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Genetic Variation for Rancidity and Grain Yield Components in Pearl Millet Seed Parents

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Abstract

Pearl millet is an important staple food crop in India, but its flour is not properly utilized due to rancidity that develops during storage. The processing methods could be many, but they are not so effective. So, the best way to increase longevity in pearl millet flour is by identifying best suitable genotypes and developing hybrids. Knowledge of genetic variability, genotypic and phenotypic coefficient of variation, heritability, correlation and analysis of path of yield contributing traits is important for efficient planning of crop improvement programmes. The present investigation was conducted to evaluate 60 seed parents of pearl millet in a Randomized Block Design with two replications. The analysis of variance revealed highly significant differences among genotypes for all the traits under investigation thereby indicating the existence of substantial genetic variation among genotypes for all the yield and its contributing traits. Vigour index exhibited high correlation coefficient, while comprehensive peroxide value on 10th day along with other traits had direct effects with respect to grain yield, in the desired direction indicated that these traits could be effective in crossing programmes to develop promising maintainer lines with longer shelf life.

***Key words*:** *Pennisetum glaucum*, Rancidity, Genetic advance, Genetic variability, Heritability, Correlation, Path analysis

Pearl millet [*Pennisetum glaucum* (L.) R. Br.; 2n=14], is an important staple food and fodder crop grown in arid and semi-arid regions of Asia and Africa. Pearl millet has very high nutritional value, in terms of energy, proteins, fat, and minerals, equivalent to, if not superior to, major cereals [1]. In India, it ranks fourth in acreage next to rice, wheat and maize.

A significant advancement in pearl millet (*Pennisetum glaucum* (L.) R.Br.) improvement was the discovery of cytoplasmic-nuclear male sterility (CMS) system and its effective use in breeding male-sterile lines of commercial hybrids where a cytoplasm that causes male sterility and a gene that restores fertility are used [2]. In this system, A, B and R lines are involved. After repeated backcrossing for 6-8 generations, A line can be formed from B lines. Then the potential male and female parents for hybrid seed production can be identified by crossing male-fertile plants (inbreds, varieties, germplasm and breeding stocks in advanced generations) to a male-sterile line (A-line) and corresponding hybrids are obtained. After that, the desired plants are selected from the F_1 hybrid and intercrossing is done to increase the level of shelf life in these genotypes, as the major problem in pearl millet is development of rancidity within a few hours to 4-5 days after milling and there is the lack of suitable molecular and biochemical markers or matrix indicating potential rancidity in pearl millet.

During the storage of pearl millet flour various complex reactions occur like lipid hydrolysis, oxidation and polymerization reaction, which results in quality failure and rancidity development. These reactions made favourable environment for the action of lipoxygenase (LOX), which is known for its deterioration in flavour and quality [3]. It is observed that lipoxygenase catalyzes the oxidation of unsaturated fatty acids, resulting in a loss of quality and flavour [4]. The production of free fatty acids during storage causes hydrolytic rancidity [5]. These free fatty acids serve as substrates for lipoxygenase (LOX), which produces lipid hydroperoxides as primary oxidative products. Peroxidases and polyphenol oxidase (PPO), two members of the cellular enzymatic machinery, then react with the free fatty acids to produce reactive oxygen species (ROS), which further breaks down the free fatty acids into more lipid hydroperoxides. The hydroperoxides undergo further non-enzymatic oxidation, resulting in secondary oxidation products, which eventually cause the flour to become rancid during its storage. During the study, two parameters indicating rancidity were monitored: acid value (indicates enzymatic rancidity) and peroxide value (indicates oxidative rancidity). Each variety's flour was stored in room temperature (25°C to 28°C) with acid and peroxide values measured at regular intervals. According to Mazumdar *et al.* [6], fat extracted from pearl millet flour increased in acid

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value and peroxide value up to the 10th day and then progressively decreased, and this was the basis for rancidity assessment in pearl millet flour. It was found that all the 60 genotypes studied had acid value and peroxide value high on the tenth day. LOX activity is dependent on available free fatty acids, and once the free fatty acid is produced by lipase, it is easily accessible to LOX, hence, the CPV values were greater than the CAV values. CPV values were found to be higher than the CAV in our experiment which was found similar to the results of Goswami *et al*. [7]. The difference in comprehensive acid value on day 1 and after 10 days of storage tells about the longevity of the flour. If the difference is low, it indicates that the genotype has longer shelf life. Similarly, for comprehensive peroxide value difference, if in genotype, the difference is low, it is found to have longer shelf life compared to other genotypes [7].

Study of genetic diversity as well as their biochemical characteristics plays a very important role in evaluating a variety's breeding potential for desired qualities and further utilization of this diversity using molecular markers might aid in the selection of germplasm for crop improvement and breeding programmes. So, the choice of parents, availability of sufficient genetic variability and the knowledge of genetic architecture is of paramount importance in the breeding programme. The effectiveness of selection is dependent upon the nature and magnitude of the variability present in the material for the desired characters and the extent to which it is heritable. Genetic parameters such as genotypic and phenotypic coefficients of variation (GCV and PCV) are crucial in detecting the amount of variability present in the genotypes. High heritability values suggest that the variables being studied are heritable, owing to the predominance of additive gene effects and are more resistant to environmental impact in their expression. Selection on the basis of grain yield character alone is usually not very effective and efficient. However, selection based on its component characters could be more efficient and reliable. So, estimates of correlation and analysis of path were also used in determining yield components both directly or indirectly. Therefore, the study of genetic variability of grain yield and its morphological and biochemical parameters associated with development of rancidity will be evaluated further for flour keeping quality, as well as processing and packaging options, in order to deliver shelf-stable pearl millet products to the consumer.

MATERIALS AND METHODS

The experimental material comprising of 60 seed parents (maintainer lines or B-lines) of pearl millet were sown in a randomized block design in two replications at the Research Area of Bajra Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana during *kharif* 2021. In each replication, genotypes were sown in a single row of four-meter length with a row to row spacing of 45 cm and plant to plant distance was kept at 10-12 cm. The recommended agronomic practices were followed for raising a good crop of pearl millet.

The observations were recorded on five representative random plants in each replication for agro-morphological traits *viz*., plant height (cm), panicle diameter (cm), panicle length (cm), number of productive tillers/plant except days to 50 per cent flowering, 1000-seed weight (g), grain yield/plant (g) and dry fodder yield/plant (g) which were recorded on plot basis. Two shelf-life traits viz., acid value (comprehensive acid value on the 1stday and 10th day and their difference i.e. CAV_0, CAV_{10} and CAV_{10}-CAV_0, respectively) and peroxide value (comprehensive peroxide value on the 1stand 10thday and their difference i.e. CPV_0, CPV_{10} and CPV_{10}-CPV_0, respectively) were estimated from bulk open-pollinated grain samples of each genotype in both replications following AOAC [8] method.

The comprehensive acid value (CAV) is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of flour, while, the comprehensive peroxide value (CPV) determines the concentration of hydroperoxide—the primary oxidation product in the flour and is an indication of the extent of oxidation suffered by the flour.

The formula to calculate CAV and CPV are:

$$CAV = 40 \times A \times N / W$$

Where; CAV is the comprehensive acid value, A denotes the volume of NaOH (mL), 40 is the constant value equivalence of mass of 0.1 N NaOH, W is the weight of the sample and N is the normality of the standard NaOH solution.

$$CPV = (A-B) \times N \times 1000 / W$$

Where; CPV is the comprehensive peroxide value, A and B denote the titration volume of Na2S2O3 (mL) in the sample and blank, respectively, W is the weight of flour in grams and N is the normality of the standard Na2S2O3 solution.

The seed parameters viz., standard germination (%), seed vigour index I, Seed vigour index II, seedling length (cm) and seedling dry weight (mg), root length(cm) and shoot length(cm) were recorded on bulk open-pollinated seed samples of each genotype in both replications.

The mean replicated data was used for analysis of variance for each character to test the difference among the genotypes. The genotypic and phenotypic coefficient of variation, which measures the magnitude of genotypic variation and phenotypic variation, respectively present in a particular character, and the heritability was calculated as per the formula suggested by Burton and de Vane [9]. The formula given by Al-Jibouri *et al*. [10] was used to evaluate the phenotypic and genotypic coefficients of correlation between two traits. Path coefficients were determined using the genotypic correlation values of yield contributing traits (independent traits) on yield (dependent trait) as proposed by Wright [11] and further illustrated by Dewey and Lu [12].

RESULTS AND DISCUSSION

Genetic variability parameters

The phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the characters representing environmental impact on these characters for total variation. High GCV and PCV were observed for the traits viz., difference between CAV on 1st day (CAV_0) and 10th day (CAV_{10}), CPV diff- difference between CPV on 1st and 10thday, grain yield /plant (g), vigour index II, dry fodder yield/ plant, Comprehensive Peroxide Value on the 1st, CPV_{10} - Comprehensive Peroxide Value on the 10thday, seedling dry weight, vigour index I, Comprehensive Acid Value on the 1stday, number of productive tillers /plant, Comprehensive Acid Value on the 10thday, seedling length, germination per cent, 1000-seed weight while moderate GCV and PCV were observed for panicle diameter, panicle length, root length, seedling length. High estimates of GCV and PCV indicate that selection can be applied to these traits to isolate a more promising line. The high magnitude of the genotypic

coefficient of variation indicated the presence of wide variation for the characters under study to allow further genetic improvement by the selection of the individual traits. Similar results for GCV and PCV were also reported by Kaushik *et al*. [13] for plant height, number of productive tillers/plants, panicle length and grain yield/plant.

Table 1 Genetic variability parameters for yield and its contributing traits in pearl millet

Traits	Mean	Range	Phenotypic coefficient of variation (%)	Genotypic coefficient of variation (%)	Heritability (%)
DF	59	51-79	10.19	7.33	51.74
PH	121.84	95-151.5	11.52	9.89	73.63
PD	2.51	1.49-3.55	17.66	11.55	42.77
PL	18.68	11.25-25.64	17.29	10.41	36.29
NPT	3	1-6	29.65	27.49	85.95
1000 SW	6.24	3.42-9.56	23.60	23.14	96.14
DRY F	33.62	7.90-94.00	52.50	50.48	92.46
CAV 0	12.32	5.93-23.93	31.66	29.29	85.59
CAV10	13.92	4.87-23.20	26.58	22.88	74.12
CPV 0	189.09	30-333.3	46.36	42.82	85.31
CPV10	218.72	45-425	36.99	33.46	81.82
CAV diff	4.50	0.53-14.13	75.38	54.79	52.82
CPV diff	91.94	10-251.67	74.17	62.43	70.85
G%	51.28	32.13-71.59	24.37	23.92	96.41
RL	11.05	6.05-15.83	18.85	18.45	95.81
SL	8.74	3.28-13.02	22.35	21.80	95.07
SEED L	19.8	12.15-28.85	16.96	16.67	96.69
DRY WT	2.95	1.02-4.95	34.65	34.40	98.55
V-I	1218.25	462.83-2096.73	35.46	35.08	97.86
V-II	184.45	41.50-437.27	50.33	49.91	98.36
GY	15.61	0.70-40.50	56.98	56.43	98.09

DF- Days to 50% flowering, PH- Plant height(cm), PD- Panicle diameter(cm), PL- Panicle length(cm), NPT- Number of productive tillers/plant, 1000SW- 1000 seed weight (g), DryF- Dry fodder yield/plant(g),CAV 0- Comprehensive Acid Value on the first day, CAV10- Comprehensive Acid Value onthe10thday,CPV0- Comprehensive Peroxide Value on the first day, CPV10- Comprehensive Peroxide Valueonthe10thday, CAVdiff-DifferencebetweenCAVon1stand10thday, CPVdiff- Difference between CPV on 1st and 10th day, G%- Germination per cent (%),RL- Root length(cm), SL- Shoot length(cm), SEEDL- Seedling length(cm), DRYwt- Seedling dry weight (mg), V-I- Vigour index I, V-II- Vigour index II, GY- Grain yield/plant(g)

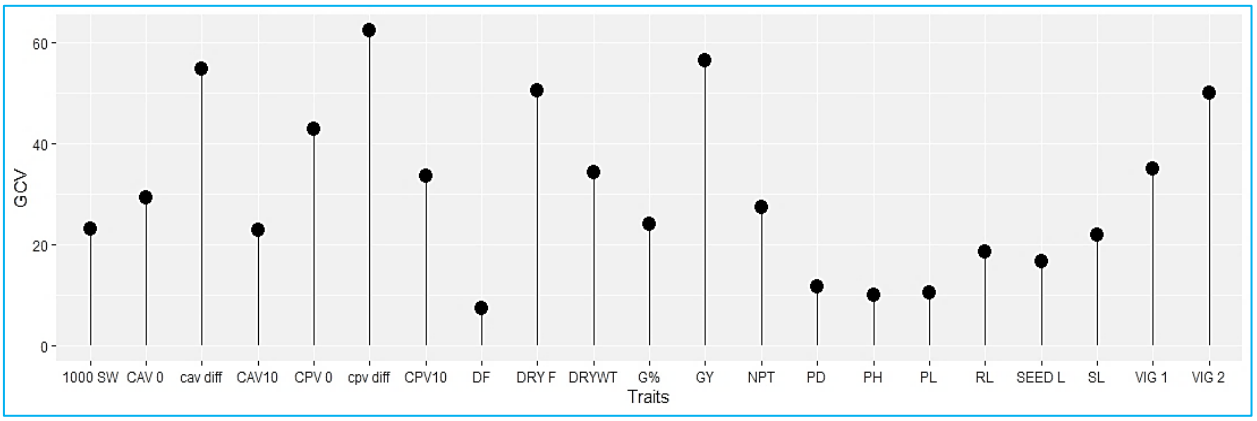

Fig 1 Estimates of GCV (%) for agro-morphological, seed and biochemical characters in pearl millet germplasm lines

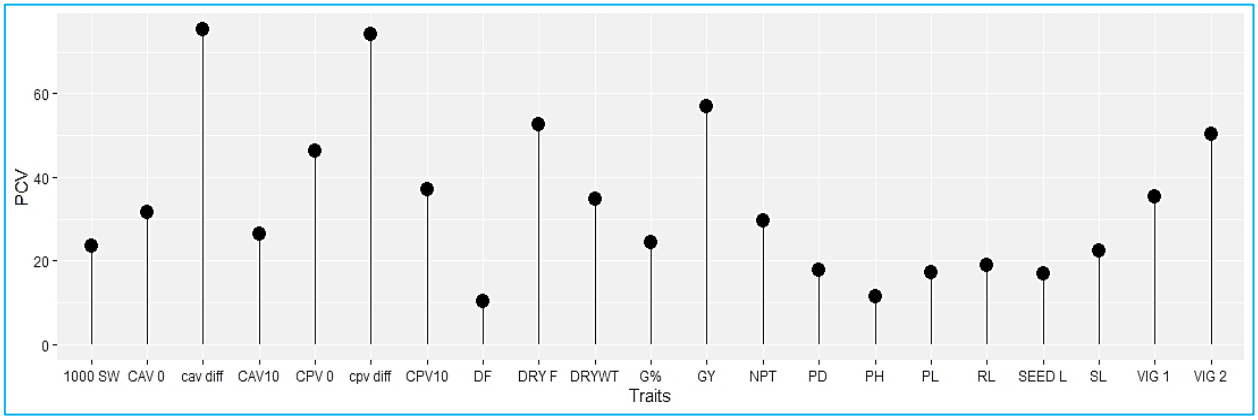

Fig 2 Estimates of PCV (%) for agro-morphological, seed and biochemical characters in pearl millet germplasm lines

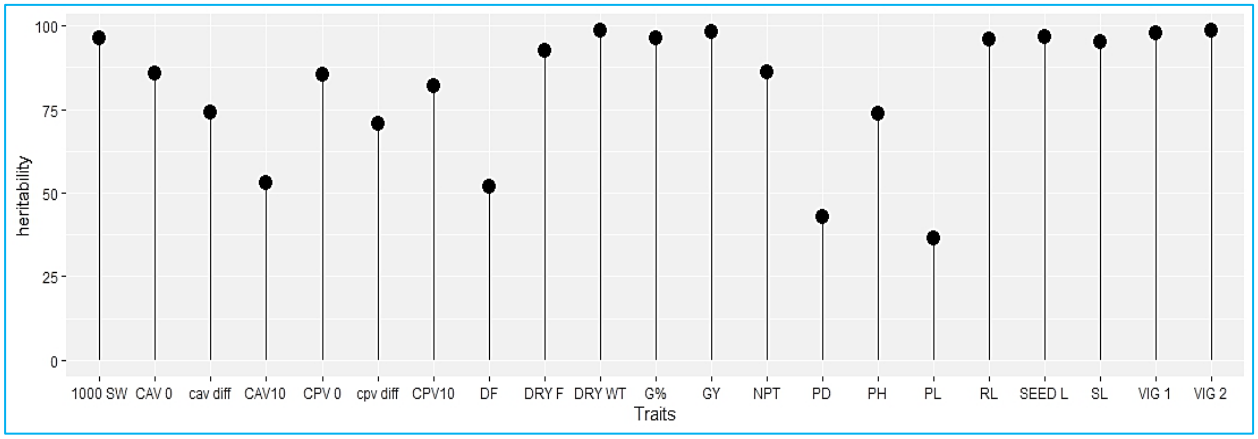

Fig 3 Estimates of heritability (h^2) (%) for agro-morphological, seed and biochemical characters in pearl millet germplasm lines

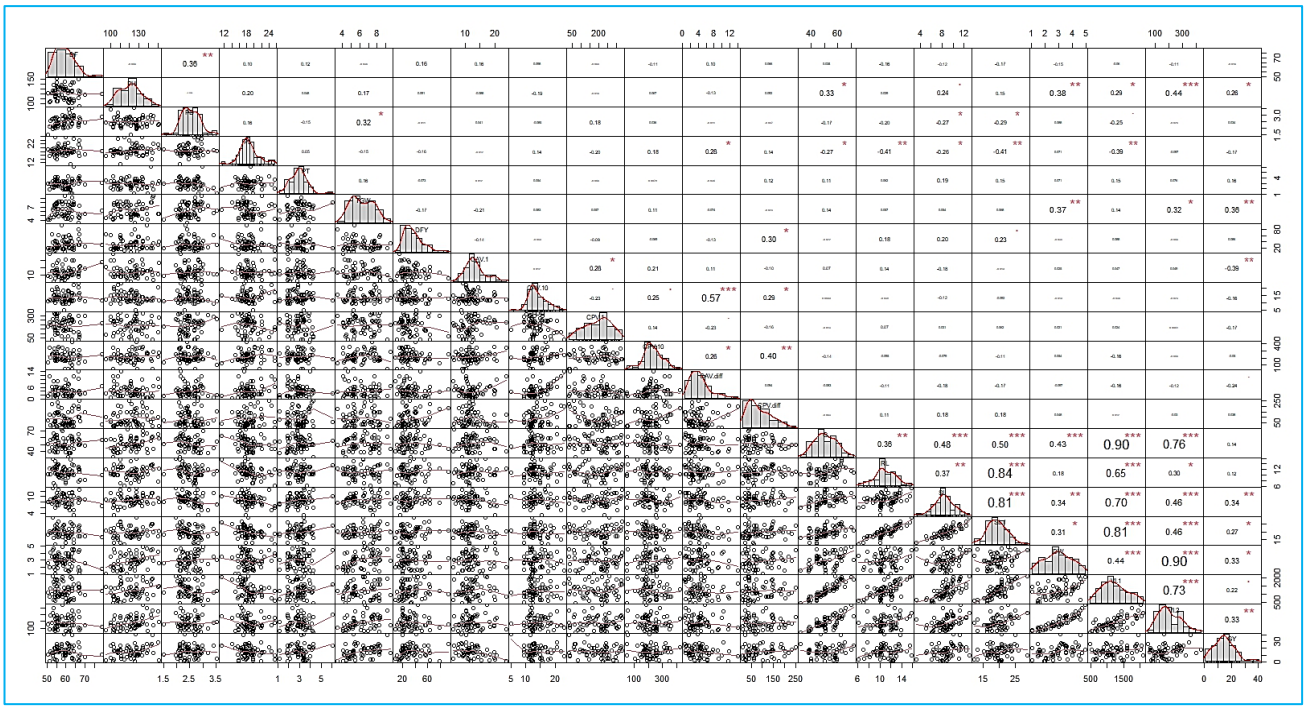

Fig 4 Estimates of genotypic and phenotypic correlations for agro morphological, seed and biochemical characters in pearl millet germplasm lines

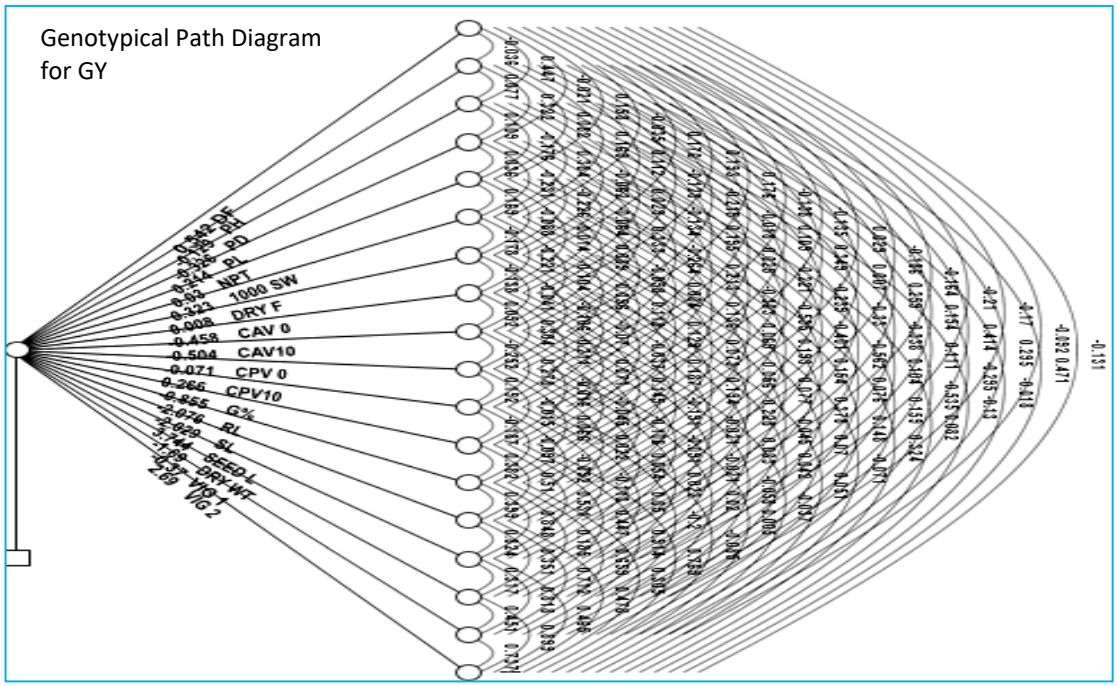

Fig 5 Estimates of genotypic path diagram for agro morphological, seed and biochemical characters in pearl millet germplasm lines

The concept of heritability is important to the breeder to determine whether the phenotypic differences observed among various individuals are due to differences in their genetic makeup or simply a result of environmental factors, and also it indicates the possibility and extent to which genetic gain is possible through selection [14]. High broad sense heritability was observed for the traits under study indicating ample additive gene action for these traits which can be relied upon for simple selection. High heritability was recorded for plant height, number of productive tillers/plants, 1000-seed weight, dry fodder yield/plant, comprehensive acid value on the 1st day, comprehensive acid value on the 10th day, comprehensive peroxide value on the 1stday, comprehensive peroxide value on the 10th day, germination percentage, shoot length, root length, seedling length, dry fodder yield/plant, seed vigour index I, seed vigour index II, grain yield/plant and the difference between comprehensive peroxide value on 1stand 10thday while rest of the traits exhibited moderate to low heritability. Sodani *et al.* [15] investigated high heritability for ear length and the number of days to flower. Sangha and Singh [16] reported that grain yield and ear length exhibited high heritability and genetic advance. High heritability and low genetic advance was observed for days to 50% flowering. These results are in close agreement with the studies performed by Ramya *et al.* [17], Chauhan *et al.* [18], Pallavi *et al.* [19].

Mazumdar *et al.* [6] examined peroxide and acid values in 56 commercial pearl millet lines (40 hybrids grown in India, 4 OPVs, and 12 hybrid parents) and discovered 13 genotypes, including 9522-B42. PM-15-NHB-1717, GHB-719, 9444, 89111-B46, and Super Boss are all available. RHB-219, PM-17-Bio-13, PM-19-Nandi-66 PM-18-12KM-80, The PV and AV (10th day) values of PM-16-JKBH-1294, GHB-538, and Raj 171 were all low, indicating that they were least susceptible to rancidity. According to Kadlag *et al.* [20], the peroxide value of the extracted oil increased for the first five days of storage before decreasing, while, Yadav [21] reported a continuous increase in the peroxide value of these materials, although HHB94 and ICMA 94222 x 78/711 were stored for 13 days.

Association between other characters

Understanding correlations with its component traits is highly helpful for the rational improvement of yield. Grain yield is a complex character and is dependent on several contributing traits. In terms of their phenotypic relationships, 1000 SW (rp=0.278), PH (rp=0.294), Germination % (rp=0.201), Shoot length (rp=0.265), Seedling length (rp=0.180), Dry weight (rp=0.242), Vigour index-I (rp=0.222), and Vigour index-II (rp=0.304) were found positively correlated with grain yield/plant. According to Dehinwal *et al.* [22], grain yield per plant showed positive and significant correlation with dry fodder yield followed by ear weight, total tillers per plant, effective tillers per plant, spike girth and plant height. However, negatively and significant correlation at phenotypic levels with grain yield/plant was observed with Comprehensive Acid Value on the 1stday (rp= -0.373), the difference between Comprehensive Peroxide Value on 1stand 10thday (rp= -0.190), Comprehensive Peroxide Value on the 10thday (rp= -0.196), and Comprehensive Peroxide Value on the 1st day (rp= -0.203). Vigour index I expressed significant positive correlation with plant height (r_p=0.263), Germination% (r_p=0.91), Root length (r_p=0.643), Shoot length (r_p=0.704), Seedling length (r_p=0.809), Dry weight (r_p=0.444), Vigour index-II (r_p=0.734), grain yield/plant (r_p=0.222) at phenotypic levels. Vigour index II expressed significant positive correlation with Plant height (r_p=0.401), 1000 Seed weight (r_p=0.309), Germination % (r_p=0.763), Root length (r_p=0.291), Shoot length (r_p=0.466), Seedling length (r_p=0.452), Dry weight (r_p=0.894), Vigour index-I (r_p=0.734), Grain yield/plant (r_p=0.304) at phenotypic levels. Singh *et al.* [23] found a correlation between yield and yield-contributing characters in ten parental lines and 45 hybrids of pearl millet. For the number of productive tillers per plant, plant height, panicle length, panicle girth, biological yield per plant, and dry fodder yield per plant with grain yield per plant, highly significant genotypic and phenotypic correlations were found in their study.

Direct and indirect effects

In the correlation tables, the indirect relationship is more complicated, less clear, and more perplexing as more factors are taken into consideration [12]. In such cases, the path coefficient analysis offers a useful method of discriminating between direct and indirect causes of association, allowing a critical evaluation of the particular forces at work to create a given correlation, and quantifies the relative significance of each causal factor. A critical examination of the results on correlation of grain yield and its component traits (Fig 4) and path analysis (Fig 5) depicting the direct and indirect effects of various traits revealed that seedling length (3.74) had the highest direct contribution towards grain yield/plant followed by vigour index-II (2.68), days to 50% flowering (0.54), 1000 seed weight (0.32), Comprehensive peroxide value on 10th day 10 (0.265) and panicle length (0.21) had direct on grain yield. Izge *et al.* [24] observed that days to 50% flowering had the high direct effects on total grain yield, according to the path analysis. Bhasker *et al.* [25] revealed that parameters like panicle length had the greatest direct effect on grain production per plant. The results were also supported by the finding of Singh *et al.* [23], Bhasker *et al.* [25], Kaushik *et al.* [13] for these characters. It was also found that root length (-2.076) and shoot length (-2.02) had very high negative direct effect which showed that selection should be done in negative direction. Path coefficient analysis highlighted seedling length and vigour index-II as the most significant contributors to grain yield.

CONCLUSION

From the results of the present study, it is inferred that the material contains a wide range of genetic variations in which most of the traits showed the high phenotypic coefficients of variation (PCV) and genetic coefficients of variation (GCV) indicating the presence of a high degree of variation for these traits among the genotypes which could be improved through selection in the desirable direction. High heritability was observed for many traits such as the difference between Comprehensive Peroxide Value on 1stand 10th day, seedling dry weight, seed vigour index and grain yield/plantindicated that these traits are governed by additive gene action. From the discussion on correlation and path coefficient analysis it could be concluded that for planning any selection criterion for improved grain yield, main emphasis should be given on seedling length, vigour index-II, days to 50% flowering, 1000 seed weight, Comprehensive Peroxide Value on the 10thday and thus direct selection of these traits could be effective in crop improvement programme of pearl millet. In terms of rancidity effects, it was found that the genotype has a longer shelf life if the difference between comprehensive acid value on day 1 and after 10 days after milling and comprehensive peroxide value between day 1 and after 10 days after milling is small as compared to other genotypes. Hence, efficient selection of pearl millet traits needs to be carried out for the development of new variety with improved shelf life and better health benefits.

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