

## CHARACTER ASSOCIATION FOR YIELD AND ITS CONTRIBUTING TRAITS IN WHEAT ((TRITICUM AESTIVUM.L)

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### Abstract:

The experimental material for the present investigation comprised of forty-five F1s developed by crossing 10 lines viz., DBW187, K1601, HD2967, HD3249, DBW321, K1317K0307, HI 1563, DBW107, and HD3059 following half diallel mating design. A total of 100 treatments with 10 parents (45 F1s and 45 F2s) were evaluated for the study of twelve quantitative characters in wheat. Phenotypic and genotypic correlations were worked out on yield and yield contributing characters in forty-five crosses and 10 parents. Correlation estimates the degree and direction of association/relationship between two or more variables. Grain yield was a complex trait and was dependent on many associated characteristics. Hence, character association was studied in the present investigation, to judge the relationships among yield, and its components for improving the usefulness of selection. Genotypic correlations are heritable associations between two variables and it was more stable and paramount relevant to the plant breeder to bring about genetic improvement in one character by selecting another character of pair that was genetically correlated. Indirect selection is more effective than the direct selection procedure when the attribute in question has low heritability and/or is not easily and precisely measured. Grain yield per plant (g) showed a significant and positive correlation with days to 75% flowering, number of tillers per plant, number of spikelets per spike, spike length, number of grains per spike and protein content while the negative and significant correlation with plant height 1000-grain weight, ear density, and duration of reproductive phase in F1 generations. In F2 generation significant and positive associations at phenotypic levels were observed for days to 75% flowering with the number of grains per spike, days to maturity 1000-grain weight, protein content, and grain yield per plant while the negative and significant correlation with duration of the reproductive phase.

**Keywords:** Genotypic Correlation, Phenotypic Correlation, Indirect selection, Heritability

### Introduction:

Wheat (*Triticum aestivum* L.,  $2n=42$ ) is the most important cereal in the world and was one of the first crops to be domesticated some 10000 years ago (Harlan and Zohary, 1966). Because of its vast acreage, excellent production, and significant position in the international food grain trade, it has been termed the "King of Cereals." Wheat has comparatively high levels of niacin and thiamin, which are the elements that make wheat so special. Gluten supplies the framework for the spongy cellular texture of bread and baked goods; therefore, wheat proteins are particularly important (Bhushan *et al.*, 2013). *T. aestivum* is a segmental allohexaploid ( $2n = 6x = 42$ , AABBDD) that originated in the

Fertile Crescent area of South-Western Asia (**Lupton 1987**), its geographical center of origin and spread globally for cultivation and consumption. Allohexaploid wheat possesses three genomes A, B, and D are three genomes. The genome "A" comes from wild einkorn wheat (*Triticum monococcum* var. *urartu*), "B" comes from an unknown species, and genome "D" comes from a weedy grass *Squarrosa Aegilops*. Hexaploid wheat (*Triticum aestivum* L.,  $2n = 42$ ) has a haploid DNA content of around  $1.7 \times 10^{10}$  bp, which is almost 40 times that of rice. (**Bennett and Smith, 1976; Amuruganathan and Earle, 1991**).

Wheat, unlike other cereals, contributes more than 30% of all calories consumed by the global population (**Peterson *et al.*, 2006**), and it contains a large level of gluten, the protein that gives bread its elasticity. Hard wheat has high protein content (10-17%) and produces gluten-rich flour, making it ideal for yeast pieces of bread. Wheat is grown on around 221.24 million hectares worldwide, with a record yield of 771.64 million tonnes of grain and productivity is 3.49 metric tons per hectare (**USDA 2023**). India has the most wheat-growing land (14 percent), followed by Russia (12.43 percent), China (11.14 percent), and the United States (6.90 percent), accounting for around 45 percent of the global total. China, on the other hand, is the world's largest wheat producer, with 136 million tonnes produced, followed by India (98.51 million tonnes), Russia (85 million tonnes), and the United States (47.35mt). Global wheat production in 2022 is predicted to decline from the 2021 record level by 0.8 per cent, reaching 771.64 million tonnes and marking the first drop in four years. Year-on-year falls in production in Australia, India, Morocco and Ukraine will likely outweigh expected increases in Canada, Iran and Russia Further; it said that in Asia, wheat production in India is forecast at 105.5 million tonnes, down nearly 4 percent from the record crop gathered in 2021. (**Business Standard 2022-23**). Part of this drop was due to a reduction in harvested area, particularly for rainfed crops. According to recent data from Brazil and the United Kingdom of Great Britain and Northern Ireland, harvests were less than expected, resulting in a slightly lower worldwide output prediction of 769.6 million tonnes, reinforcing an expected 1% year-on-year reduction. (FAO, 2021). Wheat production in 2022-23 is expected to be between 98 million and 106 million tonnes, down from 107.9 million tonnes in 2020-21 (**USDA report, 2022**). Uttar Pradesh continues to be the country's greatest producer, accounting for over 28 million tonnes, or roughly 30% of total production. Wheat demand is anticipated to rise by 50% by 2050 compared to current levels. Meanwhile, new and more aggressive pests and viruses, dwindling water resources, limited accessible land, and unpredictable weather are all threatening the crop (heat in particular). For Africa, Asia, and Latin America, the CIMMYT's Global Wheat Program is one of the most significant public sources of high-yielding, nutritious, disease-resistant, and climate-resilient wheat varieties (**Wheat Research, CIMMYT**).

The success of our wheat varieties is up to a considerable extent due to the incorporation of the Norin 10 genes *i.e.* *Rht<sub>1</sub>* and *Rht<sub>2</sub>* in wheat. These dwarfing genes changed the wheat plants type and it becomes more responsive to higher application of fertilizer and better crop management

under practices. In 1966, Dr. N.E. Borlaug, a noble laureate introduced the Mexican dwarf wheat genotypes and provides the way for the green revolution in India.

### Material and Methods:

The experiment was conducted at Oil Seed Farm, Kalyanpur, C.S. Azad University of Agriculture and Technology, Kanpur-208002 (U.P.) during Rabi, 2021-22. Geographically, this place is located between 25.280 and 26.580 N latitude, 79.310 and 80.340 E longitudes and an altitude of 125.9 m above from mean sea level. This falls in the sub-tropical climatic zone. The soil type is sandy loam. The annual rainfall is about 1270 mm. The climate of district Kanpur is semi-arid with hot summer and cold winter. The meteorological data during the crop season is presented in Table10(c). The experimental material for the present investigation comprised of forty-five F1s developed by crossing 10 lines viz., DBW187, K1601, HD2967, HD3249, DBW321, K1317K0307, HI 1563, DBW107 and HD3059 following half diallel mating design. A total of 100 treatments with 10 parents (45 F1s and 45 F2s) were evaluated for the study of twelve quantitative characters in wheat.

The material for the investigation comprised of 10 strains/varieties of wheat (*Triticum aestivum* L) selected based on the wide variability of various characters. Development F1 seed: All 10 genotypes have been grown during the Rabi 2018-19 for making crosses in half diallel fashion and resultant seeds of forty-five hybrids were harvested. Development F2 seed: Half seed of each hybrid was sent for advancement at IIWBR-Regional station, Dalang Maidan (H.P.) in off-season nursery to obtain seed for raising F2 generation. Rest half seed of each cross was procured to raise F1 generation in the final trail. The experimental materials consisted of 100 treatments (45 F1 + 45 F2 + 10 parents) were sown in Randomized Block Design with three replications. The entries were sown in a 3 m length with inter and intra-row spacing of 22.5 cm and 10 cm, respectively. All the recommended cultural practices were applied to raise a good crop. Following observations to be recorded: the sample was recorded in each treatment of all replications for the following traits. Days to 75% flowering, Plant height (cm), Number of tillers per plant, Spike length (cm), Number of spikelets/spike, Number of grains/spike, Days to maturity, 1000-grain weight (g), Ear density, duration of reproductive phase, Protein content (%) and Grain yield per plant.

### Diallel Analysis:

#### Testing the validity of the hypothesis:

To test the validity of the hypothesis, i.e., the assumptions regarding diallel analysis as proposed by Hayman (1954), such as (i) diploid segregation (ii) no maternal effect, (iii) no linkage (iv) no multiple allelism, (v) independent action of non-allelic genes and (vi) hemozygosity of parents, the  $t^2$  test was applied as suggested by Hayman (1954a):

$$t^2 = (n-2)/4 [(VarVr - VarWr)^2 / VarVr \times VarWr) - Cov^2 (Vr, Wr)]$$

which is an F test with 4 and (n-2) degrees of freedom.

A significant value of  $t_2$  would indicate the non-uniformity of  $W_r$ ,  $V_r$  and thus, invalidates the hypothesis postulated. The failure of the hypothesis is also indicated by non-significant regression coefficient.

$$b = \frac{\text{Cov}(W_r, V_r)}{\text{Var}(V_r)}$$

Where,

$$\begin{aligned} \text{Cov.}(W_r, V_r) &= \left[ \sum V_r W_r - \frac{\sum V_r \sum W_r}{n} \right] / (n-1) \text{ and} \\ \text{Var}(V_r) &= \left[ \sum V_r^2 - \frac{(\sum V_r)^2}{n} \right] / (n-1) \end{aligned}$$

The standard error of regression coefficient (b) was calculated as:

$$\text{SE}(b) = \left[ (\text{Var}W_r - b \text{Cov.} W_r - V_r) / \text{Var}V_r (n-2) \right]^{0.5}$$

Where,

N = number of parents

Now the significance of differences 'b' from zero and unity was tested by using 't' value of  $(b-0)/\text{SE}(b)$  and  $(1-b)/\text{SE}(b)$  with  $(n-2)$  degree of freedom.

(i) Variance component analysis:

The components of variance in diallel cross were computed in F1 by the use of the equation given by Hayman (1954a).

The expectation for F1 diallel crosses is as follows:

$$\begin{aligned} V_p &= \hat{D} + \hat{E} \\ V_r &= \left( \frac{1}{4} \right) \hat{D} + \left( \frac{1}{4} \right) \hat{H}_1 - \left( \frac{1}{4} \right) \hat{F} + \left[ \frac{(n+1)}{2n} \right] \hat{E} \\ W_r &= \left( \frac{1}{2} \right) \hat{D} - \left( \frac{1}{4} \right) \hat{F} + \left( \frac{1}{n} \right) \hat{E} \\ V_m &= \left( \frac{1}{4} \right) \hat{D} + \left( \frac{1}{4} \right) \hat{H}_1 - \left( \frac{1}{4} \right) \hat{H}_2 - \left( \frac{1}{4} \right) \hat{F} + \left( \frac{1}{2n} \right) \hat{E} \end{aligned}$$

Jinks (1956) and Hayman (1958) gave expectations for F2 diallel crosses. The expected statistics for F2 generation are the same as that of F1 except for the contribution of h which is halved by one generation of inbreeding. Hence, the coefficient of  $H_1$  and  $H_2$  are  $(1/4)$  of those F1 statistics while the coefficient of F is halved being second and first-degree statistics  $h_2$ , respectively (Jinks, 1956; Hayman 1958; Mather and Jinks, 1971). These expectations are as follows:

$$\begin{aligned} V_p &= \hat{D} + \hat{E} \\ V_r &= \left( \frac{1}{4} \right) \hat{D} + \left( \frac{1}{16} \right) \hat{H}_1 - \left( \frac{1}{8} \right) \hat{F} + \left[ \frac{(n+1)}{2n} \right] \hat{E} \\ W_r &= \left( \frac{1}{2} \right) \hat{D} - \left( \frac{1}{8} \right) \hat{F} + \left( \frac{1}{n} \right) \hat{E} \\ V_m &= \left( \frac{1}{4} \right) \hat{D} + \left( \frac{1}{16} \right) \hat{H}_1 - \left( \frac{1}{16} \right) \hat{H}_2 - \left( \frac{1}{8} \right) \hat{F} + \left( \frac{1}{2n} \right) \hat{E} \end{aligned}$$

Where,

$$\begin{aligned} \hat{D} &= \text{Components of variation due to additive effects of genes.} \\ &= V_0 L_0 - \hat{E} \\ \hat{H}_1 &= \text{Components of variation due to dominance effects of genes.} \\ &= V_0 L_0 - 4W_0 L_{01} + 4V_1 L_1 - (3n-2) \hat{E}/n \\ \hat{H}_2 &= \hat{H}_1 [1-(u-v)^2] = 4V_1 L_1 - 4V_0 \hat{L}_1 - 2E \end{aligned}$$

Where

$$\begin{aligned}
 U &= \text{Proportion of positive genes in the parents.} \\
 v &= \text{Proportion of negative genes in the parents} \\
 \hat{F} &= \text{The mean of } F_r \text{ over the arrays} \\
 F_r &= 2(V_0L_0 - 4W_0L_{01} + V_1L_1 - W_r - V_r) - 2(n-2) \hat{E}/n \\
 \hat{h}^2 &= \text{Dominance effects (as the algebraic sum over all loci in heterozygous phase in all crosses)} \\
 &= 4(M_{L1} - M_{L0})^2 - 4(n-1)\hat{E}/n^2 \\
 \hat{E} &= \text{the expected environmental component of variation} \\
 &= (\text{Error SS} + \text{Replication SS/d.f.})/\text{number of replication}
 \end{aligned}$$

To estimate of the accuracy of the components ( $\hat{D}$ ,  $\hat{F}$ ,  $\hat{H}_1$ ,  $\hat{H}_2$ ,  $\hat{h}^2$  and  $\hat{E}$ ) of variance, the term of main diagonal of matrix given Hayman (1954) with common multipliers  $S^2/n^5$ , was used.

Where,

$$\begin{aligned}
 S^2 &= \frac{1}{2} \text{ var. } (W_r - V_r). \text{ The formula being:} \\
 SE(\hat{D}) &= \pm [S^2 (n^5 + n^4)/n^5]^{0.5} \\
 SE(\hat{F}) &= \pm [S^2 (4n^5 + 20n^4 - 16n^3 + 16n^2)/n^5]^{0.5} \\
 SE(\hat{H}_1) &= \pm [S^2 (n^5 + 41n^4 - 12n^3 + 4n^2)/n^5]^{0.5} \\
 SE(\hat{H}_2) &= \pm [S^2 (36n^4)/n^5]^{0.5} \\
 SE(\hat{h}^2) &= \pm [S^2 (16n^2 + 16n^2 - 32n + 16n)/n^5]^{0.5} \\
 SE(\hat{E}) &= \pm [S^2 (n^4/n^5)]^{0.5}
 \end{aligned}$$

After testing the significance of the components of variation, the mean degree of dominance was calculated as  $(\hat{H}_1/\hat{D})^{0.5}$  in  $F_1$  and  $[0.25 (\hat{H}_1/\hat{D})]^{0.5}$  in  $F_2$  generation. The proportion of genes with positive and negative effects was calculated as  $H_2/4\hat{H}_1$ , the proportion of dominant and recessive genes in parents as the ration of  $[(4 \hat{D}\hat{H}_1)^{0.5} + 0.5\hat{F}] / [(4 \hat{D}\hat{H}_1)^{0.5} - 0.5\hat{F}]$  in  $F_1$  and  $[0.25 (4\hat{D}\hat{H}_1)^{0.5} + 0.5\hat{F}] / [0.25 (4\hat{D}\hat{H}_1)^{0.5} - 0.5\hat{F}]$  in  $F_2$  generation, the number of gene groups that control the character and exhibit dominance as  $\hat{h}^2 / \hat{H}_2$  and the coefficient of correlation between the parental order of dominance ( $W_r + V_r$ ) and parental measurement ( $Y_r$ ) as  $r$ .

### (iii). Correlation coefficients analysis:

The estimates of phenotypic and genotypic correlation were corked out as given under:

#### (a) Genotypic correlation

$$r_{xy}(g) = \text{Cov.}_{xy}(g) / [V_x(g) \cdot V_y(g)]^{0.5}$$

Where,

$\text{Cov.}_{xy}(g)$  = genotypic covariance between character X and y was obtained as follows:

$$\text{Cov.}_{xy}(g) = [\text{Cov.}_{xy}(p) - \text{Cov.}_{xy}(e)]/r$$

$V_x(g)$  and  $V_y(g)$  = Genotypic variance for the characters x and y respectively

$r$  = number of replications.

#### (b) Phenotypic correlation:

$$r_{xy}(p) = \text{Cov.}_{xy}(p) / [V_x(p) \cdot V_y(p)]^{0.5}$$

Where,

$\text{Cov.}_{xy}(p)$  = Phenotypic correlation between the character x and y and this was obtained as follows:

$$\text{Cov.}_{xy}(p) = \text{Cov.}_{xy}(g) + \text{Cov.}_{xy}(e)$$

$V_x(p)$  and  $V_y(p)$  = Phenotypic variance for the characters x and y, respectively.

$xy(e)$  = the error variance obtained from the ANNOVA of x and y characters.

#### Test of significance of correlation coefficients:

The significance of phenotypic coefficient was tested against 'r' values from 'r' Table of **Fisher and Yates (1938)** for (n-2) degree of freedom where 'n' is number of treatments.

#### Result and Discussion:

The data obtained from the present investigation for 12 characters viz., days to 75% flowering, plant height (cm), days to maturity, number of tillers per plant, spike length (cm), number of spikelets per spike, ear density, number of grains per spike, 1000-grain weight (g), duration of reproductive phase, protein content (%), and grain yield per plant (g) were subjected to the following statistical and biometrical analysis and relevant results were described subsequently (Table 1 and 2).

Indirect selection is more effective than the direct selection procedure when the attribute in question has low heritability and/or is not easily and precisely measured. The aim of correlation studies is primarily to know the suitability of various characters for indirect selection because selection for one or more traits results in correlated responses for several other traits (**Searle, 1965**), and the pattern of variation will also be changed (**Waddington and Robertson, 1966**). Therefore, knowledge of the genetic correlation existing between yield and its components is essential.

All possible phenotypic and genotypic correlations were worked out for twelve characters (Table 1) in ten patents, 45 F<sub>1</sub>S, 45F<sub>2</sub>S, though, the significance of genotypic correlations could not be tested as no suitable statistical test is available (**Nasr et al. 1973**) yet their magnitude is considered about the corresponding phenotypic estimates (**Fisher, 1918**).

In F<sub>1</sub> generation at genotypic correlation with positive and significant correlation was observed for grain yield per plant with days to 75% flowering, number of tillers per plant, number of spikelets per spike, spike length, number of grain per spike, and protein content while the negative and significant correlation with plant height and ear density. A positive and significant correlation was observed for grain yield per plant with days to 75% flowering, number of tillers per plant, number of spikelets per spike, spike length, number of grains per spike, and protein content while the significant negative correlation with plant height, ear density and duration of reproductive phase in F<sub>2</sub> generation. Similar results were observed by **Khan et al. (2010)**, **Rathod et al. (2019)**, **Kumar et al. (2014)**, **Kamaniet al. (2017)** and **Dhanda et al. (2018)** in cereals..

In F<sub>1</sub> generation phenotypic correlation for grain yield per plant (g) showed a significant and positive correlation with days to 75% flowering, number of tillers per plant, number of

spikelets per spike, spike length, number of grains per spike, and protein content while the negative and significant correlation with plant height. In F<sub>2</sub> generation grain yield per plant (g) showed a positive and significant correlation with days to 75% flowering, number of oh tillers per plant, spike length, number of grains per spike and protein content while negative and significant correlation with plant height. **Bayeet al. (2020), Rathodet al. (2019), Bayisaet al. (2021)** also support the same result with respect to grain yield which was significantly and positively correlated with days to maturity, 1000-grain weight.

#### **Future scope of the study:**

Main objective of this research was to develop the genotypes for the future varietal development to increase the yield if the desirable traits showing better performance in addition to existing variety at local and national level to increase the farmer income.

#### **Conflict of Interests:**

Authors have declared that no conflict of interests

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**Table 1: Phenotypic (upper half diagonal) and genotypic (lower half diagonal) Correlation among 12 characters in F1 generation in wheat.**

Phenotypic Genotypic	Days to 75 % flowering	Plant Height (cm)	Number of tillers per plant	Number of spikelets per spike	Spike length (cm)	Number of grain per spike	Days to maturity	1000- grain weight (g)	Ear density (cm)	Duration of reproductive phase	Protein content (%)	Grain yield per plant (g)
Days to 75 % flowering	1	0.02	-0.12	0.121	0.001	0.609**	0.22	0.421* *	0.14	- 0.619**	0.18	0.373* *
Plant Height (cm)	-0.01	1	-0.2	0.197	0.052	-0.12	0.21	-0.07	0.13	0.15	- 0.668* *	- 0.326* *
Number of tillers per plant	-0.13	-0.22	1	0.208	0.363* *	0.247	0.1	- 0.543* *	-0.28	0.17	0.264	0.466* *
Number of spikelets	0.14	0.21	0.21	1	0.735**	0.444**	0.405**	-0.27	0.05	0.23	-0.167	0.294* *
Spike length (cm)	0.01	0.05	0.403**	0.801**	1	0.540**	0.12	- 0.429* *	- 0.638**	0.1	0.103	0.423* *
Number of grain per spike	0.677**	-0.11	0.28	0.479**	0.596**	1	0.09	0.158	- 0.296* *	- 0.417**	0.339* *	0.536* *
Days to maturity	0.292* *	0.23	0.09	0.464**	0.165	0.113	1	-0.07	0.294* *	0.626**	- 0.314* *	0.066
1000- grain weight (g)	0.455**	-0.07	- 0.556**	-0.28	- 0.451**	0.157	-0.08	1	0.320* *	- 0.397**	-0.09	-0.188
Ear density (cm)	0.16	0.16	- 0.383**	-0.02	- 0.615**	- 0.368* *	0.362* *	0.384* *	1	0.13	- 0.326* *	-0.273
Duration of reproductive phase	- 0.605**	0.2	0.18	0.267	0.126	0.480**	0.585**	- 0.452* *	0.17	1	- 0.397* *	-0.245
Protein content (%)	0.2	- 0.695**	0.28	-0.18	0.111	0.352* *	- 0.349* *	-0.09	- 0.406**	- 0.460**	1	0.610* *
Grain yield per plant (g)	0.409**	- 0.341* *	0.481**	0.303* *	0.455**	0.551**	0.07	-0.19	- 0.338* *	-0.29	0.617* *	1

\* Significant at 5% level; \*\* significant at 1% level

**Table 2: Phenotypic (upper half diagonal) and genotypic (lower half diagonal) Correlation among 12 characters in F2 generation in wheat**

Phenotypic Genotypic	Days to 75% flowering	Plant Height (cm)	Number of tillers per plant	Number of spikelets per spike	Spike length (cm)	Number of grain per spike	Days to maturity	1000-grain weight (g)	Ear density (cm)	Duration of reproductive phase	Protein content (%)	Grain yield per plant (g)
Days to 75% flowering	1	0.07	-0.09	0.145	0.05	0.571**	0.17	0.440* ‡	0.09	-0.45	0.192	0.323* ‡
Plant Height (cm)	0.1	1	-0.18	0.2	0.086	-0.1	0.19	-0.03	0.09	0.15	- 0.629* ‡	- 0.326* ‡
Number of tillers per plant	-0.09	-0.2	1	0.192	0.363* ‡	0.247	0.06	- 0.515* ‡	- 0.295* ‡	0.12	0.268	0.456* ‡
Number of spikelets per spike	0.17	0.23	0.2	1	0.725**	0.454**	0.368* ‡	-0.25	0.06	0.21	-0.159	0.276
Spike length (cm)	0.08	0.1	0.390**	0.823**	1	0.540**	0.13	- 0.379* ‡	- 0.642**	0.09	0.102	0.408* ‡
Number of grain per spike	0.666**	-0.1	0.28	0.491**	0.601**	1	0.09	0.164	-0.28	-0.36	0.345* ‡	0.527* ‡
Days to maturity	0.356* ‡	0.28	0.14	0.560**	0.227	0.122	1	-0.04	0.23	0.71	-0.275	0.057
1000-grain weight (g)	0.498**	-0.02	-0.532**	-0.25	0.403**	0.171	0.368* ‡	1	0.27	-0.33	-0.073	-0.193
Ear density (cm)	0.1	0.13	0.391**	-0.08	0.630**	0.387**	0.478**	0.272	1	0.11	- 0.306* ‡	-0.27
Duration of reproductive phase	- 0.634**	0.22	0.22	0.298* ‡	0.162	- 0.516**	-0.07	-0.33	0.14	1	- 0.366* ‡	-0.224
Protein content (%)	0.22	0.661**	0.28	-0.18	0.109	0.355**	- 0.384**	-0.07	- 0.416**	- 0.488**	1	0.602* ‡
Grain yield per plant (g)	0.365* ‡	- 0.342* ‡	0.475**	0.293* ‡	0.439**	0.547**	0.08	-0.19	- 0.350* ‡	- 0.307* ‡	0.609* ‡	1

\* Significant at 5% level; \*\* significant at 1% level