



Association Analysis for Morpho-Physiological and Yield-Attributing Traits in Foxtail Millet (*Setaria italica* L. Beauv.)

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Abstract: In winter (rabi) 2023–2024, at the Regional Agricultural Research Station, Nandyal, research on trait association and path analysis were carried out with an emphasis on 16 morpho-physiological and yield-attributing traits among 50 foxtail millet germplasm accessions. The results revealed that the majority of genotypic correlation coefficient estimations were higher than corresponding phenotypic correlations indicating the strong inherent associations among the traits. The yield-attributing traits viz., fodder yield plant⁻¹, harvest index, plant height, panicle length, flag leaf length at flowering, 1000 grain weight, days to 50% flowering and flag leaf width at flowering had a significant and positive association with grain yield plant⁻¹, whereas abortive grain rate and number of productive tillers had displayed negative association. Grain yield plant⁻¹ was found to be significant and positively associated with physiological traits such as photosynthetic rate, stomatal conductance, transpiration rate, and relative water content, suggesting strong source and sink relationship. The strong and favorable direct impacts of fodder yield plant⁻¹, harvest index, days to 50% flowering, flag leaf length and width at flowering, photosynthetic rate, relative water content, abortive grain rate and 1000 grain weight on grain yield plant⁻¹ were found in the genotypic and phenotypic path analysis. Based on trait association and path analysis, the most important yield-attributing traits were the fodder yield plant⁻¹, harvest index, flag leaf length and width, photosynthetic rate, relative water content and the 1000-grain weight. Therefore, these attributes should be given top priority when formulating the selection criteria for the foxtail millet grain yield improvement programme.

Key words: Trait association, direct effects, photosynthetic rate, grain yield, foxtail millet.

Foxtail millet (*Setaria italica* L. (P.) Beauv) is an annual grass crop, native to China (Vavilov, 1926). It is one of the oldest cultivated grain crops, domesticated more than 8700 years ago (Pan *et al.*, 2018). It is generally cultivated on poor

and marginal lands of arid and semi-arid regions mostly belonging to tropical and sub-tropical parts of the world. It can establish quickly in warm weather and sandy to loamy soils with a pH of 5.5-7.0. Foxtail millet is an ideal crop for changing climate conditions due to its unique combination of low water requirements, drought tolerance and high photosynthetic efficiency (Vetriventhan *et al.*, 2012). It is also equipped with high water use efficiency and nutrient use efficiency which also make it unique from other cereal crops. Due to its short life cycle, C_4 metabolism and small sized genome of ~510 mb it evolved as a model system to investigate several aspects of plant architecture, genome evolution and plant physiology. (Doust *et al.*, 2009; Wang *et al.*, 2010; Bennetzen *et al.*, 2012).

The overall productivity of foxtail millet is generally low, primarily because indigenous varieties are cultivated on marginal lands in arid and semi-arid regions, resulting in suboptimal yields. This situation emphasizes the need to develop new cultivars that are highly productive, more reliable, and better adapted to these challenging conditions. Yield is a complicated and polygenically inherited trait, produced by the multiplicative interplay of its constituent traits. Consequently, the efficiency of selection depends on the direction and degree of association between yield and its attributing traits. Thus, a complete understanding of these interactions is essential for efficient selection in plant breeding. The correlation coefficients of foxtail millet traits can be misleading due to inherent associations between traits, making it difficult to determine the direct impact of one trait on another. To accurately assess the effect of a trait on another, path coefficient analysis is the only reliable method for disentangling direct and indirect effects from correlation coefficients. This approach is crucial in breeding programs, as it provides a more nuanced understanding of trait relationships, allowing for more informed selection decisions and a more effective breeding strategy.

Material and Methods

Fifty foxtail millet germplasm accessions including three checks viz., SiA 3156, SiA 3223 and SiA 3159 were evaluated during winter season (rabi) of 2023-2024, at the Regional Agricultural Research Station, Nandyal, which

is located at latitude 15°29' N, longitude 78°29' E and an altitude of 211.76 m above msl. The experiment was laid out in randomised complete block design (RCBD) with two replications by following a spacing of 22.5 cm × 10 cm. Standard agronomic practices were applied at the prime time. Five competitive plants per accession were selected randomly for recording observation on plant height (cm), number of productive tillers plant⁻¹, flag leaf length (cm), flag leaf width (cm), panicle length (cm), photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), relative water conductance (%), abortive grain rate, fodder yield plant⁻¹ (g), harvest index (%) and grain yield plant⁻¹ (g), while observations on days to 50% flowering and days to maturity were recorded on a plot basis. For 1000 grain weight, a random sample of 1000 grains were counted from the threshed seed and the weight was recorded in grams. The data subjected to ascertain the degree of association between morpho-physiological and yield-attributing traits, genotypic and phenotypic coefficients of correlations were computed in accordance with Al-Jibouri (1958). The correlation coefficient was split into direct and indirect influences on the grain yield of various traits in accordance with Dewey and Lu (1959).

Results and Discussion

Shaded correlation matrix (Table 1) illustrates the degree of association among the studied traits and the results revealed that the estimates of the genotypic correlation coefficient for the majority of the traits were higher than the corresponding phenotypic values, suggesting there were strong intrinsic associations among the traits. These low phenotypic correlations may be due to the environmental factors that mask or moderate the genetic associations between the traits, resulting in a diminished phenotypic expression. This observation is in line with the findings of Pallavi *et al.* (2020), Makwana *et al.* (2023) and Muzzayyanah *et al.* (2024). Phenotypic correlation coefficients seldom surpass their corresponding genotypic correlation coefficients viz, plant height. This may be attributed to non-genetic causes, most likely environmental inflation of the phenotypic correlation value.

Table 1. Shaded correlation matrix among 16 morpho-physiological and yield-attributing traits in 50 foxtail millet germplasm accessions

	DFF	DM	PH	NPT	FLL	FLW	PL	PR	TR	SC	RWC	AGR	TGW	FYPP	HI	GYPP
DFF	1	0.895**	0.503**	-0.274**	0.106	-0.128	-0.09	-0.045	-0.137	0.086	0.061	-0.291**	-0.115	0.579**	0.011	0.329**
DM	0.697**	1	0.633**	-0.201*	0.304**	0.120	0.103	0.015	-0.157	0.182	0.116	-0.304**	0.124	0.622**	-0.007	0.327**
PH	0.284**	0.391**	1	-0.705**	0.451**	0.128	0.383**	0.026	0.094	0.139	0.331**	-0.450**	0.329**	0.452**	0.133	0.342**
NPT	-0.053	-0.117	-0.182	1	-0.242*	-0.523**	-0.175	0.406**	0.364**	0.195	0.113	0.288**	-0.063	-0.511**	-0.084	-0.338**
FLL	0.068	0.223*	0.318**	-0.14	1	0.240*	0.754**	0.061	0.228*	0.194	0.121	-0.354**	0.386**	0.240*	0.262**	0.349**
FLW	-0.131	-0.005	0.206*	-0.156	0.324**	1	0.261**	0.113	0.276**	0.247*	0.247*	-0.066	0.445**	0.004	0.194	0.222*
PL	-0.031	0.051	0.293**	-0.068	0.636**	0.291**	1	0.156	0.319**	0.159	0.335**	-0.222*	0.448**	0.094	0.286**	0.299**
PR	-0.044	0.015	0.026	0.255*	0.044	0.087	0.124	1	0.879**	0.882**	0.370**	-0.347**	0.039	0.254*	0.405**	0.509**
TR	-0.079	-0.123	0.007	0.154	0.164	0.113	0.216*	0.761**	1	0.460**	0.173	-0.246*	0.055	0.241*	0.336**	0.458**
SC	0.023	0.063	0.077	0.068	0.214*	0.111	0.126	0.645**	0.669**	1	0.055	-0.295**	-0.016	0.058	0.475**	0.409**
RWC	0.013	0.090	0.272**	0.104	0.165	0.161	0.252*	0.338**	0.164	0.076	1	-0.172	0.344**	0.442**	0.288**	0.551**
AGR	-0.228*	-0.194	-0.341**	0.138	-0.192	-0.039	-0.220*	-0.271**	-0.204*	-0.157	-0.138	1	-0.184	-0.326**	-0.675**	-0.666**
TGW	-0.136	0.053	0.251**	-0.016	0.279**	0.253*	0.345**	0.042	0.018	-0.009	0.317**	-0.142	1	0.101	0.199	0.236*
FYPP	0.337**	0.354**	0.617**	-0.093	0.156	0.146	0.122	0.205*	0.086	0.084	0.345**	-0.284**	0.085	1	-0.047	0.782**
HI	0.044	-0.032	0.058	-0.028	0.198*	0.105	0.291**	0.352**	0.274**	0.298**	0.241*	-0.563**	0.209*	-0.099	1	0.569**
GYPP	0.231*	0.185	0.429**	-0.059	0.260**	0.203*	0.311**	0.443**	0.290**	0.316**	0.467**	-0.546**	0.240*	0.732**	0.585**	1

* Significant at 1% level and ** Significant at 5% level of probability; Above Diagonal values: Genotypic Correlation Coefficient and Below Diagonal values: Phenotypic Correlation Coefficient

Scale:

Lowest

Highest

DFF: Days to 50% flowering; DM: Days to maturity; PH: Plant height (cm); NPT: No. of productive tillers plant⁻¹; FLL: Flag leaf length at flowering (cm); FLW: Flag leaf width at flowering (cm); PL: Panicle length (cm); PR: Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); TR: Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); SC: Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); RWC: Relative water content (%); AGR: Abortive grain rate; TGW: 1000-grain weight (g); FYPP: Fodder yield plant⁻¹ (g); HI: Harvest index (%); GYPP: Grain yield plant⁻¹ (g).

Among the 16 morpho-physiological and yield-attributing traits, twelve traits *viz.*, fodder yield plant⁻¹, harvest index, relative water content, photosynthetic rate, plant height, stomatal conductance, panicle length, transpiration rate, flag leaf length at flowering, 1000 grain weight, days to 50% flowering and flag leaf width at flowering exhibited a strong positive association, both in terms of genotype and phenotype, with grain yield plant⁻¹. Similar findings for fodder yield plant⁻¹ were published by Jhansi Rani *et al.* (2021) and for harvest index were reported by Sharma and Dangi (2021). The findings of Muzzayyanah *et al.* (2024) were in consonance regarding flag leaf length and flag leaf width, Amarnath *et al.* (2018) reported similarly in case of panicle length, Pallavi *et al.* (2020) regarding 1000-grain weight and Feng *et al.* (2023) published similar results for photosynthetic rate. Alike, days to maturity also exhibited a significant positive association at genotypic level, which was similar to the findings of Jyotsna *et al.* (2016). Thus, these traits had a strong impact on foxtail millet grain yield. These traits can serve as deciding or indicating traits for the grain yield improvement of the foxtail millet. In

contrary, abortive grain rate at both phenotypic and genotypic levels and number of productive tillers plant⁻¹ at genotypic level had displayed significant negative association with grain yield plant⁻¹. These results were in accordance with the results of Amarnath *et al.* (2018) and Pallavi *et al.* (2020).

It is fascinating to note from the trait association results that physiological attributes *viz.*, photosynthetic rate, relative water content, stomatal conductance and transpiration rate showed a positive association at the phenotypic level. Grain yield increased in parallel with physiological activity. In all above said events, genotypic associations were also showed a strong and similar sign. It implied that a genetic link was the cause of phenotypic correlation. Strong positive correlation was discovered between the photosynthetic rate and the harvest index, number of productive tillers plant⁻¹, and fodder yield plant⁻¹. Similarly, there was a positive association between transpiration rate and the harvest index, fodder yield plant⁻¹, number of productive tillers plant⁻¹, panicle length, flag leaf length and width at flowering. Similar such association was observed for

stomatal conductance was also exhibited positive association with harvest index, flag leaf length and width at flowering. Whereas, relative water content showed a significant positive association with plant height, flag leaf width at flowering, panicle length, 1000-grain weight, fodder yield plant⁻¹ and harvest index. These associations clearly confirmed that superior source (length and width of flag leaf, plant height, number of productive tillers and fodder yield) stimulates physiological activities, which consequently strengthens the sink (panicle length, seed size and grain yield), which consequently contributes to enhanced economic yield. In light of the background of

drought tolerance, selecting for these traits may be beneficial to improve genetically the high potential for grain yield.

The estimates of both direct and indirect effects through path analysis at the phenotypic and genotypic levels were worked out to get an idea about the actual effect of a traits on the grain yield. In the current investigation, fifteen distinct yield-attributing and morpho-physiological traits were considered as causal factors of grain yield. The results (Table 2) revealed that the fodder yield plant⁻¹ and harvest index had high positive direct impact on grain yield plant⁻¹, which were in consistence with the

Table 2. Phenotypic (P) and genotypic (G) path coefficients among 16 morpho-physiological and yield-attributing traits of 50 foxtail millet accessions

Traits		DFF	DM	PH	NPT	FLL	FLW	PL	PR	TR	SC	RWC	AGR	TGW	FYPP	HI	GYPP
DFF	P	0.0021	-0.0059	-0.0049	0.0001	0.0007	-0.0011	0.0004	-0.0013	0.0000	-0.0002	0.0003	-0.0345	-0.0021	0.2391	0.0383	0.231*
	G	0.1523	-0.1230	-0.0510	0.0214	0.0074	-0.0010	0.0021	-0.0016	0.0048	0.0074	0.0065	-0.0323	-0.0057	0.3323	0.0089	0.329**
DM	P	0.0015	-0.0085	-0.0068	0.0001	0.0022	0.0000	-0.0007	0.0004	0.0001	-0.0005	0.0023	-0.0292	0.0008	-0.2512	-0.0281	0.185
	G	0.1363	-0.1374	-0.0642	0.0157	0.0213	0.0009	-0.0025	0.0006	0.0055	0.0157	0.0123	-0.0338	0.0061	-0.3567	-0.0058	0.327**
PH	P	0.0006	-0.0033	-0.0173	0.0002	0.0032	0.0017	-0.0039	0.0008	0.0000	-0.0006	0.0070	-0.0514	0.0038	-0.4381	0.0503	0.429**
	G	0.0766	-0.0870	-0.1015	0.0551	0.0316	0.0010	-0.0091	0.0009	-0.0033	0.0119	0.0351	-0.0500	0.0162	0.2594	0.1051	0.342**
NPT	P	-0.0001	0.0010	0.0032	-0.0011	-0.0014	-0.0013	0.0009	0.0074	-0.0001	-0.0005	0.0027	0.0208	-0.0002	-0.0659	-0.0244	-0.059
	G	-0.0417	0.0276	0.0715	-0.0782	-0.0169	-0.0040	0.0042	0.0149	-0.0128	0.0168	0.0120	0.0320	-0.0031	-0.2931	-0.0668	-0.337**
FLL	P	0.0001	-0.0019	-0.0055	0.0002	0.0100	0.0027	-0.0085	0.0013	-0.0001	-0.0017	0.0042	-0.0290	0.0042	0.1111	0.1726	0.259**
	G	0.0161	-0.0418	-0.0458	0.0189	0.0699	0.0018	-0.0179	0.0022	-0.0080	0.0167	0.0128	-0.0394	0.0191	0.1376	0.2071	0.349**
FLW	P	-0.0003	0.0000	-0.0036	0.0002	0.0032	0.0083	-0.0039	0.0025	-0.0001	-0.0009	0.0041	-0.0059	0.0038	0.1036	-0.0913	0.203
	G	-0.0195	-0.0165	-0.0130	0.0409	0.0168	0.0076	-0.0062	0.0041	-0.0097	0.0213	0.0263	-0.0073	0.0219	0.0022	-0.1533	0.222*
PL	P	-0.0001	-0.0004	-0.0051	0.0001	0.0063	0.0024	-0.0133	0.0036	-0.0001	-0.0010	0.0065	-0.0333	0.0052	0.0868	-0.2537	0.311**
	G	-0.0137	-0.0142	-0.0389	0.0137	0.0527	0.0020	-0.0238	0.0057	-0.0112	0.0137	0.0356	-0.0247	0.0221	0.0542	0.2261	0.299**
PR	P	-0.0001	-0.0001	-0.0005	-0.0003	0.0004	0.0007	-0.0017	0.0292	-0.0003	-0.0051	0.0087	-0.0409	0.0006	0.1456	-0.3072	0.443**
	G	-0.0068	-0.0021	-0.0026	-0.0317	0.0043	0.0009	-0.0037	0.0366	-0.0310	0.0760	0.0393	-0.0385	0.0019	0.1458	0.3205	0.509**
TR	P	-0.0002	0.0011	-0.0001	-0.0002	0.0016	0.0009	-0.0029	0.0222	-0.0004	-0.0053	0.0042	-0.0308	0.0003	0.0609	0.2388	0.290**
	G	-0.0209	0.0216	-0.0096	-0.0285	0.0159	0.0021	-0.0076	0.0322	-0.0352	0.0901	0.0184	-0.0274	0.0027	0.1381	-0.2660	0.458**
SC	P	0.0001	0.0005	-0.0013	0.0101	0.0021	0.0009	-0.0017	0.0188	-0.0003	-0.0080	0.0019	-0.0237	-0.0001	0.0600	0.2598	0.316**
	G	0.0131	-0.0250	-0.0140	-0.0152	0.0135	0.0018	-0.0038	0.0323	-0.0368	0.0861	-0.0158	-0.0328	-0.0008	0.0295	0.3760	0.408**
RWC	P	0.0000	-0.0008	-0.0047	-0.0001	0.0016	0.0013	-0.0034	0.0099	-0.0001	-0.0006	0.0257	-0.0208	0.0048	0.2447	0.2097	0.467**
	G	0.0093	-0.0159	-0.0336	-0.0088	0.0084	0.0019	-0.0079	0.0135	-0.0061	0.0047	0.1062	-0.0191	0.0169	-0.2534	0.2279	0.551**
AGR	P	-0.0005	0.0017	0.0059	-0.0002	-0.0019	-0.0003	0.0029	-0.0079	0.0001	0.0013	-0.0035	0.1510	-0.0021	-0.2014	-0.4906	-0.546**
	G	-0.0443	0.0418	0.0457	-0.0225	-0.0247	-0.0005	0.0053	-0.0127	0.0087	-0.0254	-0.0183	0.1111	-0.0091	-0.1870	-0.5340	-0.666**
TGW	P	-0.0003	-0.0005	-0.0043	0.0000	0.0028	0.0021	-0.0046	0.0012	0.0000	0.0001	0.0081	-0.0214	0.0151	0.0602	0.1818	0.240*
	G	-0.0175	-0.0171	-0.0334	0.0049	0.0270	0.0034	-0.0107	0.0014	-0.0019	-0.0014	0.0365	-0.0204	0.0493	0.0582	0.1576	0.236*
FYPP	P	0.0001	0.0003	-0.0010	0.0000	0.0020	0.0009	-0.0039	0.0103	-0.0001	-0.0024	0.0062	-0.0850	0.0032	0.8720	-0.0700	0.732**
	G	0.0017	0.0010	-0.0135	0.0066	0.0183	0.0015	-0.0068	0.0148	-0.0118	0.0410	0.0306	-0.0750	0.0098	0.7910	-0.0272	0.782**
HI	P	0.0007	-0.0030	-0.0107	0.0001	0.0016	0.0012	-0.0016	0.0060	0.0000	-0.0007	0.0089	-0.0428	0.0013	-0.0859	0.7100	0.585**
	G	0.0882	-0.0854	-0.0459	0.0399	0.0168	0.0000	-0.0022	0.0093	-0.0085	0.0050	0.0469	-0.0362	0.0050	-0.0375	0.5740	0.569**

Residual effect (Phenotypic) = 0.0113; Residual effect (Genotypic) = 0.0032; * Significant at 1% level and ** Significant at 5% level; Diagonal values (bold): Direct effects; Off-Diagonal values: Indirect effects.

DFF: Days to 50% flowering; DM: Days to maturity; PH: Plant height (cm); NPT: No. of productive tillers per pant; FLL: Flag leaf length at flowering (cm); FLW: Flag leaf width at flowering (cm); PL: Panicle length (cm); PR: Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); TR: Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); SC: Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); RWC: Relative water content (%); AGR: Abortive grain rate; TGW: 1000-grain weight (g); FYPP: Fodder yield plant⁻¹ (g); HI: Harvest index (%); GYPP: Grain yield plant⁻¹ (g).

findings of Jhansi Rani *et al.* (2021) and Pallavi *et al.* (2020) respectively. Consequently, these traits proved to be the primary determinants of grain yield. Breeders may consider these attributes based on phenotypic performance, as they all also showed higher genotypic path values. Low to moderate positive direct effects were exhibited by abortive grain rate at the phenotypic and genotypic levels, followed by photosynthetic rate, relative water content, 1000-grain weight, days to 50% flowering, flag leaf length at flowering and flag leaf width at flowering. Amarnath *et al.* (2018) and Ayesha *et al.* (2019) published similar results for 1000-grain weight, Prasanna *et al.* (2013) and Ashok *et al.* (2016) made similar findings regarding days to 50% flowering, the result of flag leaf length was in consonance with the reporting of Kavya *et al.* (2017) and Brunda *et al.* (2015) published similar results regarding panicle length. Similarly stomatal conductance at the genotypic level also exhibited positive direct effects. Conversely, negative direct effect on grain yield plant⁻¹, by plant height, panicle length, days to maturity, number of productive tillers plant⁻¹ and transpiration rate at both the phenotypic and genotypic levels were observed whereas the stomatal conductance showed negative direct effect at the phenotypic level. Similar results were reported for plant height and no. of productive tillers by Shingane *et al.* (2016), similarly for days to maturity was reported by Jyothsna *et al.* (2016) and by Tyagi *et al.* (2011) regarding flag leaf width.

Day to maturity, plant height, panicle length, transpiration rate and stomatal conductance were significantly positively associated with grain yield but their direct effects were found to be negative, in such scenario the traits through which indirect positive effects were displayed on grain yield, such traits viz., number of productive tillers plant⁻¹ and abortive grain rate must be given more weightage for indirect selection. While the abortive grain rate had a negative significant phenotypic and genotypic association with grain yield plant⁻¹. However, it reflected contrary in the direct effect, this indicates that some indirect traits like number of productive tillers plant⁻¹ might cause positive effects and such factors are to be considered for simultaneous selection.

In path analysis, residual values referred to the amount of the variance in the observed

variables that the model does not explain. In the present study, lower magnitudes of residual values were observed i.e., 0.0113 at phenotypic level and 0.0032 at genotypic level, suggested that potentially all significant yield-contributing traits were included and the postulated model accounts for most of the variability in the observed data.

Conclusions

Trait association and path analysis revealed that maximum direct effects as well as considerable indirect effects were exerted by fodder yield plant⁻¹, harvest index, days to 50% flowering, flag leaf length at flowering, flag leaf width at flowering, photosynthetic rate, relative water content and 1000-grain weight. These traits also showed a significant positive phenotypic and genotypic association with grain yield plant⁻¹, making them potentially the most significant yield contributing traits and that direct selection might be appropriate when breeding foxtail millet cultivars for high yielding. In contrast, the traits like abortive grain rate, number of productive tillers plant⁻¹ and panicle length must be given more weightage for indirect selection.

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