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Investigating the Association 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. All authors read and approved the final manuscript.

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ABSTRACT

Wheat is a key food crop for the world, providing a lot of the energy we need. It gives us about 55% of our carbohydrates and 20% of our calories. Many people eat cereal grains as their main food, but they often lack enough nutrients. This causes malnutrition, which is also called "hidden hunger". Micronutrient malnutrition arising from Zn and Fe deficiency has emerged as a serious health concern and it afflicts over 3 billion people globally. Zinc and Iron are very important for our health. Bio-fortification circumvents the hidden hunger by improving the micronutrient composition of food grains by either increasing its concentration in edible portions or enhancing its bio-availability. The present investigation was an attempt to decipher the association among traits and their contribution towards yield by correlation and path analysis. Findings showed that the number of grains in each

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spike and the amount of chlorophyll in the plants were strongly related to how much grain each plant produced in both F2 groups (Rajendra Genhu 4 × HD2967 and Rajendra Genhu 3 × HD2967). On the other hand, grain iron content was inversely related to the yield. The path analysis of both crosses showed that factors like plant height, days to 50 % flowering, days to maturity and chlorophyll content had a positive direct impact on the yield. However, the zinc and iron levels in the grain had a negative direct impact on the yield. These results inferred that number of grains per spike and chlorophyll content could be used in indirect selection for yield improvement.

Keywords: Wheat; yield; grain iron content; grain zinc content; association.

1. INTRODUCTION

Wheat is one of the world's major staple crops, providing a significant portion of the dietary energy. Its evolutionary journey reflects the close relationship between wheat cultivation and the progress of human civilization. In 2022-2023 global annual wheat production was recorded 789.49 million tons from an area of 221.86 million hectares and productivity 34.96 q/ha [1]. India stands third place with a share of 13% of global wheat production followed by China and the European Union. Wheat is India's second most significant crop in terms of production and utility followed by rice. In India, wheat shares 13% (31.36 mha) of total cultivable land with a production of 107.86 mt and productivity of 34.39 q/ha. In Bihar, about 2.04 m ha of agricultural land is under wheat cultivation with a production of 4.58mt and an average productivity of 28q/ha [2]. Wheat is hailed as the king of cereals and has been regarded as the essence of life for centuries. Wheat is mainly used for human nutrition and its grain contains nearly 60% starch and 11-13% protein by weight [3].

In view of nutritional supply, wheat contains 60-70% starch in whole grain, 65-75% starch in flour and 8-15% protein. It supplies around 55% of carbohydrate and 20% of the calories consumed by the global population and is recognised as the staple food of about 2 billion people in the world [4]. Nutrient deficiencies in cereal grains, which are a staple diet for the majority of people on the planet, lead to malnutrition, which is known as "hidden hunger" in the majority of cases [5]. However, the curse of hunger remained widespread with an estimated 800 million undernourished people in the developing countries [6]. According to the Global Hunger Index (GHI) report more than 2 billion people worldwide suffer from malnutrition, with the majority of those affected living in poor nations [7]. Micronutrient malnutrition arising from Zn and Fe deficiency has emerged as a serious health concern and it afflicts over 3 billion people globally and as per the estimate of WHO more than two billion people are facing hidden hunger in acute form. In human nutritional profile, Zinc and Iron play vital roles [8]. Zinc acts as a cofactor for many important enzymes and regulatory proteins. It plays significant role in nucleic acid biosynthesis and gene expression. Deficiency of Zinc in human diet results in stunted growth and neurobehavioral anomalies in children and other health issues [9]. It is also reported to be associated with anaemia as Zinc controls absorption of Iron in the intestine [10]. Likewise, Iron is well-known essential component of haemoglobin and myoglobin that are involved in oxygen transport and storage. Deficiency of Iron in human diet results in anaemia particularly in pregnant females. It also led towards infectious diseases and may even results in death [11]. Hence, in the consideration of global public health Zinc and Iron are considered as critical micronutrients.

Bio-fortification circumvents the hidden hunger by improving the micronutrient composition of food grains by either increasing its concentration in edible portions or enhancing its bio-availability. Therefore, to cope with the problem of malnutrition, breeding strategies aimed at biofortification is the best alternative approach to enrich the nutritional profile of staple crops. Comprehensive knowledge of the genetics, inheritance of pertinent traits and their contribution towards yield is required for any crop improvement program that involves parent selection and hybridization. In this context, the present investigation was an attempt to decipher the association among traits (morphophysiological and biochemical traits) and its contribution (direct or indirect) towards yield.

2. MATERIALS AND METHODS

The field experiments were conducted at wheat research farm RPCAU, Pusa (Samastipur), Bihar during the Rabi season of 2020-21, 2021-22 and 2022-23. The climate condition of Pusa is warm and temperate. It comes under the North-West Alluvial plane in the Samastipur district. The soil of Pusa is sandy loam or young alluvial in nature with moderate to high organic matter content. The average annual rainfall at Pusa is 1222.3mm. and the average annual temperature is 25.2° C. In this experiment, crosses were made to produce F1 seeds during Rabi 2020-21 and the seeds were raised and selfed in Rabi 2021-22 to produce F_2 seeds. F_2 progenies of two crosses (Rajendra Genhu 4 × HD2967 and Rajendra Genhu 3 × HD2967) were evaluated during Rabi 2022-23. A total of 10 morphophysiological traits were measured during the evaluation process. In addition, grain micronutrient content (Zinc and Iron) was measured by X-Ray Fluorescence Spectrometry at HarvestPlus Division, ICRISAT, Hyderabad.

2.1 Biometrical Analysis

The contribution of morphophysiological and micronutrient traits towards yield was calculated through correlation analysis [12]. The direct and indirect effects of contributing traits towards yield were calculated by path co-efficient analysis [13]. The analysis was carried out with the help of TNAUSTAT-Statistical package [14].

3. RESULTS AND DISCUSSION

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 was studied and their correlation matrix is presented in Tables 1 and 2.

3.1 Cross I

The correlation coefficient among traits for cross-I (Rajendra Genhu 4 × HD2967) revealed that grain yield per plant exhibited highly significant positive correlation with days to 50 % flowering, plant height, days to maturity, number of grains per spike and chlorophyll content. Days to 50 % flowering expressed highly significant positive correlation with days to maturity, number of grains per spike and a significant positive correlation with plant height, 1000 grain weight and chlorophyll content. Plant height demonstrated highly significant positive correlation with yield and a significant positive correlation with days to maturity. Days to maturity exhibited highly significant positive correlation with yield and number of grains per spike. The number of grains per spike demonstrated a significant positive correlation with chlorophyll content. 1000 grain weight exhibited significant negative correlation with NDVI and grain iron content. Chlorophyll content showed significant correlation with grain zinc content and highly significant positive correlation with grain yield. NDVI exhibited significant negative correlation with grain zinc content.

3.2 Cross II

The correlation coefficient among traits for cross-II (Rajendra Genhu 3 × HD2967) revealed that grain yield per plant exhibited highly significant positive correlation with number of grains per plant and chlorophyll content and significant positive correlation with flag leaf area. Days to 50 % flowering expressed highly significant positive correlation with days to maturity and significant positive correlation with chlorophyll content. It also exhibited significant negative correlation with grain iron content. Plant height demonstrated significant positive correlation with chlorophyll content and significant negative content with grain iron content. Number of tillers exhibited highly significant positive correlation with NNDVI. Days to maturity exhibited highly significant positive correlation with chlorophyll content. Number of grains per spike demonstrated highly significant positive correlation with grain yield and significant positive correlation with chlorophyll content. 1000 grain weight exhibited significant negative correlation with grain iron content. Chlorophyll content showed highly significant positive correlation with grain yield and a highly significant negative correlation with grain iron content. Flag leaf area demonstrated significant positive correlation with grain yield.

3.3 Path Analysis

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

Table 1. Estimates of correlation coefficient among yield attributing traits and micronutrient content for cross- I (RG – 4 × HD 2967)

Traits	DF	PH	NT	DM	NGPS	1000GW	CC	FLA	NDVI	GZC	GIC	YLD
DF	1.000											
PH	0.254*	1.000										
NT	0.090	0.121	1.000									
DM	0.750**	0.256*	-0.069	1.000								
NGPS	0.523**	0.214	-0.126	0.445**	1.000							
1000GW	0.254*	-0.139	-0.235	0.051	0.135	1.000						
CC	0.303*	0.182	-0.007	0.240	0.245*	-0.131	1.000					
FLA	0.007	0.057	0.138	0.022	-0.128	0.113	0.027	1.000				
NDVI	-0.059	0.049	0.212	-0.020	-0.123	-0.291*	0.124	-0.075	1.000			
GZC	-0.009	0.084	0.171	-0.129	-0.156	0.021	-0.277*	0.123	-0.244*	1.000		
GIC	-0.153	-0.210	0.054	-0.073	-0.195	-0.244*	-0.025	-0.172	0.237	0.139	1.000	
YLD	0.706**	0.407**	-0.062	0.629**	0.393**	0.077	0.358**	-0.053	0.121	-0.113	-0.051	1.000

** 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, 1000GW – 1000 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 micronutrient content for cross- II (RG – 3 × HD 2967)

Traits	DF	PH	NT	DM	NGPS	1000GW	CC	FLA	NDVI	GZC	GIC	YLD
DF	1.000											
PH	-0.002	1.000										
NT	-0.019	0.061	1.000									
DM	0.659**	-0.118	-0.060	1.000								
NGPS	0.143	0.210	0.026	0.072	1.000							
1000GW	0.156	0.169	-0.193	0.001	0.174	1.000						
CC	0.293*	0.254*	0.018	0.319*	0.266*	0.311	1.000					
FLA	-0.144	0.075	0.125	0.080	0.025	0.116	0.163	1.000				
NDVI	-0.006	0.001	0.372**	-0.061	0.131	-0.056	0.105	0.071	1.000			
GZC	0.213	0.194	0.024	0.222	0.023	-0.039	0.137	-0.079	-0.093	1.000		
GIC	-0.326*	-0.286*	0.186	0.002	-0.234	-0.369**	-0.398**	-0.026	-0.041	-0.056	1.000	
YLD	0.155	0.411**	0.096	0.096	0.406**	0.244*	0.416**	0.248*	0.292*	0.155	-0.363	1.000

** 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, 1000GW – 1000 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 3. Estimates of direct and indirect effects of various traits under study on grain yield for cross-I (RG – 4 × HD 2967)

Traits	DF	PH	NT	DM	NGPS	1000GW	CC	FLA	NDVI	GZC	GIC	YLD
DF	0.5820	0.0646	-0.0147	0.0869	-0.0212	-0.0006	0.0275	-0.0002	-0.0079	0.0004	-0.0109	0.7060
PH	0.1479	0.2542	-0.0197	0.0296	-0.0087	0.0003	0.0166	-0.0018	0.0066	-0.0035	-0.0150	0.4065
NT	0.0524	0.0308	-0.1627	-0.0080	0.0051	0.0006	-0.0006	-0.0044	0.0282	-0.0070	0.0039	-0.0618
DM	0.4365	0.0650	0.0112	0.1159	-0.0180	-0.0001	0.0218	-0.0007	-0.0026	0.0053	-0.0052	0.6290
NGPS	0.3044	0.0545	0.0206	0.0516	-0.0405	-0.0003	0.0223	0.0041	-0.0163	0.0064	-0.0139	0.3928
1000GW	0.1483	-0.0354	0.0382	0.0059	-0.0055	-0.0025	-0.0119	-0.0036	-0.0387	-0.0009	-0.0174	0.0765
CC	0.1762	0.0464	0.0011	0.0278	-0.0099	0.0003	0.0908	-0.0009	0.0165	0.0114	-0.0018	0.3580
FLA	0.0041	0.0145	-0.0224	0.0026	0.0052	-0.0003	0.0024	-0.0322	-0.0099	-0.0051	-0.0123	-0.0534
NDVI	-0.0345	0.0126	-0.0345	-0.0023	0.0050	0.0007	0.0113	0.0024	0.1328	0.0101	0.0169	0.1205
GZC	-0.0052	0.0214	-0.0278	-0.0149	0.0063	-0.0001	-0.0252	-0.0039	-0.0324	-0.0412	0.0099	-0.1131
GIC	-0.0889	-0.0534	-0.0088	-0.0084	0.0079	0.0006	-0.0023	0.0056	0.0315	-0.0057	0.0714	-0.0506
RESIDUE= 0.6063												

DF – Days to 50 % flowering, PH – Plant height, NT – Number of tillers, DM – Days to maturity, NGPS – Number of grains per spike, 1000GW – 1000 grain weight, CC – Chlorophyll content, FLA – Flag leaf area, NDVI – Normalized Difference Vegetation Index, GZC – Grain zinc content, GIC – Grain iron content, YLD – Yield. (Direct effects – Diagonal ; Indirect effects – above and below diagonal)

Table 4. Estimates of direct and indirect effects of various traits under study on grain yield for cross-I (RG – 3 × HD 2967)

Traits	DF	PH	NT	DM	NGPS	1000GW	CC	FLA	NDVI	GZC	GIC	YLD
DF	0.0080	-0.0005	0.0001	0.0258	0.0330	0.0100	0.0388	-0.0260	-0.0014	0.0222	0.0449	0.1548
PH	0.0000	0.2503	-0.0003	-0.0046	0.0485	0.0108	0.0335	0.0135	0.0001	0.0202	0.0393	0.4114
NT	-0.0001	0.0152	-0.0042	-0.0023	0.0060	-0.0123	0.0023	0.0225	0.0915	0.0025	-0.0256	0.0955
DM	0.0053	-0.0296	0.0003	0.0392	0.0167	0.0002	0.0422	0.0145	-0.0150	0.0231	-0.0002	0.0963
NGPS	0.0011	0.0525	-0.0001	0.0028	0.2314	0.0112	0.0352	0.0045	0.0323	0.0024	0.0322	0.4055
1000GW	0.0013	0.0422	0.0008	0.0000	0.0403	0.0640	0.0411	0.0209	-0.0137	-0.0040	0.0507	0.2436
CC	0.0023	0.0635	-0.0001	0.0125	0.0616	0.0199	0.1322	0.0294	0.0258	0.0143	0.0547	0.4161
FLA	-0.0012	0.0187	-0.0005	0.0031	0.0058	0.0074	0.0215	0.1807	0.0174	-0.0082	0.0036	0.2483
NDVI	0.0000	0.0001	-0.0016	-0.0024	0.0304	-0.0036	0.0138	0.0127	0.2462	-0.0097	0.0056	0.2917
GZC	0.0017	0.0486	-0.0001	0.0087	0.0054	-0.0025	0.0181	-0.0142	-0.0229	0.1040	0.0077	0.1545
GIC	-0.0026	-0.0715	-0.0008	0.0001	-0.0541	-0.0236	-0.0526	-0.0047	-0.0100	-0.0058	-0.1375	-0.3632
RESIDUE= 0.7384												

DF – Days to 50 % flowering, PH – Plant Height, NT – Number of Tillers, DM – Days to Maturity, NGPS – Number of Grains Per Spike, 1000GW – 1000 grain weight, CC – Chlorophyll Content, FLA – Flag Leaf Area, NDVI – Normalized Difference Vegetation Index, GZC – Grain Zinc Content, GIC – Grain Iron Content, YLD – Yield. (Direct effects – Diagonal; Indirect effects – above and below diagonal)

3.4 Cross I

From the results of cross-I (Rajendra Genhu 4 × HD2967), days to 50 % flowering (0.5820) showed highest direct effect towards yield followed by plant height, NDVI, days to maturity, chlorophyll content and grain iron content. Whereas, traits such as number of tillers, number of grains per spike, grain zinc content, flag leaf area and 1000 grain weight showed negative direct effect towards yield. In addition to the highest positive direct effect of days to 50 % flowering towards yield [15], it exhibited positive indirect effect through days to maturity (0.0869), plant height, chlorophyll content and grain zinc content. It exhibited negative indirect effect on yield through number of grains per spike, number of tillers, grain iron content, NDVI, 1000 grain weight and flag leaf area.

Plant height demonstrated the highest positive indirect effect towards yield through days to 50 % flowering (0.1479) followed by days to maturity, chlorophyll content, NDVI and 1000 grain weight. It also exhibited negative indirect effect through number of tillers, grain iron content, number of grains per spike, grain zinc content and flag leaf area. Number of tillers showed highest positive indirect effect towards yield through days to 50 % flowering (0.0524), followed by plant height, NDVI, number of grains per spike, grain iron content and 1000 grain weight. It showed negative indirect effect through days to maturity, grain zinc content, flag leaf area and chlorophyll content. Days to maturity exhibited positive indirect effect through traits such as days to 50 % flowering (0.4365), plant height, chlorophyll content, number of tillers and grain zinc content. In addition to this, it exhibited negative indirect effect through number of grains per spike (-0.0180), grain iron content, NDVI, flag leaf area and 1000 grain weight.

Number of grains per spike exhibited positive indirect effect through days to 50 % flowering (0.3044) followed by plant height, days to maturity, chlorophyll content, number of tillers, grain zinc content and flag leaf area. It exhibited highest negative indirect effect through NDVI followed by grain iron content and 1000 grain weight.1000 grain weight demonstrated highest positive indirect effect through days to flowering (0.1483) and negative indirect effect through NDVI (-0.0387) towards yield. Chlorophyll content showed positive indirect effect through days to 50 % flowering (0.1762) followed by plant height, days to maturity, NDVI, grain zinc content, number of tillers and 1000 grain weight. Chlorophyll exhibited negative indirect effect through number of grains per spike followed by grain iron content and flag leaf area. Flag leaf area showed positive indirect effect through number of grains per spike. NDVI showed positive indirect effect through grain iron content (0.0169). Grain zinc content exhibited positive indirect effect through plant height (0.0214) followed by grain iron content and number of grains per spike towards yield. Grain iron content showed positive indirect effect towards yield through NDVI (0.0315) followed by number of grains per spike, flag leaf area and 1000 grain weight.

3.5 Cross II

In cross-II (Rajendra Genhu 3 × HD2967), the trait plant height (0.2503) exhibited highest positive direct effect towards yield followed by NDVI, number of grains per spike [15], flag leaf area, chlorophyll content, grain zinc content, 1000 grain weight, days to maturity and days to 50% flowering. Traits such as grain iron content and number of tillers exhibited negative direct effect towards yield.

Days to 50 % flowering exhibited positive indirect effect towards yield through grain iron content (0.0449), chlorophyll content, number of grains per spike, days to maturity, grain zinc content, 1000 grain weight and number of tillers. Plant height showed positive indirect effect through number of grains per spike (0.0485) followed by grain iron content, chlorophyll content, grain zinc content, flag leaf area, 1000 grain weight, NDVI and days to 50 % flowering. Whereas it showed negative indirect effect through days to maturity and number of tillers towards yield. The number of tillers exhibited highest positive indirect effect towards yield through NDVI (0.0915) followed by flag leaf area, plant height, number of grains per spike, grain zinc content, and chlorophyll content. Whereas it showed negative indirect effect through grain iron content, 1000 grain weight, days to maturity and days to 50 % flowering.

Days to maturity showed positive indirect effect on yield through chlorophyll content (0.0422), grain zinc content, number of grains per spike, flag leaf area, days to 50 % flowering, number of tillers and 1000 grain weight. It also exhibited negative indirect effect through plant height, NDVI and grain iron content. The number of grains per spike showed positive indirect effect towards yield through plant height (0.0525) followed by chlorophyll content, NDVI, grain iron content, 1000 grain weight, flag leaf area, days to maturity, grain zinc content and days to 50 % flowering. 1000 grain weight exhibited highest positive indirect effect towards yield through grain iron content (0.0507), plant height, chlorophyll content, number of grains per spike, flag leaf area, days to 50 % flowering, number of tillers and days to maturity. Chlorophyll content exhibited highest positive indirect effect through plant height (0.0635). Flag leaf area exhibited highest positive indirect effect towards yield through chlorophyll content (0.0215). NDVI exhibited the highest positive indirect effect towards yield though number of grains per spike (0.0304). Grain zinc content showed highest positive indirect effect towards yield through plant height (0.0486) followed by chlorophyll content, days to maturity, grain iron content, number of grains per spike and days to 50 % flowering. Whereas it showed negative indirect effect through NDVI followed by flag leaf area, 1000 grain weight and number of tillers. Grain iron content showed negative indirect effect towards yield through plant height (-0.0715) followed by number of grains per spike, chlorophyll content, 1000 grain weight, NDVI, grain zinc content, flag leaf area, days to 50 % flowering and number of tillers.

The unearthing of contribution of specific traits, such as grain count per spike and chlorophyll content on yield, offers valuable insights for indirect selection in yield improvement. These findings have practical implications for breeding programs aimed at enhancing crop performance in diverse agro-ecological settings [16]. By combining genetic, physiological, and agronomic approaches, researchers and policymakers can create more resilient and nutritionally enriched crop varieties suited to the unique challenges of tropical agricultural systems. Ultimately, these efforts can significantly contribute to sustainable food production and address hidden hunger, benefiting both scientific knowledge and practical interventions in global agriculture and public health.

4. CONCLUSION

Grain yield per plant exhibited highly significant positive correlation with number of grains per spike and chlorophyll content in both the F_2 populations. Further yield showed negative correlation for grain iron content. Path coefficient analysis of both crosses revealed that traits such as plant height, days to 50 % flowering, days to maturity, chlorophyll content and NDVI contributes direct effected towards yield in positive way while grain zinc and iron content showed direct negative effect towards yield. These results inferred that number of grains per spike and chlorophyll content could be used in indirect selection for yield improvement.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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