^{h}	(859±38)^{c}	(908±29)	(782±46)^{i}	(751±56)^{e,f,g}	(1466±85)^{a}	(1000±62)	(5390±144)^{b,c,d}	(4851±108)^{e,f,g,h}	(5024±62)^{c,d,e,f}	(5088±105)
WH 1021	S10	(1591±14)^{c}	(656±15)^{e,f}	(788±10)^{c,d}	(1012±13)	(822±38)^{h,i}	(975±73)^{b,c}	(1436±110)^{a,b}	(1078±74)	(5072±121)^{g,h,i}	(4795±112)^{g,h}	(4580±119)^{i}	(4816±117)
PBW 771	S11	(1588±20)^{c}	(612±19)^{g,h}	(1058±23)^{a}	(1086±20)$^{#}$	(1172±35)^{b,c}	(1030±83)^{a,b}	(1247±11)^{c,d}	(1150±43)$^{#}$	(5174±106)^{e,f,g,h}	(4762±200)^{g,h}	(3967±147)^{k}	(4634±151)§
HD 3298	S12	(1086±24)^{k}	(580±19)^{h}	(957±204)^{b}	(874±83)	(1052±25)^{d}	(768±19)^{e,f}	(1271±30)^{c,d}	(1030±25)	(5334±142)^{c,d,e}	(5076±79)^{d,e}	(4348±109)^{j}	(4919±110)
WH 1124	S13	(1029±8)^{l}	(670±11)^{d,e}	(802±8)^{c,d}	(834±9)	(1057±27)^{d}	(663±42)^{g,h}	(1165±118)^{d,e}	(962±63)	(5125±146)^{f,g,h,i}	(5349±212)^{b,c}	(4928±205)^{d,e,f,g}	(5134±188)
HD 3043	S14	(1664±24)^{a}	(774±12)^{a}	(609±36)^{f,g,h}	(1016±24)	(711±28)^{j}	(746±33)^{e,f,g}	(1329±83)^{b,c}	(929±48)	(5222±26)^{d,e,f,g}	(5421±156)^{a,b,c}	(5210±165)^{b,c}	(5284±116)
PBW 644	S15		(736±40)^{b}	(564±24)^{h,i}	(890±26)	(817±25)^{h,i}	(692±44)^{f,g,h}	(705±103)^{h}	(738±58)	(5366±151)^{c,d}	(5188±93)^{c,d}	(5574±149)^{a}	(5376±131)
HI 1628	S16	(1346±9)^{f}	(696±22)^{c,d}	(684±32)^{e,f}	(909±21)	(1204±21)^{b}	(1069±47)^{a}	(829±48)^{g}	(1034±39)	(5550±147)^{b}	(5333±136)^{c}	(5409±91)^{a,b}	(5430±125)
WH 1080	S17	(1135±35)^{j}	(722±14)^{b,c}	(677±34)^{e,f,g}	(844±27)	(579±36)^{k}	(727±73)^{e,f,g}	(571±129)^{i}	(626±79)§	(5918±106)^{a}	(5638±190)^{a}	(5591±142)^{a}	(5716±146)$^{#}$
WH 1142	S18	(1286±19)^{g}	(785±22)^{a}	(744±54)^{d,e}	(938±32)	(1344±32)^{a}	(881±137)^{d}	(653±55)^{h,i}	(959±75)	(5414±150)^{b,c}	(5389±137)^{b,c}	(5193±178)^{c}	(5332±155)
NIAW 3170	S19	(1151±25)^{j}	(704±21)^{b,c}	(689±73)^{e,f}	(848±40)	(602±38)^{k}	(751±28)^{e,f,g}	(545±22)^{i}	(633±29)	(5013±106)^{h,i}	(5052±217)^{d,e,f}	(5061±144)^{c,d,e}	(5042±156)
Mean		(1322±20)	(664±20)	(664±40)	(883±27)	(932±31)	(823±52)	(998±68)	(918±50)	(5328±113)	(5039±150)	(4966±142)	(5111±135)

Mean value±SEd. (N=4). # represents highest mean value for any parameter; § represents lowest mean value for any parameter

Table 3: Contribution to variation by genotype, environment and their interaction (Gollob's Test)

Zone	Parameter	Source of Variation	Contribution (%)	Degrees of Freedom	Mean Square	F value
North Western Plains Zone	ABTS radical scavenging activity (nmols TEAC g^{-1} d. wt.)	Environment (E)	17.65	2	2788320.61	127.15**
		Genotype (G)	47.35	18	831313.16	37.91**
		G x E	35.01	36	307322.15	14.01**
	DPPH radical scavenging activity (nmols TEAC g^{-1} d. wt.)	Environment (E)	10.03	2	590676.3	157.42**
		Genotype (G)	37.29	18	243955.5	65.02**
		G x E	52.68	36	172318.4	45.92**
	Soluble Phenolic content (µg GAE g^{-1} d. wt.)	Environment (E)	81.71	2	10977417.61	7358.72**
		Genotype (G)	6.70	18	99961.07	67.01**
		G x E	11.59	36	86514.44	57.99**
Central Zone	ABTS radical scavenging activity (nmols TEAC g^{-1} d. wt.)	Environment (E)	31.33	2	14952235.36	529.97**
		Genotype (G)	34.68	11	3009136.53	106.66**
		G x E	33.99	22	1474705.27	52.27**
	DPPH radical scavenging activity (n mols TEAC g^{-1} d. wt.)	Environment (E)	51.97	2	10603105.39	2316.51**
		Genotype (G)	28.44	11	1054889.11	230.47**
		G x E	19.59	22	363425.94	79.40**
	Soluble Phenolic content (µg GAE g^{-1} d. wt.)	Environment (E)	24.16	2	125698.05	85.09**
		Genotype (G)	41.62	11	39375.74	26.66**
		G x E	34.22	22	16183.77	10.96**

**indicates the statistical significance at one percent level of probability



1a Soluble Phenolics 1b DPPH 1c ABTS

Fig. 1 The average environment coordination view of the GGE biplot for North-Western Plains Zone showing mean performance and stability of the genotypes

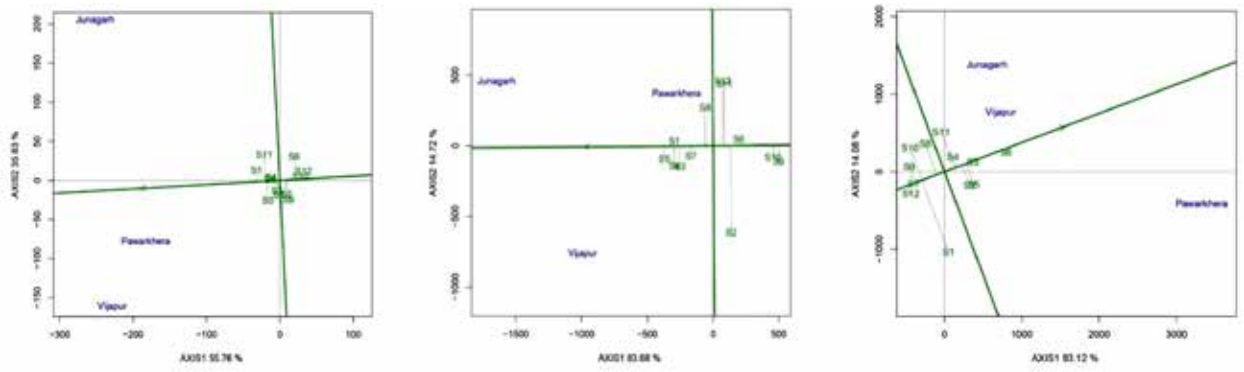
3.2 Effect of location on the content of soluble phenolics and antioxidant capacity of wheat genotypes representing CZ

Similarly, the varieties of central zone grown at three different locations were evaluated for their total antioxidant activity and soluble phenol content. The data for phenolic content, ABTS and DPPH radical scavenging activities of whole meal flour has been elaborated in Table 4. The phenolic content of whole meal flour ranged from 709±35 (DDW 47; S12) to 891±39 (HI 1634; S1) µg GAE g^{-1} d. wt. basis (Table 4). Overall, Junagarh location had shown higher mean phenolic content (861±37) compared to Powarkhera (783±34) and Vijapur (765±34) locations in terms of µg GAE g^{-1} d. wt. basis. The DPPH TEAC of phenolic extract ranged from 326±36 (HI 8627; S9) to 1252±57 (CG 1029; S5) n mols TEAC g^{-1} d. wt. basis (Table 4). For CZ environment, the Junagarh location had the highest mean DPPH activity (1391±66), followed by Vijapur (584±54), and Powarkhera (570±56) locations expressed as nmols TEAC g^{-1} d. wt. basis. Interestingly, the DPPH activity was higher (>2 folds) in varieties grown at Junagarh location as compared to the varieties grown at both Powarkhera and Vijapur locations. The ABTS activity of phenolic extract ranged from 3915±141 (DDW 47; S12) to 5554±143 (GW 322; S6) nmols TEAC g^{-1} d. wt. basis (Table 4). The Powarkhera location (3881±200) showed lower mean values of ABTS as compared to Vijapur (4849±110) and Junagarh (4847±167) locations expressed as nmols trolox equivalent g^{-1} d. wt. basis. Pearson's correlation studies showed a significant positive correlation of phenol with DPPH (r = 0.598**) and ABTS (r = 0.186*) radical scavenging activities. However, there was no significant correlation among ABTS and DPPH activities (r = 0.155).

The analysis of variance based on additive main effects and multiplicative interaction (AMMI) indicated highly significant environment, genotype and genotype-environment (GxE) interactions, contributing 31.33, 34.68 and 33.99% of variations, respectively for ABTS; the corresponding variations were 51.97, 28.44 and 19.59%, for DPPH radical scavenging activity (Table 3). However, in case of CZ, contributions of environment, genotype and genotype-environment interactions were 24.16, 41.62 and 34.22% (Table 3) for soluble phenolic content, respectively.

The GGE biplots "Mean vs. stability" of phenol (2a), DPPH (2b) and ABTS (2c) for CZ are given in Fig. 2. On the basis of the performance of various genotypes with respect to their phenolic content, DPPH and ABTS activities, most stable genotypes across environments found respectively, were MP 3288 (S8), HI 1634 (S1) and MP 3336 (S3) (Fig. 2). The highest mean values for phenolic content, DPPH and ABTS activities were recorded with genotypes HI 1634 (S1), CG 1029 (S5) and GW 322 (S6), respectively. The genotypes with highest mean values for phenol (HI 1634) and DPPH (CG 1029) were 3rd next for their respective parameters while just next in stability for ABTS (GW 322) to their respective stable genotypes (Fig. 2; Table 4).

The soluble phenolic content in the present study ranged from 457-1664 µg GAE g^{-1} d. wt basis for whole wheat flour across the environment. Vitaglione *et al.* (2008) reported range of phenolic content from 305-1505 µg g^{-1} for whole wheat flour while Yu*et al.* (2003) reported total phenols in the lower range of 177-257 µg g^{-1}. However, Adom *et al.* (2003) reported phenol content in the higher range of 1207-1463 µg g^{-1} in a set of whole wheat flours



2a Soluble Phenolics 2b DPPH 2c ABTS

Fig. 2 The average environment coordination view of the GGE biplot for Central Zone showing mean performance and stability of the genotypes

of 11 diverse wheat varieties and experimental lines. The observed differences may be due to the diverse genetic backgrounds as well as the effect of the environment differing in soil type, solar radiation, average rainfall and temperature *etc.* Adom *et al.* (2003) and Moore *et al.* (2006) also reported significant effect of environmental factors towards variation in phenolic content as well as antioxidant activity. Likewise, variation in antioxidant activity might fall prey to assay methods used (Moore *et al.,* 2006).

In this investigation for NWPZ genotypes, there was a significant positive correlation of phenol (r= 0.278**) with ABTS activity while non-significant (r= 0.117) with DPPH radical scavenging activity. The ABTS and DPPH activities showed a significant negative correlation (r= -0.213**). On the other hand, for CZ genotypes, there was a significant positive correlation of phenol content with DPPH (r = 0.598**) activity and ABTS (r = 0.186*) radical scavenging activities. The ABTS and DPPH activities, however, showed a non-significant correlation (r = 0.155). Both negative and positive correlations have been reported between phenolic content and antioxidant activity. Gammoh *et al.*(2017) found a negative correlation between antioxidant activity (DPPH) and total phenols (r= -0.067). There was a strong negative correlation (r= -0.828) between total phenolic content and DPPH in the case of *Terminalia sericea* Burch (Anokwuru *et al.,* 2018). A similar negative but significant correlation has been reported by Al-Laith *et al.*(2019) and Thoo *et al.* (2013) between total, free and bound phenols with ABTS and DPPH free radical scavenging activities in three medicinal plants and *Andrographis paniculata* extracts, respectively. On the other hand, Verma *et al.*(2008) found a positive correlation between free, bound and total phenols with antioxidant activity (r=0.8, p<0.05) in wheat. Moore *et al.*(2006) have also reported a highly significant positive correlation with ABTS but a negative correlation with DPPH *vs.* total phenols in hard winter wheat. Contrary to various negative and positive correlations by various researchers, Yu *et al.*(2003) reported no correlation between total phenolic content with DPPH and ABTS antioxidant activities in hard winter wheat varieties (Akron, Trego and Platte).

Overall, a wide variation in correlations between phenolic extract *vs.* their antioxidant activity may be explained on the basis that phenols alone might not be responsible for all the antioxidant activity and there might be some other secondary metabolites (flavonoids, anthocyanin *etc.*) contributing to total antioxidant activity (Shahidi and Ambigaipalan, 2015; Žilić, 2016). It has been reported that DPPH radical can screen mostly lipophilic compounds, while ABTS radical can be used to screen both lipophilic as well as hydrophilic samples (Sadeer *et al.*2020) and that might be the probable cause of higher ABTS activity reported for all samples tested compared to their DPPH scavenging activity, as extraction was done with 80% methanol in the current investigation. The yield of phenol extraction and total consequent antioxidant activity may also vary depending upon the type of extracting solvent and other factors like extraction time, sample-to-solvent ratio *etc.* Particularly methanol happens to be quite efficient in extracting lower molecular weight polyphenols (Dai and Mumper, 2010).

Table 4: Soluble phenolic content and trolox equivalent antioxidant activity of various genotypes from three different locations of Central Zone

Genotype	Sample code	Soluble Phenolic content (µg GAE g d. wt.)				DPPH radical scavenging activity (n mols TEAC g^{-1} d. wt.)				ABTS radical scavenging activity (n mols TEAC g^{-1} d. wt.)			
		Junagarh	Powarkhera	Vijapur	Mean	Junagarh	Powarkhera	Vijapur	Mean	Junagarh	Powarkhera	Vijapur	Mean
HI 1634	S1	(1004±68)^{a}	(833±31)^{b,c}	(834±19)^{a,b}	(891±39)$^{\#}$	(1926±42)^{a}	(689±58)^{b}	(881±120)^{b,c}	(1165±73)	(4013±223)^{f}	(4260±231)**c**	(4104±98)^{g}	(4126±184)
HD 2932	S2	(783±67)^{d,e}	(757±35)^{d,e,f}	(798±23)^{a,b,c}	(779±42)	(887±23)^{g}	(421±23)^{d,e}	(905±107)^{b}	(738±51)	(4855±115)^{c,d}	(4783±225)^{b}	(4820±76)^{b,c,d,e}	(4819±139)
MP 3336	S3	(830±10)^{c,d}	(843±42)^{b}	(787±21)^{b,c}	(820±24)	(1801±106)^{b,c}	(523±70)^{c}	(938±16)^{b}	(1087±64)	(4944±119)^{c}	(4824±192)^{b}	(5324±59)^{a}	(5030±123)
HD 2864	S4	(914±26)^{b}	(783±36)^{c,d,e}	(803±19)^{a,b}	(833±27)	(1902±101)^{a,b}	(466±37)^{c,d,e}	(958±28)^{b}	(1109±55)	(5201±76)^{a,b}	(4136±263)^{c}	(4803±102)^{c,d,e,f}	(4713±147)
CG 1029	S5	(830±35)^{c,d}	(899±53)^{a}	(843±17)^{a}	(857±35)	(2006±23)^{a}	(689±91)^{b}	(1059±58)^{a}	(1252±57)$^{\#}$	(4976±149)^{b,c}	(4924±224)^{b}	(4709±154)^{e,f}	(4870±176)
GW 322	S6	(885±19)^{b,c}	(739±10)^{e,f,g}	(613±24)^{e}	(746±18)	(1073±47)^{f}	(495±34)^{c,d}	(329±24)^{e}	(632±35)	(5345±153)^{a}	(5935±186)^{a}	(5383±90)^{a}	(5554±143)$^{\#}$
HI 1544	S7	(808±43)^{d,e}	(834±24)^{b,c}	(797±53)^{a,b,c}	(813±40)	(1691±87)^{c}	(501±75)^{c,d}	(791±52)^{c}	(995±71)	(4361±186)^{e}	(2856±299)^{e,f}	(4880±86)^{b,c,d}	(4032±190)
MP 3288	S8	(914±61)^{b}	(753±22)^{d,e,f}	(834±33)^{a,b}	(834±39)	(1566±61)^{d}	(814±106)^{a}	(491±77)^{d}	(957±81)	(5081±211)^{b,c}	(3134±75)^{e}	(4965±145)^{b,c}	(4393±143)
HI 8627	S9	(756±23)^{e}	(744±32)^{e,f,g}	(813±45)^{a,b}	(771±33)	(464±19)^{h}	(383±55)^{e}	(130±33)^{f}	(326±36)§	(4631±166)^{d}	(2651±241)^{f}	(4846±71)^{b,c,d,e}	(4042±159)
UAS 466	S10	(787±31)^{d,e}	(713±58)^{f,g}	(669±46)^{d}	(723±45)	(518±53)^{h}	(514±54)^{c}	(182±60)^{f}	(404±55)	(5029±328)^{b,c}	(2633±181)^{f}	(4735±154)^{d,e,f}	(4132±221)
DBW 110	S11	(1028±37)^{a}	(801±41)^{b,c,d}	(747±58)^{c}	(859±45)	(1418±181)^{e}	(657±47)^{b}	(168±29)^{f}	(748±86)	(5411±168)^{a}	(3653±113)^{d}	(4974±132)^{a}	(4679±138)
DDW 47	S12	(793±26)^{d,e}	(697±25)^{g}	(638±53)^{d,e}	(709±35)§	(1438±51)^{e}	(692±28)^{b}	(171±39)^{f}	(767±40)	(4320±110)^{e}	(2785±164)^{f}	(4641±149)^{f}	(3915±141)§
Mean		(861±37)	(783±34)	(765±34)	(803±35)	(1391±66)	(570±56)	(584±54)	(848±59)	(4847±167)	(3881±200)	(4849±110)	(4526±159)

Mean value ± SEd. (N=4). # represents highest mean value for any parameter; § represents lowest mean value for any parameter

Conclusions

A wide variation in correlations between soluble phenolic extract *vs*. their antioxidant activities has been observed in Indian wheat genotypes grown at multi-locations. The NWPZ genotypes had higher overall mean values for soluble phenols, DPPH and ABTS activities compared to CZ genotypes. Significant differences in the antioxidant activity and phenol content were observed among the genotypes. Overall, the Karnal location of NWPZ was shown to have higher phenolic content and ABTS activity compared to genotypes grown at different locations of both zones. There was significant effect by the environment, genotype and their interactions on the phenolic content and antioxidant activities on genotypes of both the zones. The genotypes HI 1628 and PBW 771 of NWPZ and HI 1634, CG 1029 and GW 322 of CZ exhibited higher mean phenolic content and antioxidant potential and were found comparatively stable across their environments with respect to the parameters tested. The identified genotypes can be used in breeding as well for cultivation in respective zones for higher phenolic content and antioxidant potential.

Acknowledgements

The authors are thankful to the Indian Council of Agricultural Research and Indian Institute of Wheat and Barley Research for providing the necessary infrastructure and resources for conducting the research. The authors acknowledge the support of the various centers for conducting the trials across different locations in India. The contribution of Dr. Arun Gupta for providing pedigree of the genotypes is acknowledged.

Funding

This research was funded by Indian Council of Agricultural Research under institutional project (IXX15473) and Consortium Research Project on Bio-fortification (1006422).

Author contributions

Conceptualization of research (SK, Priti & SR); Designing of the experiments (SK, OPG & VP); Contribution of experimental materials (SK, SR & OPG); Execution of field/lab experiments and data collection (SK, Priti & SR); Analysis of data and interpretation (SK & SR); Preparation of the manuscript (SK, Priti & GPS).

Conflict of interest: No

Declaration

The authors declare no conflict of interest.

References

1. Adom KK, M Sorrells and RH Liu (2003) Phytochemical profiles and antioxidant activity of wheat varieties. *J Agric Food Chem* 51:7825–7834. https://doi.org/10.1021/jf030404l
2. Al-Laith AA, J Alkhuzai and A Freije (2019) Assessment of antioxidant activities of three wild medicinal plants from Bahrain. *Arab J Chem* 12:2365-2371. http://dx.doi.org/10.1016/j.arabjc.2015.03.004
3. Anokwuru C, M Sigidi, M Boukandou, P Tshisikhawe, A Traore and N Potgieter (2018) Antioxidant activity and spectroscopic characteristics of extractable and non-extractable phenolics from *Terminalia sericea* Burch. ex DC. *Molecules* 23: Article 1303. https://doi.org/10.3390/molecules23061303
4. Beta T, S Nam, JE Dexter and HD Sairstein (2005) Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chem* 82(4):390–393. https://doi.org/10.1094/CC-82-0390
5. Braun HJ, G Atlin and T Payne (2010) Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds MP (ed) Climate change and crop production, CABI, Oxfordshire, UK, pp.115-138. https://www.cabi.org/cabebooks/ebook/20103205645
6. Dai J and RJ Mumper (2010) Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15:7313-7352. https://doi.org/10.3390/molecules15107313
7. Gammoh S, MH Alu'datt, MN Alhamad, T Rababah, K Ereifej and A Almajwal *et al* (2017) Characterization of phenolic compounds extracted from wheat protein fractions using high performance liquid chromatography/liquid chromatography mass spectrometry in relation to antiallergenic, anti-oxidant, anti-hypertension, and anti-diabetic properties. *Int J Food Properties* 20(10):2383-2395. https://doi.org/10.1080/10942912.2016.1238832
8. Gu C, K Howell, FR Dunshea and HAR Suleria (2019) LC-ESI-QTOF/MS characterisation of phenolic acids and flavonoids in polyphenol-rich

fruits and vegetables and their potential antioxidant activities. Antioxidants 8: Article 405. https://doi.org/10.3390/antiox8090405

9. Kumar S and S Malhotra (2008) Partial purification of superoxide dismutase and peroxidase from ber (*Zizyphus mauritiana* Lamk.) fruit using anion exchange chromatography. *Physiol Mol Biol Plants* 14(3):167-172. https://dx.doi.org/10.1007%2Fs12298-008-0015-1
10. Kumar S, P Yadav, V Jain and SP Malhotra (2011) Oxidative stress and antioxidative system in ripening ber (*Ziziphus mauritiana* Lam.) fruits. *Food Technol Biotechnol* 49(4):453–459
11. Laddomada B, M Durant, F Minervini, A Garbetta, A Cardinalli and I D'Antuono *et al* (2015) Phytochemical composition and anti-inflammatory activity of extracts from the whole-meal flour of Italian durum wheat cultivars. *Int J Mol Sci* 16(2):3512-3527. https://doi.org/10.3390/ijms16023512
12. Moore J, JG Liu, KQ Zhou and LL Yu (2006) Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *J Agric Food Chem* 54:5313–5322. https://doi.org/10.1021/jf0603811
13. Narwal S, V Thakur, S Sheoran, S Dahiya, S Jaswal and RK Gupta (2014) Antioxidant activity and phenolic content of the Indian wheat varieties. *J Plant Biochem Biotechnol* 23(1):11-17. http://dx.doi.org/10.1007/s13562-012-0179-1
14. Opitz SEW, S Smrke, BA Goodman and C Yeretzian (2014) Methodology for the measurement of antioxidant capacity of coffee: A validated platform composed of three complementary antioxidant assays. In: Preedy V (ed) Processing and impact on antioxidants in beverages, Academic Press, San Diego, CA, USA, pp 253–264. https://doi.org/10.1016/B978-0-12-404738-9.00026-X
15. Re R, N Pellegrini, A Proteggente, A Pannala, M Yang and C Rice-Evans (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol Med* 26:1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3
16. Regoli F and GW Winston (1999) Quantification of total oxidant scavenging capacity of antioxidants for peroxynitrite, peroxyl radicals and hydroxyl radicals. *Toxicol Appl Pharmacol* 56:96-105. https://doi.org/10.1006/taap.1999.8637
17. Sadeer BN, D Montesano, S Albrizio, G Zengin and MF Mahomoodally (2020) The versatility of antioxidant assays in food science and safety–Chemistry, applications, strengths, and limitations. Antioxidants, Article 709. https://doi.org/10.3390/antiox9080709
18. Sedej I, M Sakac, A Mandic, A Misan, V Tumbas and M Hadnadev (2011) Assessment of antioxidant activity and rheological properties of wheat and buckwheat milling fractions. *J Cereal Sci* 54:347–353. http://dx.doi.org/10.1016/j.jcs.2011.07.001
19. Shahidi F and P Ambigaipalan (2015) Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review. *J Func Foods* 18 (Part B):820-897. https://doi.org/10.1016/j.jff.2015.06.018
20. Singleton VL, R Orthofer and RM Lamuela-Raventos (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteureagent. *Methods Enzymol* 299:152–178. https://doi.org/10.1016/S0076-6879(99)99017-1
21. Tan BL, ME Norhaizan and W Liew (2018) Nutrients and oxidative stress: Friend or foe? Oxidative Medicine and Cellular Longevity, Article 9719584. https://doi.org/10.1155/2018/9719584
22. Thoo YY, SY Ng, MZ Khoo, WM Wan Aida and CW Ho (2013) A binary solvent extraction system for phenolic antioxidants and its application to the estimation of antioxidant capacity in *Andrographis paniculata* extracts. *Int Food Res J* 20(3):1103-1111.
23. Verma A and GP Singh (2021) Stability, adaptability analysis of wheat genotypes by AMMI with BLUP for restricted irrigated multi location trials in Peninsular zone of India. *Agric Sci* 12:198-212. http://dx.doi.org/10.4236/as.2021.123013
24. Verma B, P Hucl and RN Chibbar (2008) Phenolic content and antioxidant properties of bran in 51 wheat cultivars. *Cereal Chem* 85(4):544-549. https://doi.org/10.1094/CCHEM-85-4-0544
25. Vitaglione P, A Napolitano and V Fogliano (2008) Cereal dietary fibre: a natural functional ingredient

to deliver phenolic compounds into the gut. *Trends Food Sci Technol* 19:451—463. http://dx.doi.org/10.1016/j.tifs.2008.02.005

26. Yan W and NA Tinker (2006) Biplot analysis of multi-environment trial data: Principles and applications. *Can J Plant Sci* 86:623–645. http://dx.doi.org/10.4141/P05-169

27. Yu LL, J Perret, M Harris, J Wilson and S Haley (2003) Antioxidant properties of bran extracts from 'Akron' wheat grown at different locations. *J Agric Food Chem* 51:1566–1570. https://doi.org/10.1021/jf020950z

28. Žilić S (2016). Phenolic compounds of wheat their content, antioxidant capacity and bioaccessibility. *MOJ Food Process Technol.* 2(3):85-89. https://doi.org/10.15406/mojfpt.2016.02.00037

29. Žilić S, A Serpen, G Akillioğlu, M Jancović and V Gökmen (2012) Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. *J Cereal Sci* 56(3):652–658. http://dx.doi.org/10.1016/j.jcs.2012.07.014

30. Žilić S, VH Šukalović, D Dodig, V Maksimović, M Maksimović and Z Basić (2011) Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. *J Cereal Sci* 54:417–424. https://doi.org/10.1016/j.jcs.2011.08.006