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Genetic studies on association and inter-relationship of green fodder yield and fodder quality traits in hybrids of fodder pearl millet [*Pennisetum glaucum* (L.) r. br.]

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Abstract
Pearl millet is an ideal fodder crop having highly shiny, lush, palatable, nutrient-dense, excellent silage and biomass production potential and bestowed with various essential nutrients required for the livestock. The study material consists of 54 fodder pearl millet hybrids obtained through the hybridisation between 15 selected superior parents from ICRISAT gene-pool as well as gene pool of Department of Forage Crops, TNAU, Coimbatore. For the better understanding of the relationship between green fodder yield per plant, its contributing traits and various quality traits, genetic parameters such as genotypic and phenotypic correlation as well as path coefficients were analysed. Correlation studies revealed that out of all the seventeen biometric traits studied, all the traits are shown positive correlation with green fodder yield per plant whereas fifteen traits are found to have significant positive correlation with green fodder yield per plant. Ash content (r_g =0.855), dry fodder yield per plant (r_g =0.850), leaf-stem ratio (r_g =0.710) have shown very strong significant positive correlation and plant height (r_g =0.641), days to green fodder harvest (r_g =0.630), stem girth (r_g =0.616) and days to fifty per cent flowering (r_g =0.606) have recorded with moderately strong significant positive correlation with green fodder yield per plant. Number of tillers per plant (0.859), internode length (0.574), ash content (0.421) and dry fodder yield per plant (0.372) revealed highly positive direct effects with green fodder yield per plant. Hence, selection for traits exhibiting positive association and direct effects for green fodder yield would help the breeder to enhance it further.

Keywords: Biometric traits, correlation, direct-indirect effects, fodder pearl millet, green fodder yield, hybridisation

Introduction
Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an excellent crop species extensively grown in India since ancient times. It has somatic chromosome number (2n) of 14 and belongs the genus *Pennisetum* in the family Poaceae. It is otherwise known as Bajra and Bajri in North India, Cumbu in Tamil Nadu and gero, sanio, babala, dukhin, mahangu, nyoloti, cat-tail millet, bulrush millet, dark millet, candle millet etc in various countries in the world. It is a multipurpose crop originated in Africa meant for both food and fodder for humans and livestock respectively. It is a drought-tolerant highly tillering cross-pollinated, hardy annual C_4 grass that usually grows to a height range of 120-350 centimetres. The stems usually have a diameter of 1–3 cm or more. Depending on spacing, management, and cultivar, the plant can tiller anywhere from one to twenty culms (FAO, 2020).
 Pearl millet contributes approximately fifty per cent of total millet production globally. Thirty-one million hectares of land is devoted to this crop worldwide for grain as well as fodder production (ICRISAT, 2020) [11]. With an area and output of 43.3 and 42 per cent, India is a major pearl millet producing country in the globe (Santosh *et al.,* 2018). This crop is grown in all parts of India except some north-eastern states having very low temperatures unsuitable for the crop.

As per the fourth Advanced Estimates (2019-2020), the production of Pearl millet in India is increased to 10.28 million tonnes from 8.66 million tonnes in previous year 2018-2019. Madhya Pradesh has the largest productivity in Pearl millet (2458kg/ha) followed by Gujarat (2101kg/ha) and Haryana (2068kg/ha) as per the Agricultural Statistics data published by the Government of India, 2020. The demand has been tremendously increased in recent years due to its nutritious qualities and tolerance to a wide range of climate situations (Tako et al., 2015) [27].

Pearl millet is considered as an anytime forage having excellent silage source particularly in the locations where dry spells occurs during the wet season. Unlike fodder Sorghum, Pearl millet can be grazed, chopped and supplied to livestock at any stage of its growth since it does not have the colourless toxic Hydrogen cyanide (HCN) or Prussic acid which causes dyspnea, urinary incontinence, incoordination of hind limbs etc. finally can even lead to the death of animals (Shashikala et al., 2013) [24].

Pearl millet consumes less amount of water per unit forage production since it is a heat and drought tolerant crop. Hence it is a gifted agricultural crop especially in tropical areas where millions of poor rural farmers and their livestock rely on it for food, feed, stover or dry fodder, and fuel (Hanna and Cardona, 2001). Therefore, it has been widely growing in locations where the environment, particularly rainfall, temperature and soil fertility, are too harsh for other cereals to thrive (Khairwal et al., 2009).

Materials and Methods

The present experiment was carried out at the New area farm under Department of Forage crops, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during Rabi 2020 and Kharif 2021.The experimental location is situated at an altitude of 427 m above MSL, latitude of 11° N and a longitude of 77° E. The average annual rainfall received at this location is around 700 mm. Fifty-four hybrids of fodder pearl millet were produced from hybridisation between nine lines (female parents) and six testers (male parents) through line x tester mating pattern. The parents were phenotypically observed and were selected from ICRISAT gene-pool as well as gene pool of Department of Forage Crops, TNAU, Coimbatore during the Rabi season (2020). Later hybrids were evaluated genotypically and phenotypically in the Kharif season (2021).

The experimental trial was laid out in randomized block design with two replications under irrigated conditions. Data of randomly selected five competitive plants per entry in a replication such as days to 50 per cent flowering, days to green fodder harvest, number of tillers per plant (cm), plant height (cm), number of leaves per plant (cm),internode length (cm), stem girth (cm), leaf length (cm), leaf breadth (cm), leaf –stem ratio, crude protein (g/100g)),crude fibre (g/100g), crude fat (g/100g), ash content (%), dry matter percentage (%), dry fodder yield per plant (g) and green fodder yield per plant (g) were recorded on single plant basis.

Sowing was done in a row per entry of 4 m length and 3m breadth having two replications with a spacing of 60 x 15 cm and all the recommended package of practices were followed to raise the crop. Data was analysed using TNAUSTAT Software and GENRES 7.01 software package. Data obtained were subjected to correlation and path coefficient analysis using Statistical Software R package ''corrplot'' (Taiyun Wei and Viliam Simko, 2017).

The Analysis Of Variance (ANOVA) was studied with the replication mean values of all the genotypes (Panse and Sukhatme, 1964) [21]. Correlation coefficient and path coefficient were calculated as per the method proposed by Searle (1961) [23] and Dewey and Lu (1959) [4], respectively. The measurement of the direct and indirect effects was characterized based on the scale given by Lenka and Misra (1973) [18] as negligible (0.00 to 0.09), low (0.10 to 0.19), moderate (0.20 to 0.29), high (0.30 to 0.99) and very high (>1.00). Both genotypic and phenotypic correlation coefficients were calculated from the genotypic and phenotypic components of variance and covariance as described by Singh and Choudhary (1985).

The Analysis Of Variance (ANOVA) was

Performed with the replication mean values of all the Genotypes (Panse and Sukhatme, 1964) [21]. The correlation coefficients were estimated as described by Singh and Chaudhary (1979) [25].

Results and Discussion

Green fodder yield is a complex polygenic trait controlled by various components which reflect positive and negative effects on it. It is important to note that whenever two traits are correlated, selecting for one would ensure selection for the other trait, therefore selecting for the best of the traits that correlated with yield in this study would result in increased yields. Analysis of Variance (ANOVA) represented in Table.1a and Table.1b for all the seventeen biometric traits including green fodder yield was studied. The mean sum of squares due to genotypes were showed significance except number of tillers per plant, stem girth, leaf breadth, leaf-stem ratio and crude fibre content (at P=0.01).

Genotypic and phenotypic correlation coefficients

Correlation indicates the useful attributes that could be used as a selection index and provides a measure of genetic relationship between the characters under study. Table 2 shows the knowledge of correlation coefficients in all known combinations of yield characters that were estimated at the genotypic and phenotypic levels. Yield contributing traits such as number of tillers per plant (r_p =0.583, r_g =0.641, plant height (r_p =0.532, r_g =0.546) which is similar to the results given by Kumari and Nagarajan, (2008) [17], number of leaves per plant (r_p =0.372, r_g =0.538), internode length (r_p =0.361, r_g =0.545), stem girth (r_p =0.472, r_g =0.567), leaf length (r_p =0.528, r_g =0.616),leaf breadth (r_p =0.676, r_g =0.710), leaf-stem ratio (r_p =0.335, r_g =0.402), crude protein (r_p=0.829, r_g =0.855),dry matter percentage (r_p=0.555, r_g =0.577) and dry fodder yield per plant (r_p =0.834, r_g =0.850) have shown highly significant positive correlation with green fodder yield. This result is in harmony with Paroda et al., 1975 [22]. It is interestingly noted that crop developmental characters such as days to 50 % flowering and days to green fodder harvest had also reported highly significant positive correlation with green fodder yield as like the experimental results formulated by Kumari and Nagarajan, (2008) [17]. The graphical representation of correlation coefficients and relation with different variables are represented in the "corrplot" representation obtained from ' R Studio' software (Figure 1).

The genotypic correlation coefficient is a measure of genetic relationship between features that aids in selecting which characters should be examined for enhanced green fodder production. The genotypic correlation coefficients (r_g) were

greater in magnitude than the phenotypic correlation coefficients (r_p) for all the seventeen traits studied. This says that the genetic factors are mostly contributed to the trait association than environmental interactions. Similar results were obtained by Kote *et al.,* (2014) [15]. Lower phenotypic correlations could be due to the environment's modifying effect on the genetic relationship of traits (Pandey and Gritton, 1975) [20].

Out of four quality parameters studied, only ash content and crude protein depicted highly positive significant correlation while rest of them indicated positive correlation with green fodder yield (Kapoor *et al.*, 2015) [12]. Ash content in fodder crop is the availability of inevitable anions and cations such as chloride, sulphate, calcium and magnesium required for the growth and better hygiene of livestock. Similarly crude protein is a mandatory quality trait for the overall growth process of livestocks.

Path coefficient analysis for direct and indirect effects on green fodder yield:
When there are fewer variables to consider in a selection programme, correlation analysis alone can be sufficient. But when the number of variables are increased, there arises a complex situation. To overcome this complexity, path analysis (Wright, 1921) [29] was done to partition the correlation into direct and indirect effects, so that the effect of each trait is established and hence their number is reduced in selection programs. It measures the direct and indirect contribution of various independent characters on a dependent character. The results of path analysis were presented in Table 3. Residual effect was found to be 0.20, which indicated that around 80% of the total variation were explained by the traits taken for study. Hence, selection on these studied characters might be useful in genetic improvement for yield.

Path analysis revealed that number of tillers per plant (0.859), internode length (0.574), ash content (0.421), stem girth (0.396) and dry fodder yield per plant (0.372) had highly positive direct effects on green fodder yield per plant. Kara *et al.*, (1999) [13], Kumar and Singh (2004) and Gallais *et al.*, (1976) were also interpreted that the green fodder yield/plant is significantly and positively associated with dry fodder yield/ plant and yield components such as plant height, number of leaves/plant, and stem diameter or girth.

At the same time, days to 50% flowering (0.015), days to green fodder yield (0.109), number of leaves per plant (0.065), leaf length (0.009) and crude fibre content (0.199) were exhibited negligible positive direct effects on green fodder yield per plant. While considering the traits having traits having positive direct effect with green fodder yield per plant, the other traits with indirect effects to yield should also be noticed to focus only needed traits for yield improvement and upcoming breeding programs. Furthermore, plant height (-0.320), leaf breadth (-0.269), leaf-stem ratio (-0.228), crude protein (-0.092), crude fat (-0.277) and dry matter percentage (-0.200) was observed having negative direct effects with green fodder yield per plant. These findings were found similar with Kapoor *et al.*, 2015 [12].

All the yield contributing traits except quality traits such as crude fibre and crude protein contented exhibited highly indirect positive effects with green fodder yield. Days to 50% flowering expressed highly indirect positive association with number of tillers per plant (0.536) and green fodder yield per plant (0.606). Days to green fodder harvest (0.503), plant height (0.605), number of leaves per plant (0.593), leaf length (0.446), leaf breadth (0.466), leaf-stem ratio (0.552), crude protein (0.688), ash content (0.429), dry matter percentage (0.340) and dry fodder yield per plant (0.442) exhibited highly indirect positive effects with number of tillers per plant along with green fodder yield per plant. Also, dry fodder yield had shown positive indirect effect with number of tillers per plant (0.442) and ash content (0.314). Hence it is obvious that number of tillers per plant should be one of the prime criteria in selecting superior plants for enhanced green fodder yield. The indirect effects of number of tillers per plant to green fodder yield was also found by Subbulakshmi *et al.*, 2018) [26] and Govindaraj and Selvi (2012) [9].

Another noticeable fact is even though plant height predicted strong positive correlation with green fodder yield per plant, association studies marked its relation as highly negative significance with green fodder yield. This interesting and confusing fact was already noted by Kumari and Nagarajan, (2008) [17]. As per Paroda *et al.*, (1975) [22], Gupta and Nanda (1971) due to the reduction of internodes and denser packing of broad and long leaves per plant, the negative effect of plant height revealed that it may not be required to look for a very tall plant, as even plants of intermediate height could give more forage supply. Similarly leaf breadth and leaf-stem also found to have negative direct effect with green fodder yield even if these characters have strong significant correlation with green fodder yield.

For a proper leaf development, necessary stem girth is inevitable which in turn accounts for higher green fodder production (Paroda *et al.*, 1975) [22]. In a nutshell if fodder pearl millet is concerned, plant height, stem girth, leaf characters and leaf-stem ratio together decides the final green fodder yield. The undesirable indirect effects may be the reason for the negative direct effects towards yield by some of these characters.

Summary
For the enhanced green fodder yield in fodder Pearl millet under research will be significant if plants having more number of tillers with longer leaves, longer internode with thicker stems are the prime criteria for selection. From the investigation above, all the traits are highly directly or indirectly contributes to enhanced green fodder production. At the same time, selection for traits like plant height or thicker leaf or leaf-stem ratio alone, which have a strong negative correlation with green fodder yield, may hamper progress in breeding for increased green fodder yield as per the results obtained.
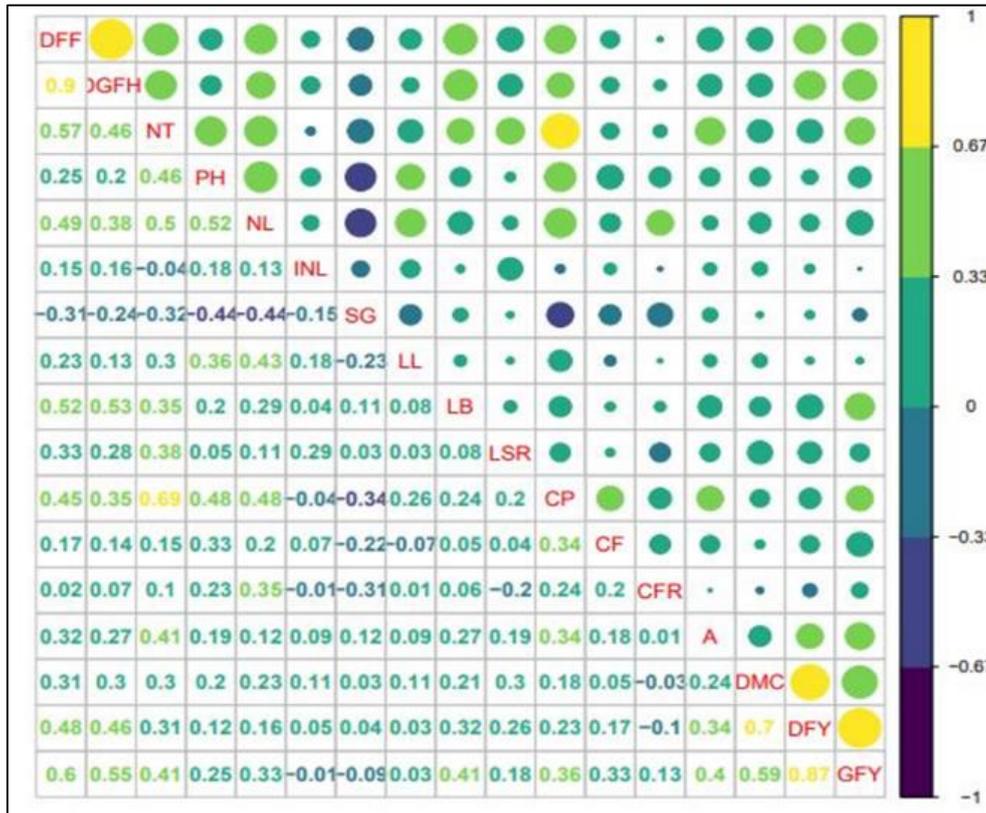
Acknowledgements

Fig 1: Heatmap for Correlation coefficients (*Corrplot*) in fodder pearlmillet hybrids

Correlation coefficient values ranges from -1 to +1. The values closer to zero means there is no linear trend between the two variables. Value close to 1, the correlation is said to be more positively correlated; that is as one increases so does the other and the closer to one, stronger the relationship. In this case, Yellow circles (closer to one) indicates that there exists a significant positive correlation between DFY and GFY (0.850), DMC and GFY (0.86). Green shaded circles depicts positive correlation. The correlation closer to -1 is similar, but instead of both increasing, one variable decreases as the other increases. Here, Blue shaded circles indicates negative correlation with. The diagonals shows perfect correlation. The size of the circle varies from very minute dots to larger circles) depending on the correlation coefficient values.

Table 1: Analysis of variance (ANOVA) for green fodder yield, its contributing traits and fodder quality traits in fodder pearl millet

Variation	Degrees of freedom	Leaf-stem ratio	Crude protein	Crude fibre	Crude fat	Ash content	Dry matter percentage	Dry fodder yield	Green fodder yield
Replication	1	0.08	0.18	0.07	0.38	1.06	0.27	2599.55	7652.44
Genotypes	53	0.001	4.30**	0.02	2.84**	3.47**	15.23 **	3911.00**	83981.29**
Error	53	0.005	0.75	0.001	0.30	0.18	0.55	80.25	378.47

Note: **- 1% significance level,'t' test with (n-2) degrees of freedom where 'n' represents the number of observations

Table 2: Phenotypic and Genotypic correlation coefficients for green fodder yield and its attributing traits in fodder pearl millet

	DFF	DGFH	NTPP	PH	NLPP	IL	SG	LL	LB	LSR	CP	CFBR	CF	ASH	DMP	DFY	GFY
r_p	1	0.975	0.571	0.370	0.369	0.246	-0.009	0.312	0.502	0.561	0.417	0.016	0.126	0.399	0.288	0.469	0.593**
r_g	1	0.998	0.624	0.385	0.517	0.385	-0.009	0.359	0.602	0.590	0.495	0.002	0.155	0.416	0.300	0.490	0.606**
r_p		1	0.516	0.323	0.337	0.274	0.066	0.307	0.529	0.559	0.349	0.006	0.118	0.401	0.276	0.489	0.603**
r_g		1	0.586	0.348	0.433	0.401	0.088	0.360	0.633	0.603	0.434	-0.013	0.152	0.428	0.296	0.517	0.630**
r_p			1	0.636	0.436	0.054	0.006	0.413	0.411	0.554	0.614	0.065	0.244	0.432	0.352	0.460	0.583**
r_g			1	0.705	0.690	0.148	0.042	0.519	0.543	0.642	0.801	0.072	0.307	0.499	0.395	0.514	0.641**
r_p				1	0.545	0.332	-0.033	0.428	0.350	0.341	0.469	0.133	0.251	0.431	0.319	0.399	0.532**
r_g				1	0.786	0.458	-0.039	0.536	0.383	0.366	0.560	0.184	0.278	0.446	0.337	0.416	0.546**
r_p					1	0.273	-0.065	0.347	0.276	0.282	0.375	0.204	0.127	0.349	0.271	0.223	0.372**
r_g					1	0.360	-0.160	0.550	0.392	0.442	0.576	0.338	0.270	0.527	0.403	0.313	0.538**
r_p						1	0.009	0.195	0.217	0.304	0.053	-0.008	0.223	0.238	0.259	0.317	0.361**
r_g						1	0.007	0.279	0.296	0.454	0.012	0.129	0.326	0.364	0.401	0.516	0.545**
r_p							1	0.155	0.321	0.179	-0.089	-0.156	-0.056	0.176	0.119	0.290	0.306**
r_g							1	0.184	0.516	0.264	-0.205	-0.235	-0.011	0.236	0.137	0.326	0.354**
r_p								1	0.354	0.359	0.272	-0.044	-0.076	0.459	0.512	0.481	0.472**
r_g								1	0.496	0.450	0.346	-0.089	-0.079	0.600	0.596	0.583	0.567**

r_p								**1**	0.381	0.201	0.001	0.135	0.328	0.236	0.453	0.528**
r_g								**1**	0.442	0.292	0.072	0.093	0.387	0.275	0.519	0.616**
r_p									**1**	0.355	0.040	0.161	0.498	0.450	0.539	0.676**
r_g										0.445	0.065	0.170	0.533	0.504	0.587	0.710**
r_p										**1**	0.212	0.144	0.233	0.172	0.190	0.335**
r_g										**1**	0.289	0.236	0.355	0.185	0.229	0.402**
r_p											**1**	0.031	0.125	-0.006	-0.063	0.125
r_g											**1**	0.143	0.180	-0.019	-0.072	0.170
r_p												**1**	0.150	-0.211	0.061	0.163
r_g												**1**	0.149	-0.200	0.069	0.174
r_p													**1**	0.520	0.711	0.829**
r_g													**1**	0.556	0.745	0.855**
r_p														**1**	0.646	0.555**
r_g														**1**	0.660	0.577**
r_p															**1**	0.834**
r_g															**1**	0.850**
r_p																**1**
r_g																**1**

Note:*-5% significance level:**-1% significance level, where ' **rp** ' – Phenotypic correlation coefficient and ' **rg** ' – Genotypic correlation coefficient and DFF- Days to 50% flowering, DGFH- Days to green fodder harvest, NTPP- No. of tillers per plant, PH- Plant height, NLPP- No. of leaves per plant, IL- Internode length, SG- Stem girth, LL- Leaf length, LB- Leaf breadth, LSR- Leaf-stem ratio, CP-Crude protein, CFR- Crude fibre, CF-Crude fat, ASH-Ash content ,DMP-Dry matter percentage, DFYPP-Dry fodder yield per plant, GFYPP-Green fodder yield per plant. Diagonal bolded values represents the direct effects of studied traits over green fodder yield.

Table 3: The genotypic direct and indirect effects of the component traits over yield

Traits	DFF	DGFH	NTPP	PH	NLPP	IL	SG	LL	LB	LSR	CP	CFR	CF	ASH	DMP	DFYPP	GFYPP
DFF	**0.015**	0.109	0.536	-0.123	0.033	0.221	-0.004	0.003	-0.162	-0.135	-0.045	0.000	-0.043	0.175	-0.060	0.084	0.606**
DGFH	0.015	**0.109**	0.503	-0.112	0.028	0.230	0.035	0.003	-0.170	-0.138	-0.040	-0.003	-0.042	0.180	-0.059	0.089	0.630**
NTPP	0.010	0.064	**0.859**	-0.226	0.045	0.085	0.017	0.005	-0.146	-0.146	-0.073	0.014	-0.085	0.210	-0.079	0.188	0.641**
PH	0.006	0.038	0.605	**-0.320**	0.051	0.263	-0.015	0.005	-0.103	-0.084	-0.051	0.037	-0.077	0.188	-0.068	0.271	0.546**
NLPP	0.008	0.047	0.593	-0.252	**0.065**	0.206	-0.063	0.005	-0.105	-0.101	-0.053	0.067	-0.075	0.222	-0.081	0.154	0.538**
IL	0.006	0.044	0.127	-0.147	0.023	**0.574**	0.003	0.003	-0.080	-0.103	-0.001	0.026	-0.090	0.153	-0.080	0.089	0.545**
SG	0.004	0.010	0.036	0.012	-0.010	0.004	**0.396**	0.002	-0.138	-0.060	0.019	-0.047	0.003	0.099	-0.027	0.056	0.354**
LL	0.006	0.039	0.446	-0.172	0.036	0.160	0.073	**0.009**	-0.133	-0.103	-0.032	-0.018	0.022	0.253	-0.119	0.100	0.567**
LB	0.009	0.069	0.466	-0.123	0.025	0.170	0.204	0.005	**-0.269**	-0.101	-0.027	0.014	-0.026	0.163	-0.055	0.189	0.616**
LSR	0.009	0.066	0.552	-0.117	0.029	0.260	0.105	0.004	-0.119	**-0.228**	-0.041	0.013	-0.047	0.224	-0.101	0.101	0.710**
CP	0.008	0.047	0.688	-0.179	0.037	0.007	-0.081	0.003	-0.078	-0.101	**-0.092**	0.057	-0.065	0.149	-0.037	0.039	0.402**
CFR	0.005	-0.001	0.062	-0.059	0.022	0.074	-0.093	-0.001	-0.019	-0.015	-0.026	**0.199**	-0.040	0.076	0.004	-0.012	0.170
CF	0.002	0.017	0.264	-0.089	0.017	0.187	-0.004	-0.001	-0.025	-0.039	-0.022	0.028	**-0.277**	0.063	0.040	0.012	0.174
ASH	0.006	0.047	0.429	-0.143	0.034	0.209	0.093	0.006	-0.104	-0.122	-0.032	0.036	-0.041	**0.421**	-0.111	0.128	0.855**
DMP	0.005	0.032	0.340	-0.108	0.026	0.230	0.054	0.006	-0.074	-0.115	-0.017	-0.004	0.055	0.234	**-0.200**	0.213	0.577**
DFYPP	0.008	0.056	0.442	-0.133	0.020	0.296	0.129	0.005	-0.139	-0.134	-0.021	-0.014	-0.019	0.314	-0.132	**0.372**	0.850**

Note:*-5% significance level:**-1% significance level, where DFF- Days to 50% flowering, DGFH- Days to green fodder harvest, NTPP- No. of tillers per plant, PH- Plant height, NLPP- No. of leaves per plant, IL- Internode length, SG- Stem girth, LL- Leaf length, LB- Leaf breadth, LSR- Leaf-stem ratio, CP-Crude protein, CFR- Crude fibre, CF-Crude fat, ASH-Ash content ,DMP-Dry matter percentage, DFYPP-Dry fodder yield per plant, GFYPP-Green fodder yield per plant. Diagonal bolded values represents the direct effects of studied traits over green fodder yield

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