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Development of a Five-Parameter Model to Facilitate the Estimation of Additive, Dominance, and Epistatic Effects with a Mediating Using Bootstrapping in Advanced Generations of Wheat (*Triticum aestivum* L.)

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Abstract: As a result of two crosses among three local varieties of wheat, five populations (P$_1$, P$_2$, F_5, F_6 and F_7) were used as parents and grown during two successive seasons; 2016/2017 and 2017/2018. To estimate five types of gene action (e.g., mean effects, additive, dominance, additive × additive, and dominance × dominance), five formulas were developed from with algebraic solution, algebraic proof, and mathematical proof. Besides, to test adequate of a simple additive-dominance model, three formulas *A*, *B*, and *C* scaling test were developed. The path analysis method by PROCESS Macro, AMOS, and Bootstrapping was employed to assess the relationships between grain yield/plant (GYP) as the dependent variable and each one of the number of spikes (NS) and 1000-grain weight (TW) as the independent variables. The results show that there are eight validated equations used to estimate the scaling test (*A*, *B* and *C*) and five types of gene effects (m, a, D, I and L), respectively. Confidence interval using Bootstrapping results indicate that TW was played as the partial mediator between NS as an exogenous variable and GYP as an endogenous variable. Generation means analysis is a relatively simple and statistically reliable tool suitable for the fundamental estimation of different genetic influences.

Keywords: gene action; generation mean analysis; path analysis; mediation; bootstrapping

1. Introduction

Bread wheat (*Triticum aestivum* L.), as one of the paramount crops globally and in Egypt, occupies a cultivated area of about one million hectares. In Egypt, wheat production is around 9.5 million tons, which cover less than 60% of the local consumption. Wheat accounts for about 10% of the total agricultural output value and about 20% of total agricultural imports [1].

Grain yield is a complex polygenic trait that occurs due to the relation between different innate characters and the environment. Via indirect selection based on yield components, wheat grain yield can be improved [2]. Combinations of favorable yield-contributing traits will ameliorate productivity; also, the key selection criteria for wheat breeding are high grain yield, number of spikelets per spike, weight of a thousand kernels, and grain yield, among others [3,4]. The method of selecting pedigree is effective for tillers per plant, spike kernels, and grain yield per plant [5]. Besides, data in [6] explained that

the method of selecting pedigree is more effective than the other methods in improving the yield and its components. It has been noted that a 13.5% or 12.6% increase in grain yield per plant is associated with a direct selection of 1000-kernel weight and grain yield per plant [7]. Based on the evaluated genetic parameters, El-Ameen et al. [8] demonstrated the efficacy of late pedigree selection in the F_3, F_4, F_5 and F_6 generations in developing high-yielding genotypes from bread wheat.

Selection in progressive generations can be successful for the number of kernels per spike and the grain yield due to dominance and epistatic effects [9]. The concept of generation means analysis was to estimate the gene components of variance [10,11]. In plant breeding, analysis of generation means is a robust method for estimating important genetic influences (dominance and additive) and their digenic interactions (additive × additive, additive × dominance, and dominance × dominance) responsible for the inheritance of quantitative traits [12]. A reasonably simple and statistically accurate method for a fundamental quantification of the influences of different genes is an analysis of generation means [13].

Several researchers have used a five-parameter model to regulate the form of genetic action in which the inheritance of yields and their components are controlled in wheat [14–18]. Using the generated advanced filial data, the gene influences are estimated [19]. To estimate genetic influences, many genetic models have been developed, with most of these models supposing certain basic prerequisites. Almost all models [10,11] have been developed only to assess the influences of both non-allelic additives and the dominant gene, whereas after Fisher's paper [20], the effects of an epistatic gene are expected to be minimal.

This research aimed at establishing a novel model of five criteria for estimating the mean generation in advanced filial. Three pedigree selection cycles were used to develop high-yielding wheat lines in advanced generations (e.g., F_5, F_6 and F_7).

2. Materials and Methods

In the current study, three advanced populations of F_5, F_6, and F_7 of two crosses ($P_1 \times P_2$ and $P_1 \times P_3$) of wheat-derived from three local cultivars were used as parents (P_1; Sakha 93, P_2; Gimmiza 5, and P_3; Sids 1). The P_1 (the cultivar Sakha 93) showed moderate in the grain yield per plant and was selected as the female parent. The P_2 (the cultivar Gimmiza 5) and P_3 (the cultivar Sids 1) are the lowest and the highest in the grain yield per plant, respectively, and were selected as the male parents for their components.

During two consecutive seasons (2016/2017 and 2017/2018), five populations (P_1, P_2, F_5, F_6 and F_7) of both the first and second crosses were grown in the experimental farm (Southeast Fayoum; 29° 17′ N; 30° 53′ E), Agriculture College, Fayoum University, Egypt. For each cross, the five mentioned populations were sown and evaluated using a randomized complete block design with three replications. In each replication, there were 3 rows of each parent, 6 rows of F_5, 5 rows of F_6, and 4 rows of F_7 populations. Each row was 3.0 m long with 20 cm between-row spacing and 10 cm within-row spacing. The number of plants selected varied according to the generation, as follows: 15, 25, 20, and 20 for parents, F_5, F_6, and F_7 respectively. For each cross, grain yield per plant, number of spikelets per spike, and weight of 1000-grain were measured and analyzed.

Variance analysis and mean values of different generations were performed by using SPSS software.

As authors of this work, the analysis of generation mean was conducted using a five-novel, improved formula. In the case of the adequacy of the additive-dominance model, population genetic components mean (Table 1):

$$\overline{P}_1 = m + a,$$

where P_1 (AA) is consider the as large parent

$$\overline{P}_2 = m - a,$$

where P$_2$ (aa) is consider the small parent

$$\overline{F}_5 = m + 1/16\, D,$$

$$\overline{F}_6 = m + 1/32\, D$$

and

$$\overline{F}_7 = m + 1/64\, D$$

Algebraic Proof: Components of population genetic means if non-allelic gene actions are involved (Table 1):

$$\overline{P}_1 = m + a + I,$$

where P$_1$ (AA) is consider as the large parent

$$\overline{P}_2 = m - a + I,$$

where P$_2$ (aa) is consider as the small parent

$$\overline{F}_5 = m + \frac{1}{16}D + \frac{1}{256}\,L,$$

$$\overline{F}_6 = m + \frac{1}{32}D + \frac{1}{1024}L$$

and

$$\overline{F}_7 = m + \frac{1}{64}D + \frac{1}{4096}L$$

Scaling test formulas:

Table 1. Coefficients of the parameters used in the genetic model for generation means analysis.

Populations	Gene Actions				
	Additive-Dominance Model			**Non-Allelic (Epistasis)**	
	m	**a**	**D**	**I = aa**	**L = DD**
$\overline{P}_1$	1	+1	0	+1	0
$\overline{P}_2$	1	−1	0	+1	0
$\overline{F}_5$	1	0	+1/16	0	+1/256
$\overline{F}_6$	1	0	+1/32	0	+1/1024
$\overline{F}_7$	1	0	+1/64	0	+1/4096

where: $\overline{P}_1$ = mean of the large parent, $\overline{P}_2$ = mean of the small parent, $\overline{F}_5$ = mean of the 5th filial genera tion, $\overline{F}_6$ = mean of the 6th filial generation, $\overline{F}_7$ = mean of the 7th filial generation, [m] = mean effects; [a] = additive; [D] = dominance; [I] = additive × additive and [L] = dominance × dominance.

To assess the existence of non-allelic gene interaction, the additive-dominance model scaling tests *A*, *B*, and *C* were applied to evaluate the appropriate genetic model formulas (*A*, *B*, and *C*) and their variances. The scales were calculated as follows:

The primary principle of scaling is to develop the relationship between population means. If the means of generation depend only on the additive and dominance effects of genes, that is, no non-allelic interactions exhibited and no differential viability or fertility differential, then it is evident that simple relationships between the predicted values of means of different generations can be identified.

From the mean of F_6 generation formula, it was noted that the mean of F_6 generation would be expected to deviate from the mid-parent value by half the amount that the mean of the F_5 generation deviates from the mid-parent value under these circumstances. That is:

$$\overline{F}_6 - \frac{1}{2}\left(\overline{P}_1 + \overline{P}_2\right) = \frac{1}{2}\left[\overline{F}_5 - \frac{1}{2}\left(\overline{P}_1 + \overline{P}_2\right)\right]$$

$$\overline{F}_6 = \frac{1}{2}\left[\overline{F}_5 - \frac{1}{2}\left(\overline{P}_1 + \overline{P}_2\right)\right] + \frac{1}{2}\left(\overline{P}_1 + \overline{P}_2\right)$$

$$\overline{F}_6 = \frac{1}{2}\overline{F}_5 - \frac{1}{4}\overline{P}_1 - \frac{1}{4}\overline{P}_2 + \frac{1}{2}\overline{P}_1 + \frac{1}{2}\overline{P}_2$$

In various ways, this can be rewritten, with one of the most beneficial being F_6:

$$\overline{F}_6 = \frac{1}{2}\overline{F}_5 + \frac{1}{4}\overline{P}_1 + \frac{1}{4}\overline{P}_2$$

by multiple formula sides of two by four:

$$4\overline{F}_6 = 2\overline{F}_5 - \overline{P}_1 - \overline{P}_2$$

this can be rephrased in scale E as follow:

$$A = 4\overline{F}_6 - 2\overline{F}_5 - \overline{P}_1 - \overline{P}_2$$

by the same way we can prove B

$$B = 4\overline{F}_7 - 2\overline{F}_6 - \overline{P}_1 - \overline{P}_2 \ \square$$

Scaling test formulae:

$$A = 4\overline{F}_6 - 2\overline{F}_5 - \overline{P}_1 - \overline{P}_2$$
$$B = 4\overline{F}_7 - 2\overline{F}_6 - \overline{P}_1 - \overline{P}_2$$
$$C = \frac{8}{3}\ \overline{F}_7 - 2\overline{F}_6 + \frac{1}{3}\ \overline{F}_5 - \frac{1}{2}\overline{P}_1 - \frac{1}{2}\ \overline{P}_2$$

Formulas for estimating genetic influences:

Both the five-population means were used not only to detect the influences of non-allelic interactions on these means but also to measure their magnitude. Since five genetic parameters (e.g., m, a, D, I and L) were found for earlier generations, at least five family means are needed to be estimated [21].

The algebraic solution for the deduction of five (m, a, D, I, and L) parameters:

The vital point in this study is to find the values of both unknown D and L in terms of population means of $\overline{F}_5$ and $\overline{F}_6$ filial generation means as follows:

$$\overline{F}_5 = m + \frac{1}{16}D + \frac{1}{256}\ L$$

$$\overline{F}_6 = m + \frac{1}{32}D + \frac{1}{1024}L$$

$$D = 64\overline{F}_6 - 16\ \overline{F}_5 - 48\ m$$

$$L = 512\ \overline{F}_5 - 1024\overline{F}_6 + 512\ m$$

To estimate m, compensate D and L in the following formula:

$$\overline{F}_7 = m + \frac{1}{64}D + \frac{1}{4096}L$$

$$m = \frac{8}{3}\ \overline{F}_7 - 2\overline{F}_6 + \frac{1}{3}\ \overline{F}_5$$

To estimate L, we compensated m $\left(m = \frac{8}{3}\ \overline{F}_7 - 2\overline{F}_6 + \frac{1}{3}\ \overline{F}_5\right)$ in the following formula:

$$L = 512\ \overline{F}_6 - 1024\overline{F}_6 + 512\ m$$

So,

$$L = \left(\frac{2048}{3}\right)\overline{F}_5 - 2048\overline{F}_6 + \left(\frac{4096}{3}\right)\overline{F}_7$$

For estimating D we compensated m $\left(m = \frac{8}{3}\overline{F}_7 - 2\overline{F}_6 + \frac{1}{3}\overline{F}_5\right)$ in next formula

$$D = 64\overline{F}_6 - 16\,\overline{F}_5 - 48\,m$$

So,

$$D = 160\overline{F}_6 - 32\,\overline{F}_5 - 128\,\overline{F}_7$$

The equation to estimate the Additive × Additive effect (I):
To estimate I by summing

$$\overline{P}_1 + \overline{P}_2$$

$$I = \frac{1}{2}\overline{P}_1 + \frac{1}{2}\overline{P}_2 - m$$

Then, compensated m $\left(m = \frac{8}{3}\overline{F}_7 - 2\overline{F}_6 + \frac{1}{3}\overline{F}_5\right)$ in next formula:

$$I = \frac{1}{2}\overline{P}_1 + \frac{1}{2}\overline{P}_1 - m$$

So,

$$I = \frac{1}{2}\overline{P}_1 + \frac{1}{2}\overline{P}_2 - \frac{8}{3} \times \overline{F}_7 + 2 \times \overline{F}_6 - \frac{1}{3} \times \overline{F}_5$$

a = Additive gene effect a is half of the difference between two parents.

$$a = \frac{1}{2}\left(\overline{P}_1 - \overline{P}_2\right)$$

Algebraic proof of scaling test formulas (*A*, *B* and *C*):
　　Note that the following proof is determined by the additive-dominance model only which means that no non-allelic interaction (epistasis).

$$A = 4\overline{F}_6 - 2\overline{F}_5 - \overline{P}_1 - \overline{P}_2$$
$$= 4(m + \tfrac{1}{32}D) - 2\,(m + \tfrac{1}{16}D) - (m + a) - (m - a) = \text{Zero}$$

$$B = 4\overline{F}_7 - 2\overline{F}_6 - \overline{P}_1 - \overline{P}_2$$
$$= 4(m + \tfrac{1}{64}D) - 2\,(m + \tfrac{1}{32}D) - (m + a) - (m - a) = \text{zero}$$

$$C = \tfrac{8}{3}\,\overline{F}_7 - 2\overline{F}_6 + \tfrac{1}{3}\,\overline{F}_5 - \tfrac{1}{2}\overline{P}_1 - \tfrac{1}{2}\,\overline{P}_2$$
$$= 8/3\,(m + \tfrac{1}{64}D) - 2\,(m + \tfrac{1}{32}D) + 1/3\,(m + \tfrac{1}{16}D) - 1/2\,(m + a) - 1/2\,(m - a) = \text{zero}$$

Algebraic proof of m, a, D, I, and L:
　　Note that the following proof is determined by the non-allelic gene actions model which means that non-allelic interactions (epistasis) are involved.

$$m = \tfrac{8}{3}\,\overline{F}_7 - 2\overline{F}_6 + \tfrac{1}{3}\,\overline{F}_5$$
$$= 8/3\,(m + \tfrac{1}{64}D + \tfrac{1}{4096}L) - 2\,(m + \tfrac{1}{32}D + \tfrac{1}{1024}\,L) + 1/3\,(m + \tfrac{1}{16}D + \tfrac{1}{256}\,L) = m$$

$$a = \tfrac{1}{2}\,(\overline{P}_1 - \overline{P}_2)$$
$$= 1/2\,(m + a + I) - 1/2\,(m - a + I) = a$$

$$D = 160\,\overline{F}_6 - 32\,\overline{F}_5 - 128\,\overline{F}_7$$
$$= 160\,(m + \tfrac{1}{32}D + \tfrac{1}{1024}L) - 32\,(m + \tfrac{1}{16}D + \tfrac{1}{256}\,L) - 128\,(m + \tfrac{1}{64}D + \tfrac{1}{4096}L)$$
$$= D$$

$$I = \tfrac{1}{2}\,\overline{P}_1 + \tfrac{1}{2}\,\overline{P}_2 - \tfrac{8}{3} \times \overline{F}_7 + 2 \times \overline{F}_6 - \tfrac{1}{3} \times \overline{F}_5$$
$$= 1/2(m+a+I) + 1/2(m-a+I) - 8/3\left(m + \tfrac{1}{64}D + \tfrac{1}{4096}\,L\right) + 2\left(m + \tfrac{1}{32}D + \tfrac{1}{1024}L\right) - 1/3\left(m + \tfrac{1}{16}D + \tfrac{1}{256}\,L\right)$$
$$= I$$

$$L = \left(\tfrac{2048}{3}\right)\overline{F}_5 - 2048\,\overline{F}_6 + \left(\tfrac{4096}{3}\right)\overline{F}_7$$
$$= (2048/3)\left(m + \tfrac{1}{16}D + \tfrac{1}{256}\,L\right) - 2048\left(m + \tfrac{1}{32}D + \tfrac{1}{1024}L\right) + (4096/3)\left(m + \tfrac{1}{64}D + \tfrac{1}{4096}L\right)$$
$$= L$$

The following notation for gene influences was utilized in [12] methodology: (m), mean; (a), additive; (D), dominance; (I), additive × additive; and (L), dominance × dominance.

Statistical analysis:

Using SPSS version 25 tools, variance analysis and mean values of different generations were tested. A t-test checked the significance of gene effects.

In addition to the use of correlation coefficient path analysis results are important when direct and indirect effects are interpreted. Analysis of path coefficients was performed following the procedure developed by PROCESS Macro, IBM SPSS AMOS 24 and Bootstrapping. Dewey and Lu [22] and Arbuckle [23] were employed to assess the relationships between grain yield/plant (GYP) as the dependent variable and each one of the number of spikes (NS) as the independent variables and 1000-grain weight (TW) as mediator variable.

3. Results

For three traits studied over two seasons, the mean square values computed in two crosses revealed highly significant differences between populations (Table 2). Over two seasons, three characteristics under study showed a large effect size, where the smallest eta square > 86% is greater than 40% of the critical eta square values.

Table 2. Mean squares and effect size (η^2 Eta square) for three wheat traits studied in two crosses over two seasons.

Source of Variation	df	Mean Square					
		N. Spikelet		1000-Weight		Grain y/Plant	
Cross I ($P_1 \times P_2$)		2017	2018	2017	2018	2017	2018
Rep.	2	5.48 ns	1.75 ns	1.11 ns	0.23 ns	0.59 ns	0.18 **
Populations	4	226.97 **	160.80 **	620.50 **	532.54 **	341.12 **	353.90 **
Error	61	1.85	1.71	0.76	0.68	0.56	0.25
Effect Size η^2%		89.00	89.3	98.2	96.7	97.5	94.8
Cross II ($P_1 \times P_3$)							
Rep.	2	0.09 ns	1.26 ns	2.63 ns	0.02 ns	1.09 ns	3.17 ns
Populations	4	220.16 **	242.21 **	496.20 **	511.42 **	338.01 **	370.12 **
Error	61	1.73	1.39	1.125	0.77	1.23	0.96
Effect Size η^2%		86.00	91.9	99.10	97.7	98.1	96.20

ns: non-significant at $p < 0.05$; ** indicates the different significance at $p < 0.01$ level.

In cross two of the first season, the high value of eta square was reported by 1000-grain weight 99.1% which means that from five populations (P_1, P_2, F_5, F_6, and F_7), 99.1% of the variance of 1000-grain weight trait was predictable. In cross one of the first season, 1000-grain weight also showed eta square 98.2% high value while spikelet/spike number showed 89 and 86% lowest value in cross one and two, respectively, in the first season.

In both crosses, $P_1 \times P_2$ and $P_1 \times P_3$, spikelet/spike, weight of 1000-grain and grain yield/plant were evaluated in two seasons. For each cross and each season, sample size, means, and standard errors of the three traits for the five generations were presented separately (Table 3). To evaluate the significance between parents, the T-test was used. The findings showed that, as shown by the "t" test in two seasons, for all of the three characters studied, the differences between each parental pair in two crosses were highly significant.

Table 3. Mean performance of the three traits studied in five generations of wheat in two seasons.

Generations	No.	Traits					
		N. Spikelet (n)		1000-Weight (g)		Grain Yield/Plant (g)	
		Mean $\pm$ S.E		Mean $\pm$ S.E		Mean $\pm$ S.E	
Cross I ($P_1 \times P_2$)		2017	2018	2017	2018	2017	2018
Sakha93 (P_1)	10	20.00 ± 0.45	20.20 ± 0.54	37.96 ± 0.28	38.60 ± 0.15	15.97 ± 0.08	15.72 ± 0.21
Gimmiza5 (P_2)	10	16.60 ± 0.65	17.80 ± 0.74	33.90 ± 0.31	34.30 ± 0.30	12.56 ± 0.14	12.47 ± 0.15
t-value		4.29 **	2.49 **	9.65 **	12.99 **	20.95 **	12.74 **
F_5	18	25.00 ± 0.27	24.17 ± 0.26	48.30 ± 0.24	47.57 ± 0.24	22.99 ± 0.29	23.20 ± 0.13
F_6	15	26.73 ± 0.32	25.97 ± 0.13	49.28 ± 0.23	48.85 ± 0.24	24.33 ± 0.16	24.24 ± 0.11
F_7	15	26.76 ± 0.34	26.47 ± 0.08	49.69 ± 0.15	49.31 ± 0.11	24.53 ± 0.11	24.50 ± 0.09
Cross II ($P_1 \times P_3$)							
Sakha93 (P_1)	10	19.80 ± 0.44	19.60 ± 0.60	38.10 ± 0.28	37.90 ± 0.23	15.83 ± 0.12	15.93 ± 0.19
Sids1 (P_3)	10	21.80 ± 0.33	22.00 ± 0.33	43.90 ± 0.31	43.79 ± 0.29	20.03 ± 0.12	19.77 ± 0.18
t-value		3.64 **	3.49 **	13.84 **	15.82 **	24.15 **	14.77 **
F_5	18	27.60 ± 0.33	28.00 ± 0.25	51.40 ± 0.29	51.67 ± 0.23	25.57 ± 0.30	26.40 ± 0.30
F_6	15	28.29 ± 0.32	29.27 ± 0.25	52.77 ± 0.25	52.82 ± 0.24	28.02 ± 0.39	28.30 ± 0.28
F_7	15	29.29 ± 0.34	29.54 ± 0.25	53.10 ± 0.30	52.92 ± 0.17	28.30 ± 0.27	28.55 ± 0.28

** indicates the different significance at $p < 0.01$ level.

Means and standard errors of the number of spikelet/spike, weight of 1000-grain, and grain yield/plant of the two wheat crosses studied over two seasons of the five populations (P_1, P_2, F_5, F_6, and F_7) were reported in Table 3. All three traits, spikelet/spike number; 1000-grain weight and grain yield/plant in cross one over two seasons were higher for P_2 than P_1. While in cross two over two seasons P_1 had spikelet/spike number, weight of 1000-grain, and grain yield/plant lower than P_3.

The number of spikelet/spike, weight of 1000-grain, and grain yield/plant means of selected lines in two crosses over two seasons showed that grand means was statistically higher and significant than three parents in F_5, F_6, and F_7.

Spikelet/spike number, weight of 1000-grain and grain yield/plant means of selected parents showed that in F_5 the overall mean was lower than in F_6 and F_7 and statistically significant. In F_7, the highest value was recorded over two seasons in two crosses. In addition, the mean of F_6 for two crosses over two seasons was lower, but not statistically significant, than the mean of F_7.

The joint scaling test was used to assess the mean (m), additive effect (a), dominance effect (D), additive $\times$ additive (I), and dominance $\times$ dominance (L) values in two crosses over two seasons, as well as the epistasis type (Table 4).

Table 4. Scaling test, gene actions and epistasis for three quantitative traits in two crosses of wheat over two seasons.

Generations	No.	Traits					
		N. Spikelet		$1000 - $Weight		Grain y/Plant	
		Mean		Mean		Mean	
Cross I		2017	2018	2017	2018	2017	2018
Scaling test	A	20.31 **	17.55 **	28.66 **	27.38 **	22.81 **	22.36 **
	B	16.97 **	15.94 **	28.34 **	26.63 **	20.93 **	21.33 **
	C	−8.74 **	−8.41 **	−18.08 **	−18.52 **	−5.18 **	−4.98 **
Gene Actions	m	26.23 **	26.70 **	50.05 **	49.64 **	24.42 **	24.59 **
	a	1.70 **	1.20 **	2.03 **	2.15 **	1.71 **	1.63 **
	D	51.41 ^{ns}	−6.25 ^{ns}	−21.12 ^{ns}	−17.28 ^{ns}	17.28 ^{ns}	−0.11 ^{ns}
	I	−7.93 **	−7.70 **	−14.12 **	−13.19 **	−10.15 **	−10.49 **
	L	−1137.78 ^{ns}	−548.86 ^{ns}	−109.23 ^{ns}	−254.86 ^{ns}	−641.71 ^{ns}	−352.71 ^{ns}
Cross II							
Scaling test	A	18.92 **	19.48 **	26.28 **	26.24 **	25.08 **	24.69 **
	B	17.70 **	18.01 **	24.86 **	24.34 **	21.30 **	21.91 **
	C	−9.75 **	−9.19 **	−22.07 **	−22.59 **	−7.30 **	−7.11 **
Gene Actions	m	29.45 **	29.56 **	53.19 **	52.70 **	27.95 **	28.34 **
	a	−1.00 **	−1.2 **	−2.09 **	−2.95 **	−2.10 **	−1.92 **
	D	−3.25 ^{ns}	6.51 ^{ns}	1.6 ^{ns}	24.00 ^{ns}	42.56 ^{ns}	28.31 ^{ns}
	I	−8.65 **	−8.76 **	−12.19 **	−11.85 **	−10.02**	−10.49 **
	L	−416.63 ^{ns}	−502.90 ^{ns}	−484.69 ^{ns}	−648.53 ^{ns}	−1290.24 ^{ns}	−949.36 ^{ns}

^{ns}: non-significant at $p < 0.05$; ** significant at $p < 0.01$.

The findings of the two-season A, B, and C scaling test for the two wheat crosses have shown that the significance of both of these measurements implies the existence of epistasis on the measurement scale used. The findings of the scaling test demonstrated that the additive–dominance model was inadequate to clarify the inheritance of all characters tested, implying the existence of non-allelic gene interaction over two seasons in two crosses.

The mean parameters (m) showed that the contribution was highly significant beccause of the overall mean plus the mean influences and interaction of the fixed loci for three studied traits of two crosses over two seasons (Table 4). The additive gene effect (a) was significant over two seasons for three traits in two crosses. In the second cross of the first season and cross one in the second season, the presence of complementary gene action for spikelet/spike, weight of 1000-grain in cross one over two seasons, and grain yield/plant in cross one in the second season suggests that parents selected for crossing are varied.

A-five parameter model was proposed in [10]. When dominance (D) and dominance $\times$ dominance (L) have the same sign, results are complementary while different signs indicated duplicated epistasis [12].

The path analysis method was used to determine the relationships between (GYP) as the dependent variable and each of (NS) and (TW) variables as the independent. In Figure 1 and Table 5, TW testing was provided as a mediator between NS and GYP. Results showed that in the first and second seasons, a positive and direct effect was found between NS and GYP (0.92 and 0.91, respectively). In both the first and second seasons, as well as a positive and direct effect was observed between TW with NS and GYP by 0.96 and 0.98, respectively.
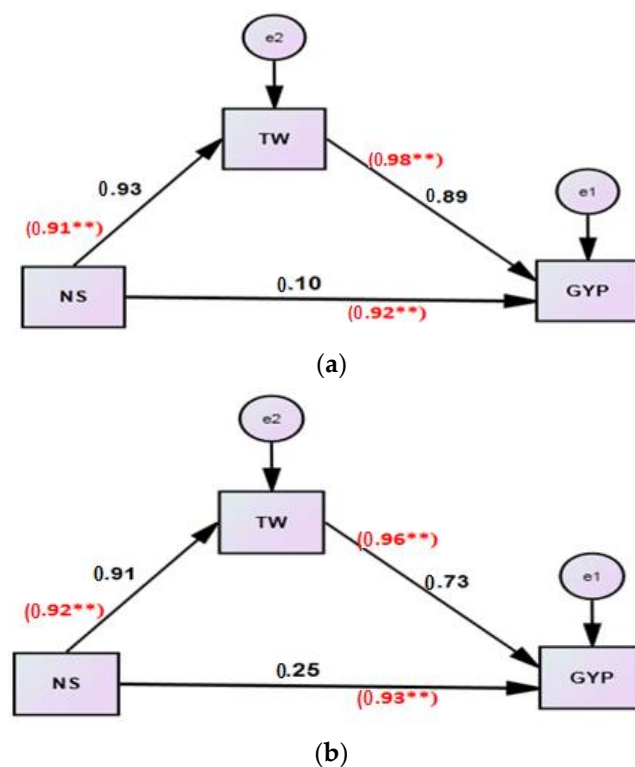
(**a**)

(**b**)

Figure 1. (**a**) The path analysis for direct and indirect effects and Person correlation (r) of (NS) as independent variable and (TW) as mediator variable and (GYP) as dependent variable of first season. (**b**) The path analysis for direct and indirect effects and Person correlation (r) of (NS) as independent variable and (TW) as mediator variable and (GYP) as dependent variable of second season (** indicates the different significance at $p < 0.01$ level).

Table 5. Direct, indirect, total effects, type of mediation and confidence interval by bootstrapping of Tw and NS traits on grain yield per plant over all five populations of two crosses of wheat.

Effect	Traits	Season	Value	Confidence Interval Using Bootstrapping		*p*-Value by Bootstrapping	Sig.
				Lower	Upper		
Direct Effect via	TW	First	0.89	0.80	0.96	0.003	**
		Second	0.73	0.64	0.83	0.008	**
	NS	First	0.10	0.17	0.19	0.04	*
		Second	0.25	0.15	0.35	0.006	**
Indirect Effect of NS via	TW	First	=(0.93) (0.89) =0.83	0.73	0.92	0.04	*
		Second	=(0.91) (0.73) =0.66	0.56	0.77	0.003	**
Total effect	TW	First	=0.89 + 0.83 =1.72	0.91	0.93	0.015	*
		Second	=0.73 + 0.66 =1.39	0.87	0.92	0.005	**
	NS	First	0.1	0.89	0.94	0.007	**
		Second	0.25	0.88	0.93	0.006	**
Type of mediation	First season		Partial mediation				
	Second season		Partial mediation				

* indicates the different significance at $p < 0.05$ level; ** indicates the different significance at $p < 0.01$ level.

In the two seasons, TW possessed the greatest direct effect on GYP. In the first and second seasons, NS showed an indirect positive effect on GYP via TW through 0.83 and 0.66. Person correlation between all combinations is shown in Figure 1a,b).

In both seasons, respectively, GYP had a positive significant and strong correlation between TW (0.98 and 0.96) and NS (0.92 and 0.93). NS demonstrated a significant positive and strong correlation with TW (0.91 and 0.92) in the first and second seasons, respectively.

In the case of TW as a mediator variable in the relationship between NS as an independent or exogenous variable, GYP was used as a dependent or endogenous variable in a model process [24] tools in SPSS (Figure 1 and Table 5).

4. Discussion

The above findings indicate a wide level of variability between parents and hybrids for three characteristics and the possibility of selection in advanced generations. In wheat, genetic variation was found for weight of 1000-grain and grain yield/plant [25–27]. For three traits over two seasons, the effect size was reported using the eta square (η^2) index [28–30]. In the dependent variable, the proportion of variance illustrated by the independent variable analysis is defined as Eta square [31]. Over two seasons, three characteristics under study showed large effect size, where the smallest eta square >86% is greater than 40% of critical eta square values.

To evaluate the significance between parents, the T-test was used. The findings showed that, as shown by the "t" test in two seasons, for all of the three characters examined, the differences between each parental pair in two crosses were highly significant. Thus, the use of these three wheat varieties showed a reasonable amount of genetic variability, valid to deciding types of gene behavior and interactions controlling the inheritance of the characters under study. In addition, because of the various generation responses to environmental influences, variability expressed as standard error, which was mostly lower in advanced generations than parents, varied from season to another due to the different generation responses. The number of spikelet/spike, weight of 1000-grain, and grain yield/plant means of lines selected in two crosses over two seasons showed that the grand means were statistically higher and more significant than three parents in F_5, F_6, and F_7.

The joint scaling test was used to assess the mean (m), additive effect (a), dominance effect (D), additive × additive (I), and dominance × dominance (L) values in two crosses over two seasons, as well as epistasis type. The results of the two seasons A, B, and C scaling test for the two wheat crosses showed that the significance of both tests measurements indicated the presence of epistasis on the measurement scale used.

For three traits in two crosses, the additive gene effect (a) was significant over both seasons. These findings revealed that, in these crosses, the three characters were under the control of simple gene pathways involving major genes of great effect, and it may be more effective to improve the output of these traits by using the pedigree selection program. Although estimation of the dominance gene action (D) was not significant over two seasons for the three traits in two characters, indicating a lack of heterozygosity and homogeneity of the lines.

The presence of (D) for different traits but not significant suggested that selection should not be delayed if population heterozygosity was reduced. For all studied traits over two seasons, the epistatic (I) effect (additive × additive) was significant in the two crosses, confirming the important role of gene interaction (additive × additive) in the genetic system.

A five-parameter model was proposed in [10]. When dominance (D) and dominance × dominance (L) have the same sign, the results are complementary despite different signs of duplicated epistasis appearing [12]. The path analysis method was used to determine the relationships between (GYP) as the dependent variable and each variable (NS) and (TW) as the independent. In two seasons, TW possessed the greatest direct influence on (GYP). In the first and second seasons, NS showed an indirect positive effect on (GYP) via (TW) through 0.83 and 0.66. Person correlation between all combinations is shown in

Figure 1. In the case of TW as a mediator variable in the relationship between NS as an independent or exogenous variable and its GYP as a dependent or endogenous variable in a model process [24] tools were used in SPSS (Figure 1 and Table 5).

The above test endorsed the mediation test and partial mediation, in which the direct and indirect effects were significant over two seasons. To verify the effect of the direct and indirect effect, Bootstrapping's confidence interval was used [32,33]. The Bootstrapping confidence interval revealed that in two seasons TW was played as a partial mediator between NS and GYP as dependent variables.

5. Conclusions

Most of the estimation methods for estimating five types gene action (m, a, D, I, and L) focused on the means of earlier filial generation (P_1, P_2, F_1, F_2, and F_3) in case backcross progenies do not exist. The defect of this design is that it is limited for estimating only single gene effects. Such classical designs are impractical for full analysis of metric traits if any of the earlier five populations is absent or its size is not pretties enough for acceptable analysis. On the other hand, in practice, breeders may have different advanced filial populations which are adequate for perfect investigation. In this paper, five novel formulas were developed from five advanced populations mean (P_1, P_2, F_5, F_6, and F_7) along with three formulas as scaling test (A, B, and C) with algebraic solution, algebraic proof, and mathematical proof approach to quantify the influences of genes. Path analysis was applied to estimate the direct and indirect influences between the studied traits using PROCESS Macro, AMOS, and Bootstrapping. The results suggest that five parameter models can be particularly useful for estimating gene effects (m, a, D, I, and L). The confidence interval using Bootstrapping results indicates that TW was played as a partial mediator between NS as an exogenous variable and GYP as an endogenous variable.

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