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Deciphering the Contribution of Traits Influencing Yield and Micronutrient Content in F2 Generation of Wheat (*Triticum aestivum* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. Author JPP designed and performed the study, wrote the protocol, and wrote the first draft of the manuscript. Author NR performed the statistical analysis and wrote the first draft of the manuscript. Authors SKS, Nilanjaya and PP managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Wheat, a crucial food crop, provides substantial dietary energy and has been intertwined with human civilization throughout its cultivation history. It meets 20 % of daily protein and calorie requirement worldwide. However, cereal grains, which form the staple diet for most people with limited content of certain essential nutrients, leading to a condition often referred to as "hidden hunger." Micronutrients like zinc and iron are vital for human health, and deficiencies in these elements contribute to micronutrient malnutrition, a significant health concern. To combat

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malnutrition, biofortification emerges as a promising strategy to enhance the nutritional quality of staple crops. In this study, we explored the relationship between morphophysiological and biochemical traits and their impact on yield. Notably, the grain yield per plant exhibited a positive correlation with both plant height and chlorophyll content in the F2 populations resulting from crosses between HD2733 × Rajendra Genhu 4 and Rajendra Genhu 1 × PBW Zn 1. Further analysis revealed that traits such as days to 50 % flowering, days to maturity, number of tillers, chlorophyll content, and NDVI directly contributed to yield improvement. However, grain zinc and iron content had a negative effect on yield. These findings suggest that plant height and chlorophyll content could serve as indirect selection criteria for enhancing crop yield.

Keywords: Biofortification; morphophysiological traits; biochemical traits; correlation; indirect selection.

1. INTRODUCTION

Wheat is a major food crop that provides a large amount of dietary energy. It has evolved along with human civilization and its cultivation. In 2022-2023, the global wheat production was 789.49 million tons from 221.86 million hectares of land, with an average yield of 34.96 g/ha [1]. India ranks third in the world in wheat production, after China and the European Union. Wheat is the second most important crop in India, after rice. It occupies 13 % (31.36 mha) of the total arable land, with a production of 107.86 mt and a yield of 34.39 g/ha. In Bihar, wheat is grown on 2.04 m ha of land, with a production of 4.58 mt and a yield of 28 q/ha [2]. Wheat is known as the king of cereals and has been valued as the essence of life for centuries. Wheat is mainly consumed by humans and its grain contains about 60 % starch and 11-13 % protein by weight [3].

Wheat provides 60-70% starch in whole grain, 65-75% starch in flour and 8-15% protein. It accounts for about 55% of the carbohydrates and 20% of the calories consumed by the global population and is the staple food for about 2 billion people in the world [4]. However, cereal grains, which are the main diet for most people on the planet, lack some essential nutrients, leading to malnutrition, which is often called "hidden hunger" [5]. The problem of hunger is still widespread, with an estimated 800 million people in the developing countries suffering from undernutrition [6]. According to the Global Hunger Index (GHI) report, more than 2 billion people worldwide are malnourished, with most of them living in poor countries [7]. Zinc and Iron deficiency. which causes micronutrient malnutrition, is a serious health issue that affects over 3 billion people globally and according to WHO, more than two billion people are facing acute hidden hunger. Zinc and Iron are important

micronutrients for human health [8]. Zinc is a cofactor for many enzymes and regulatory proteins. It is essential for nucleic acid synthesis and gene expression. Zinc deficiency in human diet causes stunted growth and neurobehavioral problems in children and other health complications [9]. It also affects anaemia, as Zinc regulates Iron absorption in the intestine [10]. Iron is a key component Similarly, of haemoglobin and myoglobin, which are involved in oxygen transport and storage. Iron deficiency in human diet causes anaemia, especially in pregnant women. It also increases the risk of infectious diseases and may even lead to death [11]. Therefore, Zinc and Iron are critical micronutrients for global public health.

Bio-fortification is a way to overcome the hidden hunger by improving the micronutrient content of food grains by either increasing its concentration in the edible part or enhancing its bio-availability. issue of malnutrition. To address the biofortification is the best alternative strategy to improve the nutritional guality of staple crops. For any crop improvement program that involves parent selection and hybridization, it is necessary to have a comprehensive understanding of the genetics, inheritance and contribution of relevant traits to yield. The aim of this study was to relationship the explore among traits (morphophysiological and biochemical traits) and their direct or indirect effect on yield.

2. MATERIALS AND METHODS

This study aimed to examine the relationship between yield and its related characteristics, such as morpho-physiological and biochemical features, in bread wheat. The experiments took place at the wheat research farm of RPCAU, Pusa (Samastipur), Bihar, during the Rabi seasons of 2020-21, 2021-22 and 2022-23. Pusa has a warm and mild climate. It is part of the North-West Alluvial plain in the Samastipur district. The soil of Pusa is alluvial or sandy loam. with a moderate to high amount of organic matter. The mean annual rainfall and temperature of Pusa are 1222.3mm. and 25.2° C, respectively. In this experiment, F1 seeds were produced by crossing different varieties in Rabi 2020-21, and then self-pollinated in Rabi 2021-22 to obtain F2 seeds. F2 offspring of two crosses (HD2733 × Rajendra Genhu 4 and Rajendra Genhu 1 x PBW Zn 1) were assessed in Rabi 2022-23. Ten morphophysiological traits were recorded during the assessment. Moreover, grain micronutrient content (Zinc and Iron) was analyzed by X-Ray Fluorescence Spectrometry at the Harvest Plus Division, ICRISAT, Hyderabad.

2.1 Biometrical Analysis

Correlation analysis was made to measure the relationship between yield, morphophysiological and micronutrient characteristics [12]. Path coefficient analysis was carried out [13] to estimate the direct and indirect influences of these characteristics on yield. The analysis was carried out using the TNAUSTAT-Statistical package [14].

3. RESULTS AND DISCUSSION

3.1 Association Analysis

Association between two or more traits in terms of degree and direction can be defined by correlation. In the present study correlation among different characters of F_2 population of two crosses were studied and their correlation matrix is presented in Tables 1 and 2.

3.1.1 Cross I

The correlation coefficient among traits for cross-I (HD2733 × Rajendra Genhu 4) revealed that traits such as plant height and grain iron content showed highly significant positive contribution towards grain yield. Whereas traits such as, number of grains per spike chlorophyll content and grain zinc content showed significant positive contribution towards grain yield [15]. Days to 50 % flowering exhibited highly significant positive correlation with days to maturity and significant positive correlation with chlorophyll content. Plant height demonstrated highly significant positive correlation with chlorophyll content, grain yield and grain iron content. Whereas it exhibited significant positive correlation with grain zinc content. Number of tillers showed significant positive correlation with chlorophyll. Days to maturity showed significant negative correlation with thousand grain weight. Thousand grain weight showed significant positive correlation with grain iron content. Chlorophyll content showed highly significant correlation with grain iron content and significant correlation with grain zinc content and grain yield.

3.1.2 Cross II

The correlation coefficient among traits for cross-II (Rajendra Genhu 1 × PBW Zn 1) revealed that traits such as days to 50 % flowering, days to maturity and chlorophyll content showed exhibited highly significant positive correlation grain yield [15]; significant positive with correlation with thousand grain weight. Grain zinc content showed significant negative correlation towards grain vield. Days to 50 % flowering showed highly significant correlation with days to maturity and significant positive correlation with grain iron content. Plant height showed significant positive correlation with days to maturity, thousand grain weight and chlorophyll content, whereas it exhibited highly significant negative correlation with grain zinc content. Number of tillers showed significant correlation with number of grains per spike. Days to maturity showed highly significant correlation with thousand grain weight, chlorophyll content and grain iron content.

3.2 Path Analysis

Path matrix among different characters of F_2 population from two crosses is presented in Tables 3 and 4.

3.2.1 Cross I

Plant height (0.4219) exhibited highest direct effect followed by grain iron content, number of grains per spike [16], grain zinc content and number of tillers towards grain yield. Traits such as flag leaf area (-0.2051) exhibited highest negative direct effect followed by days to 50 % flowering, NDVI, thousand grain weight, chlorophyll content and days to maturity towards grain yield.

Days to 50 % flowering demonstrated highest indirect effect through thousand plant height (0.0542) followed by grain iron content. Number of tillers, NDVI, thousand grain weight, grain zinc content and number of grains per spike. Whereas it exhibited highest negative indirect effect through flag leaf area followed by days to maturity and chlorophyll content. Plant height showed highest positive indirect effect towards through grain iron content (0.0715) followed by grain zinc content, number of grains per spike, flag leaf area, number of tillers and days to Days to 50 % flowering (-0.0243) maturity. exhibited highest negative indirect effect towards grain yield followed by chlorophyll content, NDVI and thousand grain weight. Number of tillers exhibited highest positive indirect effect towards yield through plant height (0.0704) followed by grain iron content, NDVI, flag leaf area, number of grins per spike, grain zinc content and days to maturity. Plant height also exhibited negative indirect effect through days to 50 % flowering followed by chlorophyll content and thousand grain weight.

Days to maturity demonstrated highest positive indirect effect towards yield through thousand grain weight (0.0211) followed by flag leaf area, grain zinc content and chlorophyll content. It also exhibited negative indirect effect through days to 50 % flowering followed by plant height, grain iron content, number of grains per spike, number of tillers and NDVI. Number of grains per spike exhibited highest positive indirect effect towards yield through plant height (0.0948) followed by grain iron content, flag leaf area, grain zinc content, number of tillers, days to maturity and NDVI. It also showed negative indirect effect through thousand grain weight followed by days to 50 % flowering and chlorophyll content. Thousand grain weight showed highest positive indirect effect towards yield through grain iron content (0.0504) followed by plant height, days to 50 % flowering, number of grains per spike, NDVI, grain zinc content, days to maturity and number of tillers. It also showed negative indirect effect through flag leaf area and chlorophyll content. Chlorophyll content exhibited highest positive indirect effect towards yield through plant height (0.2555) followed by grain iron content, grain zinc content, number of grains per spike, number of tillers and days to maturity. It also showed negative indirect effect through days to 50 % flowering followed by NDVI, thousand grain weight and flag leaf area.

Flag leaf area showed highest positive indirect effect towards yield through NDVI (0.0142) followed by days to maturity. NDVI exhibited positive indirect effect towards grain yield through plant height (0.0460). Grain zinc content showed positive indirect effect through plant height (0.1411) followed by grain iron content, NDVI, number of grains per spike, number of tillers and flag leaf area. It also exhibited negative indirect effect through days to 50 % flowering followed by chlorophyll content, thousand grain weight and days to maturity. Grain iron content showed positive indirect effect towards yield through plant height (0.1725) followed by flag leaf area, number of grains per spike, grain zinc content, number of tillers, NDVI and days to maturity. It also exhibited negative indirect effect through thousand grain weight followed by days to 50 % flowering and chlorophyll content.

Table 1. Estimates of correlation coefficient among yield attributing traits and micronutrient content for cross- I (HD 2733 × RG 4)

Traits	DF	PH	NT	DM	NGPS	TGW	CC	FLA	NDVI	GZC	GIC	GY
DF	1											
PH	0.129	1										
NT	0.154	0.167	1									
DM	0.650***	-0.147	-0.017	1								
NGPS	0.029	0.225	0.063	-0.184	1							
TGW	-0.118	0.100	0.033	-0.322*	0.175	1						
CC	0.289*	0.606***	0.289*	-0.130	0.181	0.158	1					
FLA	0.131	-0.076	-0.121	-0.062	-0.129	0.065	0.029	1				
NDVI	-0.063	0.109	-0.257	0.003	-0.013	-0.081	0.146	-0.107	1			
GZC	0.080	0.334*	0.070	0.038	0.114	0.081	0.305*	-0.016	-0.214	1		
GIC	0.076	0.409**	0.237	-0.259	0.245	0.289*	0.461***	-0.215	-0.062	0.223	1	
GY	-0.128	0.520***	0.215	-0.230	0.288*	0.080	0.292*	-0.315*	-0.101	0.292*	0.426**	1

* and **: Significance at 5% and 1% respectively.

DF – Days to 50 % flowering, PH – Plant Height, NT – Number of Tillers, DM – Days to Maturity, NGPS – Number of Grains Per Spike, TGW – Thousand Grain Weight, CC – Chlorophyll Content, FLA – Flag Leaf Area, NDVI – Normalized Difference Vegetation Index, GZC – Grain Zinc Content, GIC – Grain Iron Content, YLD – Yield

Table 2.	Estimates of correlation coefficient among yield attributing traits and micronutrier	It
	content for cross- II (RG 1 × PBW Zn 1)	

Traits	DF	PH	NT	DM	NGPS	TGW	CC	FLA	NDVI	GZC	GIC	GY
DF	1											
PH	0.183	1										
NT	0.095	0.030	1									
DM	0.436**	0.308*	0.154	1								
NGPS	0.257	0.178	0.280*	0.161	1							
TGW	0.246	0.322*	0.004	0.441**	0.140	1						
CC	0.256	0.317*	0.045	0.445**	0.096	0.110	1					
FLA	-0.114	-0.118	0.095	0.013	0.037	-0.068	-0.138	1				
NDVI	0.001	-0.041	-0.035	0.045	0.034	0.039	-0.036	0.038	1			
GZC	-0.175	-0.520***	-0.106	-0.166	-0.170	-0.182	-0.170	0.159	0.018	1		
GIC	0.281*	-0.072	-0.104	0.449**	0.106	0.083	0.172	-0.048	-0.080	0.104	1	
GY	0.556***	0.379**	0.012	0.504***	0.084	0.309*	0.486***	-0.233	0.128	-0.415**	0.260	1

* and **: Significance at 5% and 1% respectively. DF – Days to 50 % flowering, PH – Plant Height, NT – Number of Tillers, DM – Days to Maturity, NGPS – Number of Grains Per Spike, TGW – Thousand Grain Weight, CC – Chlorophyll Content, FLA – Flag Leaf Area, NDVI – Normalized Difference Vegetation Index, GZC – Grain Zinc Content, GIC – Grain Iron Content, YLD – Yield

3.2.2 Cross II

Traits such as days to 50 % flowering (0.3593) followed by chlorophyll content, NDVI, grain iron content, days to maturity and plant height exhibited positive direct effect towards grain yield. Traits such as grain zinc content (-0.2672) followed by number of grains per spike, flag leaf area and number of tillers showed negative direct effect towards yield.

Days to 50 % flowering showed highest indirect effect towards yield through chlorophyll content (0.0671) followed by grain zinc content, days to maturity, grain iron content, thousand grain weight, plant height, flag leaf area and NDVI. It also showed negative indirect effect through number of grains per spike and number of tillers. Plant height demonstrated highest indirect effect towards yield through grain zinc content (0.1388) followed by chlorophyll content, days to 50 % flowering, days to maturity, thousand grain weight and flag leaf area. It showed negative indirect effect towards grain yield through number of grains per spike followed by grain iron content, NDVI and number of tillers. Number of tillers exhibited highest positive indirect effect through days to 50 % flowering (0.0342) followed by grain zinc content, days to maturity, chlorophyll content, plant height and thousand grain weight. It also showed negative indirect effect through number of grains per spike followed by grain iron content, flag leaf area and NDVI.

Days to maturity showed highest positive indirect effect towards yield through days to 50 % flowering (0.1568) followed by chlorophyll

content, grain iron content, grain zinc content, thousand grain weight, plant height and NDVI. It exhibited negative indirect effect through number of grains per spike followed by number of tillers and flag leaf area. Number of grains per spike demonstrated positive indirect effect towards grain yield through days to 50 % flowering (0.0925) followed by grains zinc content, chlorophyll content, days to maturity, grain iron content, plant height, thousand grain weight and NDVI. It showed negative indirect effect through number of tillers and flag leaf area. Thousand grain weight showed highest positive indirect effect towards yield through days to 50 % flowering (0.0884) followed by grain zinc content, days to maturity, chlorophyll content, plant height, grain iron content, flag leaf area and NDVI. Whereas it showed negative indirect effect through number of grains per spike and number of tillers.

Chlorophyll content showed positive indirect effect towards grain yield through days to 50 % flowering (00092) followed by grain zinc content, days to maturity, plant height, grain iron content, flag leaf area and thousand grain weight. Flag leaf area showed positive indirect effect towards yield through NDVI (0.0059) followed by days to maturity. NDVI exhibited positive indirect effect through days to maturity (0.0045) followed by thousand grain weight, number of tillers and days to 50 % flowering towards grain yield. Grain zinc content demonstrated positive indirect effect towards grain yield through number of grains per spike (0.0214) followed by grain iron content, NDVI and number of tillers. Grain iron content demonstrated positive indirect effect towards grain yield through days to 50 % flowering

Traits	DF	PH	NT	DM	NGPS	TGW	CC	FLA	NDVI	GZC	GIC	GY
DF	-0.1893	0.0542	0.0105	-0.0097	0.0037	0.0077	-0.0072	-0.0268	0.0084	0.0072	0.0133	-0.128
PH	-0.0243	0.4219	0.0113	0.0022	0.0284	-0.0065	-0.0151	0.0156	-0.0145	0.0301	0.0715	0.5204
NT	-0.0292	0.0704	0.068	0.0003	0.008	-0.0022	-0.0072	0.0248	0.0343	0.0063	0.0415	0.215
DM	-0.1231	-0.0622	-0.0011	-0.0149	-0.0232	0.0211	0.0032	0.0127	-0.0003	0.0034	-0.0452	-0.2297
NGPS	-0.0056	0.0948	0.0043	0.0027	0.1263	-0.0115	-0.0045	0.0265	0.0017	0.0102	0.0428	0.2879
TGW	0.0224	0.0422	0.0022	0.0048	0.0222	-0.0654	-0.0039	-0.0134	0.0108	0.0073	0.0504	0.0796
CC	-0.0548	0.2555	0.0196	0.0019	0.0229	-0.0103	-0.0249	-0.0059	-0.0195	0.0274	0.0805	0.2924
FLA	-0.0247	-0.032	-0.0082	0.0009	-0.0163	-0.0043	-0.0007	-0.2051	0.0142	-0.0015	-0.0376	-0.3152
NDVI	0.0119	0.046	-0.0175	0.0001	-0.0016	0.0053	-0.0036	0.0219	-0.1335	-0.0193	-0.0108	-0.1012
GZC	-0.0152	0.1411	0.0048	-0.0006	0.0144	-0.0053	-0.0076	0.0033	0.0286	0.090	0.039	0.2924
GIC	-0.0144	0.1725	0.0161	0.0039	0.031	-0.0189	-0.0115	0.0441	0.0082	0.0201	0.1747	0.4259

Table 3. Estimates of direct and indirect effects of various traits under study on grain yield for cross-I (HD 2733 × RG 4)

RESIDUE= 0.7318

DF – Days to 50 % flowering, PH – Plant Height, NT – Number of Tillers, DM – Days to Maturity, NGPS – Number of Grains Per Spike, TGW – Thousand Grain Weight, CC – Chlorophyll Content, FLA – Flag leaf area, NDVI – Normalized Difference Vegetation Index, GZC – Grain Zinc Content, GIC – Grain Iron Content, YLD – Yield

Traits	DF	PH	NT	DM	NGPS	TGW	CC	FLA	NDVI	GZC	GIC	GY
DF	0.3593	0.0114	-0.0018	0.0435	-0.0322	0.0183	0.0671	0.0109	0.0001	0.0469	0.0321	0.5556
PH	0.0656	0.0624	-0.0006	0.0308	-0.0223	0.024	0.0831	0.0114	-0.0063	0.1388	-0.0082	0.3786
NT	0.0342	0.0019	-0.0185	0.0154	-0.0351	0.0003	0.0117	-0.0091	-0.0055	0.0284	-0.0119	0.0118
DM	0.1568	0.0192	-0.0029	0.0998	-0.0202	0.0328	0.1165	-0.0012	0.0069	0.0444	0.0513	0.5036
NGPS	0.0925	0.0111	-0.0052	0.0161	-0.1253	0.0105	0.0252	-0.0036	0.0053	0.0455	0.0122	0.0843
TGW	0.0884	0.0201	-0.0001	0.044	-0.0176	0.0744	0.0289	0.0066	0.006	0.0486	0.0095	0.3088
CC	0.092	0.0198	-0.0008	0.0444	-0.0121	0.0082	0.2620	0.0133	-0.0056	0.0455	0.0196	0.4863
FLA	-0.0409	-0.0074	-0.0018	0.0013	-0.0046	-0.0051	-0.0362	-0.0962	0.0059	-0.0424	-0.0055	-0.2327
NDVI	0.0002	-0.0026	0.0007	0.0045	-0.0043	0.0029	-0.0095	-0.0037	0.1542	-0.0048	-0.0091	0.1284
GZC	-0.063	-0.0324	0.002	-0.0166	0.0214	-0.0135	-0.0446	-0.0152	0.0028	-0.2672	0.0118	-0.4147
GIC	0.1009	-0.0045	0.0019	0.0448	-0.0133	0.0062	0.0449	0.0047	-0.0123	-0.0277	0.1143	0.2599

RESIDUE= 0.6357

DF – Days to 50 % flowering, PH – Plant Height, NT – Number of Tillers, DM – Days to maturity, NGPS – Number of Grains Per Spike, TGW – Thousand Grain Weight, CC – Chlorophyll Content, FLA – Flag Leaf Area, NDVI – Normalized Difference Vegetation Index, GZC – Grain Zinc Content, GIC – Grain Iron Content, YLD – Yield.

(0.1009) followed by chlorophyll content, days to maturity, thousand grain weight, flag leaf area and number of tillers. IT also showed negative indirect effect through grain zinc content followed by number of grains per spike, NDVI and plant height.

This study reveals how certain characteristics, such as the height of plants and the amount of chlorophyll they have, can help in indirectly selecting crops that produce more yield. This has important consequences for breeding programs that aim to improve how crops grow in different environmental conditions [17]. By using a combination of genetic, physiological, and agronomic methods, researchers and policymakers can develop crop varieties that are more adaptable and nutritious, and that can cope with the specific problems of tropical farming systems. This way, they can support sustainable food production and reduce hidden hunger, advancing both scientific understanding and practical actions in global agriculture and public health.

4. CONCLUSION

The grain yield per plant showed a significant positive correlation with both plant height and chlorophyll content in both F₂ populations. Path coefficient analysis of both crosses indicated that traits such as plant height, days to 50% flowering, days to maturity, number of tillers, chlorophyll content, and NDVI directly contributed to yield improvement in a positive manner. Conversely, grain zinc and iron content had a direct negative effect on yield. These findings suggest that plant height and chlorophyll content could serve as indirect selection criteria for enhancing yield.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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