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RESEARCH ARTICLE

Genetic Variability, Character Association and Heterosis Studies in Intraspecific F_1 Hybrids and Open-Pollinated Progenies of *Moringa oleifera* for Seed Germination, Seedling Growth and Nutritional Characteristics

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Abstract

The present study aimed to assess intraspecific F_1 hybrids along with the open-pollinated progenies of *Moringa oleifera* for seed germination (%), seedling growth and nutritional characteristics at Punjab Agricultural University, Ludhiana, during 2021-22. Significant variations were noted among hybrids and open-pollinated progenies for the studied parameters. First and last germination traits were found to be highly influenced by the environment and others are highly heritable traits for which we can select the hybrids for further evaluation. The majority of nursery and seedling traits had a positive significant correlation, and nutritional and physiological traits had a negative significant correlation. However, some have a significant negative correlation with total carotenoids. Two out of 22 components showed an Eigenvalue greater than unity and elucidated a significant amount of variation, i.e., approximately. 100% of the total variation existed among the hybrids and progenies. Only two hybrid progenies, i.e., *PKM-1* × *Bhagya* (H_1) and *PKM-1*× *PKM-2* (H_2), had the maximum heterosis for the majority of traits. Hybrid H_1 exhibited maximum magnitude of heterosis for seedling height (cm), leaf width (cm), and chlorophyll content (mg/g FW), carbohydrates (g/100 g DM), crude protein (g/100 g DM) and crude fibre (g/100 g DM). Hybrid H_2 exhibited maximum magnitude of heterosis for seedling height, collar diameter, chlorophyll content, crude protein, crude fibre and crude fat. Thus, only two hybrid progenies of crosses H_1 and H_2 showed higher economic heterosis for a majority of traits.

Keywords: *Moringa oleifera*, Open-pollinated progenies, F_1 hybrids, Genetic variations, heterosis.

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Introduction

Moringa oleifera Lam. (syn. *M. ptreygosperma* Gaertn.; n=14) is one of the best-known and most commonly utilized and naturalized tree species that has a place in the monogeneric family Moringaceae (Nadkarni, 1976; Ramachandran *et al.,* 1980). It is native to the western and sub-Himalayan tracts, including India and Pakistan; Asia Minor, Africa and Arabia (Somali *et al.,* 1984), but now is distributed in several countries of Asia and Africa (Morton, 1991; Pradheep *et al.,* 2011). Among 13 species in the genus moringa, *M. oleifera* is well known and widely adapted to the tropics and subtropics owing to its fast growth, capabilities to survive in humid, dry hot climates with poor soils (Arora and Onsare, 2014). Next in importance are the *M. concanensis, M. sternoptala, M. peregrina, M. optera, M. arabica* and *M. zyleynica*. *M. oleifera* is grown on approximately. 43,600 hectares of land in India, yielding 12 lakh tonnes of pods annually and India is the world's largest producer of moringa (Sandeep *et al.,* 2019). Andhra Pradesh is the leading Indian state with the maximum area under moringa cultivation (15,665 ha), followed by Tamil Nadu (13,042 ha), Karnataka (10,280 ha) and the remaining 4,613 ha area occupied by other states (Sannagoudar *et*

al., 2022). Tamil Nadu is the pioneering state as it initiated its genetic improvement works with local selections and introductions from Sri Lanka.

The moringa is referred to as a "wonder-tree" due to its multiple uses, especially for nutritional, mineral and important phytochemical ingredients (Verma and Nigam, 2014). Besides its wider cultivation as quality fodder to small size livestock in south and central Indian states, *M. oleifera* is utilized for edible pods, therapeutic significance and seed oil (Fugli, 2005). *M. oleifera* is an important nutrient-rich plant and is normally considered versatile for food that can be eaten in different recipes (Kumssa *et al.,* 2017). Moringa leaves has adequate amount of nutrients (g/100g DM) like crude protein (14.80 ± 0.18; 24.50 ± 0.35), crude fibre (17.81 ± 0.36; 24.72 ± 0.52), total carbohydrates (71.46 ± 0.20; 59.19 ± 0.39), crude fat (3.52 ± 0.01; 5.01 ± 0.01) and total soluble sugars (9.69 ± 0.02; 6.43 ± 0.03) in tender and mature leaves, respectively (Dhakad *et al.,* 2024). Moringa is one of the significant perennial vegetables growing in India. Because it contains vital nutrients including vitamins, minerals, phytochemicals, and other substances, all of its parts, including its leaves, roots, flowers, pods, and seeds, exhibit a surprising range of characteristics (Dhakad *et al.,* 2019).

Genetic diversity is a crucial need for choosing parents for hybridization and developing high yielding genotypes in any crop breeding programme. Transgressive segregants are more likely to be produced when there is a higher genetic variety between the parents. The drumstick plant's genetic diversity may be caused by a variety of agro-ecological conditions, genetic drift, gene flow, introduction and interchange of genetic stocks at the national and international research institutes, as well as natural and artificial selection among the wild and cultivated cultivars. During the late 90s, three varieties (*PKM-1; PKM-2* and *ODC-3*) have been developed in Tamil Nadu, India, while, Bhagya was developed from UHS Bagalkot, Karnataka in 2011 to improve leaf biomass and pod production (Sannagoudar *et al.,* 2022). Morphological and nutritional characterization is a fundamental tool for the selection, conservation, breeding and creation of new varieties (Popoola *et al.,* 2016). The study of accessions under homogeneous environmental conditions allows detecting the variability in the growth, flowering, number and size of leaves and fruits and allows identifying the resistance to various types of biotic and abiotic stress (Resmi *et al.,* 2005). Despite the great adaptability of the moringa plant, deciduous populations have been found in subtropical climates (Folkard *et al.,* 1999).

M. oleifera is cross-pollinated. Therefore, high heterogeneity in morphological, physiological and quantitative traits is anticipated. This tree has a variety of forms, from semi-spreading to upright, annual to perennial. Additionally, some trees flower twice a year, while others flower throughout the year (Raja *et al.,* 2013). Depending on the variety and location, flowers take a wide range of times to mature. In regions with little seasonal fluctuation in temperature or precipitation, flowering may be more or less constant. Insects pollinate flowers, some birds, and bees (Jyothi *et al.,* 1990; Morton, 1991) leads to the chances of incorporating the characters of widely planted trees in an ecosystem. *M. oleifera* has extremely low fruit set rates, 0.31% in the wet season and 1.5% in the dry (Krieg *et al.,* 2017) and the cause of this is still unknown. Usually, the fruit set by open pollination is 11 to 15%, while artificial/controlled pollination yields 62 to 100% (Kanthaswamy, 2005).

Knowledge of morphological diversity in moringa can become a resource for its breeding through the selection of elite varieties adapted to local conditions and having high nutritional value which is directly correlated to the leaf biomass production (Leone *et al.,* 2015). It is a fact that the south Indian varieties produced more leaf biomass with only high cultivation practices and good management, while north Indian cultivars especially from the Punjab region produced limited leaf biomass with a high amount of essential minerals, medicinally important secondary metabolites, dry matter digestibility (Singh, 2021). Punjab local cultivars are well adapted under extreme summers and winters, and can withstand poor management and cultivation practices. Even though *M. oleifera* is widely cultivated under various agro-ecological systems, large-scale commercial plantations are still not reported in the northern Indian states including Punjab possibly due to less biomass potential of locally adapted sources. But these salient findings, i.e. biomass and nutritional contents, observed in different cultivars should bring together in one cultivar before recommending it at a commercial scale in sub-tropical climatic conditions of Punjab. Keeping in view the above facts and discrepancies, the present study aimed to evaluate intraspecific F_1 of *M. oleifera* with their open pollinated progenies for seed germination, growth and nutritional characteristics.

Materials and Methods

The experiment was conducted at the Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana, Punjab, since 2020-21. The study area falls in the central zone of Punjab (30°54′ N latitude and 75°48′ E longitude, with 247 m above mean sea level) and is characterized by sub-tropical to tropical, semi-arid type of climate. The minimum temperature may go down to 4°C or even less, while the maximum temperature may be as high as more than 45°C during the summer season. The occurrence of frost is not common. The soils are deep, well-drained, sandy loam in texture with low humus content. The soil pH is neutral. The average annual rainfall in Ludhiana is 760 mm, about three-fourths of which is contributed by the south-west monsoon during July to September.

The experimental was carried out in nursery evaluation of 10 open pollinated progenies of *M. oleifera* and its 4 F_1 hybrids of *PKM-1* × *Bhagya* (H_1), *PKM-1*× *PKM-2* (H_2), *PKM-2* × *ODC-3* (H_3) and *PKM-2* × Mandya local, Karnataka (H_4) established since July 2022 in polythene bags in three replications with plot size of 5 seeds following complete randomized design (CRD). The data on germination parameters, seedling growth and nutritional characteristics of seedling leaves were recorded. The data for leaf morphometric traits were recorded as per the procedure laid down for AVRDC-GRSU characterization record sheet developed by the World Vegetable Centre, Taiwan. The observations for seedlings growth traits and leaf characteristics were recorded on plants having 3-months of growth. Only healthy, mature and complete leaves were subjected to data collection and further nutritional and physiological evaluation.

The data collected from the seed sources of *M. oleifera* on different parameters were subjected to statistical analysis. Coefficient of variability at phenotypic and genotypic level (%), heritability (%) in broad sense, expected genetic advance (%) and genetic gain (%) expressed as genetic advance per cent of mean were calculated as per the formula suggested by Burton and De Vane (1953). Genotypic and phenotypic correlation coefficients were calculated according to the methods suggested by Goulden (1952). Genotypic and phenotypic parameters were statistically analyzed using OPSTAT software. SPSS software, version 16.0, was used to perform principal component analysis on data recorded for germination parameters, seedling growth and nutritional characteristics. The latent root criterion (eigenvalues greater than one) was applied for estimating the number of principal components. A bi-plot display of the first three principal components was used for grouping genotypes, illustrating the relationship between genotypes and indices (Yang and Kang, 2003) using JMP Pro 10.0 software. The superiority of F_1 hybrids over the standard commercial variety/cultivar, i.e. *PKM-1, PKM-2, ODC-3*, PAU local and Mandya local, known as standard heterosis or economic heterosis, were estimated by using the heterosis for replicated data with check(s) function of TNAUSTAT software (Nadarajan *et al.,* 2016).

Results and Discussion

Estimates of genetic variability

A key to progress in tree improvement and breeding programmes is the degree and extent of variation present in a species and germplasm. For planning of strategies for improvement and breeding of any species, the coefficient of variation (%) is most importance. Heritability (%) and genetic advance give information of the variations present in planting material as well as environmental influences on those traits. The components of variance with high genetic gain and high heritability can be used to generate an idea and scope for response of selection to various characters (Chauhan *et al.,* 2004).

In general, the phenotypic coefficient of variation was higher than the corresponding genotypic coefficient of variation for all characters, reflecting the sufficient genetic variations for the characters studied among hybrid and open-pollinated progenies (Table 1). High genotypic variance was observed in seed weight (29.85%), followed by crude fat (21.88%), survival percent (21.84%), and leaf length (20.22%). Total energy value (1.337%), followed by total carbohydrates (4.91%), ash content (5.318%) and crude fiber (4.82%) had the lowest amount of genotypic variance across progenies. A high heritability indicates that much of the variation for a given characteristic observed in the population is genetic in origin. The majority of the traits had very high heritability (>60%) except first and last germination (<30%) traits, which were highly influenced by environmental conditions. It means that the traits were under the strong influence of additive gene action, and selection would be quite effective.

In the current study, the trend of genetic advance as per cent of the mean shows a huge scope for genetic improvement in *M. oleifera*. In fact, moderate genetic gain was observed in survival percent (19.60%), followed by germination percent (18.89%), while the minimum was observed in total soluble energy (0.015%), first germination (0.468%) and last germination (0.722%). Similar results were reported by Verma *et al.* (2019), when compared to other characteristics, pod weight (g) had moderate heritability (51.32%). And genetic advance as a percentage of mean was observed lowest for number of seeds per pod (4.49) and pod girth 6.21, and a similar result was found by Balaguru *et al.* (2020) genetic advance as percentage of mean was observed lowest for number of seeds per pod and pod girth in *M. oleifera*.

Correlation studies

Correlation provides an insight to the deep complexity and the amount of inter-relationship existing between different traits. Because some traits are inter-linked related to one another, knowledge of associations is important when choosing one or more traits. It provides an excellent base for indirect selection, particularly for below-ground or biomass traits which require destructive sampling. The amount of genetic gain obtained from indirect selection, *i.e.* correlated response gives a better understanding of degree and magnitude of genetic association and must be given the due weightage while formulating the selection criteria in any tree improvement programme.

Significant correlations were recorded for the traits studies and presented in Tables 2 and 3. The majority of nursery and seedling traits had a positive significant, and nutritional and physiological traits had a negative significant correlation. However, some have a significant negative correlation with total carotenoids. Carotenoids had positive significant correlation with seedling weight (0.658**), survival percent (0.468**), plant percent (0.342**), leaf length

Table 1: Genetic variability estimates for seed germination, seedling growth and nutritional characteristics of open-pollinated progenies and F_1 hybrids of *Moringa oleifera* Lam.

Parameter	*Mean*	*Range*	*Coefficient of variation*		*Heritability (%)*	*Genetic advance (%)*	*Genetic gain (% of mean)*
			Phenotypic (%)	*Genotypic (%)*			
SW	16.01 ± 0.67	03.64-31.91	31.77	29.84	88.22	11.32	57.75
FG	06.82 ± 0.91	06.00-8.00	28.96	10.39	12.86	0.46	07.67
LG	06.82 ± 0.91	10.00-15.00	26.21	09.29	12.56	0.72	06.78
GP	54.50 ± 3.06	28.57-66.66	14.96	14.52	94.24	18.89	29.05
SP	34.72 ± 1.72	14.28-42.85	22.71	21.84	92.44	19.60	43.26
PP	23.96 ± 2.29	00.00-36.66	20.30	18.70	84.82	10.06	35.48
SH	60.09 ± 1.49	47.00-82.00	16.71	15.85	89.91	17.19	30.96
CD	07.22 ± 0.59	04.88-09.24	19.52	14.85	57.90	01.46	23.29
LL	20.18 ± 2.28	15.00-30.80	23.81	20.21	72.09	06.84	35.36
LW	12.31 ± 0.90	09.00-21.80	25.22	19.78	61.52	03.68	31.97
PL	06.93 ± 0.46	04.75-09.50	20.59	17.31	70.72	02.02	30.00
NoP	06.66 ± 0.55	06.00-08.20	17.20	13.09	57.89	01.55	20.51
DM	24.64 ± 0.29	22.98-25.74	09.19	08.98	95.39	04.43	18.07
CP	37.39 ± 0.27	32.17-40.63	10.54	10.47	98.63	07.43	21.42
CFt	04.60 ± 0.19	03.33-05.43	22.38	21.87	95.52	02.05	44.04
Ash	10.24 ± 0.10	09.47-10.93	05.86	05.31	82.15	01.02	09.93
CFb	22.24 ± 0.14	20.48-23.37	06.50	05.85	81.23	02.48	10.87
TCb	12.55 ± 0.19	12.07-13.06	08.04	07.99	98.84	08.24	16.38
TEV	382.03 ± 0.84	370.8-388.4	01.38	01.33	93.38	10.16	02.66
TSS	00.04 ± 0.00	0.03-0.04	18.04	17.50	94.12	00.01	34.99
TCH	47.77 ± 0.19	12.07-13.06	05.21	04.91	88.65	01.19	09.52
CART	38.59 ± 0.27	33.39-42.50	08.21	08.09	96.99	06.46	16.42

Note 1: SW- Seed Weight (g/100seeds); FG- First Germination (%); LG- Last Germination (%); GP- Germination Percentage (%); SP- Survival Percentage (%); PP- Plant Percentage (%); SH- Seedling Height (cm); CD- Collar Diameter (mm); LL- Leaf Length (cm); LW- Leaf Width (cm); PL- Petiole Length (cm); NOP- Number of Pinnae; DM- Dry Matter (g/100g); CP- Crude Protein (g/100g DM); CFt-Crude Fat (g/100g DM); Ash- Ash Content (g/100g DM); CFb- Crude Fiber (g/100g DM); TCb- Total Chlorophyll (mg/g FW); TEV- Total Energy Value (Mcal/kg); TSS- Total Soluble Sugars (g/100g DM); TCH- Total Carbohydrates (g/100g DM): CART- Total Carotenoids (g/100g DM).

(0.305*), petiole length (0.345*), total carbohydrates (0.517**), while significant negative correlation with all nutritional traits and all physiological traits except total carbohydrates at phenotype level. However, carotenoids showed only a positive significant correlation with seedling weight (0.711**), survival percent (0.494**), and total carbohydrates (0.542*) at the genotype level. Seedling weight showed positive correlations with germination percentage (0.471**), seedling height (0.479**), leaf width (0.484**), petiole length (0.434**), number of pinnae (0.500**) and total carotenoids (0.658**) at the phenotype level. Seedling weight showed positive correlations with germination percent (0.519**), survival percent (0.620**), seedling height (0.531**), leaf length (0.601**), leaf width (0.625**), petiole length (0.505), number of pinnae (0.786**), total crude fibre (0.356**) and total carotenoids (0.711**) at genotype level. Germination percent had a high correlation with survival percent, plant percentage and a low correlation with petiole length at the phenotype level. Germination percentage had a high correlation with survival percent, number of pinnae and a low correlation with petiole length at the genotype level. Maximum correlations show crude fat with total energy value at the phenotype level. At the genotype level, maximum correlations show first germination and last germination.

It was clearly evident from the Table 2 and 3 that the magnitude of genotypic correlation was higher than the corresponding phenotypic correlation which might be due to strong inherent linkage of traits at gene level or pleiotropic effect of a gene, suggested that any change in the gene locus of one trait may alter the genetic expression of the associated traits. The results are in agreement with the findings of Lastin *et al.* (2019), who observed that the genotypic correlation coefficient was higher than the

Table 2: Genotypic Correlations among seed germination, seedling growth and nutritional characteristics of open-pollinated progenies and F_1 hybrids of *M. oleifera* Lam.

	SW	*FG*	*LG*	*GP*	*SP*	*PP*	*SH*	*CD*	*LL*	*LW*	*PL*	*NoP*	*DM*	*CP*	*CFt*	*Ash*	*CFb*	*TCb*	*TEV*	*TSS*	*TCH*
FG	-0.052^{NS}																				
LG	-0.402**	2.800**																			
GP	0.519**	-0.343*	-0.613**																		
SP	0.620**	-0.431**	-0.733**	0.824**																	
PP	0.377*	-0.204^{NS}	-0.341*	0.616**	0.666**																
SH	0.531**	0.364*	0.009^{NS}	0.193^{NS}	0.011^{NS}	-0.188^{NS}															
CD	-0.166^{NS}	1.063**	0.908**	-0.097^{NS}	-0.510**	-0.414**	0.737**														
LL	0.601**	0.376*	0.037^{NS}	0.069^{NS}	0.131^{NS}	-0.374*	0.844**	0.503**													
LW	0.625**	0.050^{NS}	0.130^{NS}	0.071^{NS}	0.272^{NS}	-0.379*	0.660**	0.293^{NS}	1.065**												
PL	0.505**	-0.029^{NS}	-0.167^{NS}	0.017^{NS}	0.212^{NS}	-0.303^{NS}	0.805**	0.299^{NS}	0.904**	0.877**											
NoP	0.786**	0.105^{NS}	-1.058**	0.648**	0.591**	-0.004^{NS}	0.712**	0.047^{NS}	0.749**	0.792**	0.704**										
DM	-0.490**	-0.119^{NS}	-0.036^{NS}	0.371*	0.017^{NS}	-0.020^{NS}	-0.312*	0.098^{NS}	-0.483**	-0.434**	-0.643**	-0.166^{NS}									
CP	-0.054^{NS}	0.317*	0.578**	0.004^{NS}	-0.230^{NS}	0.230^{NS}	0.509**	0.696**	0.130^{NS}	-0.110^{NS}	0.016^{NS}	-0.165^{NS}	0.035^{NS}								
CFt	0.207^{NS}	0.566**	-0.378*	0.383*	0.315*	0.250^{NS}	0.130^{NS}	-0.070^{NS}	0.138^{NS}	0.289^{NS}	0.019^{NS}	0.312*	0.130^{NS}	-0.022^{NS}							
Ash	0.312*	-0.304^{NS}	-0.577**	0.455**	0.138^{NS}	0.333*	0.198^{NS}	0.000^{NS}	-0.148^{NS}	-0.133^{NS}	-0.348*	0.136^{NS}	0.328*	0.399**	0.209^{NS}						
CFb	0.356*	-0.058^{NS}	-0.145^{NS}	0.172^{NS}	0.254^{NS}	0.255^{NS}	-0.038^{NS}	-0.308*	0.125^{NS}	0.137^{NS}	0.247^{NS}	0.055^{NS}	-0.503**	-0.249^{NS}	-0.173^{NS}	-0.299^{NS}					
TCb	-0.046^{NS}	-0.388*	-0.348*	-0.162^{NS}	0.109^{NS}	-0.317*	-0.519**	-0.610**	-0.132^{NS}	0.045^{NS}	0.028^{NS}	0.052^{NS}	-0.109^{NS}	-0.951**	-0.261^{NS}	-0.550**	0.309*				
TEV	0.072^{NS}	0.695**	-0.128^{NS}	0.186^{NS}	0.255^{NS}	0.106^{NS}	0.044^{NS}	-0.070^{NS}	0.202^{NS}	0.345*	0.169^{NS}	0.253^{NS}	-0.011^{NS}	-0.194^{NS}	0.907**	-0.222^{NS}	-0.044^{NS}	-0.024^{NS}			
TSS	0.098^{NS}	0.193^{NS}	-0.037^{NS}	-0.390*	-0.408**	-0.158^{NS}	-0.030^{NS}	-0.119^{NS}	-0.050^{NS}	-0.155^{NS}	-0.104^{NS}	-0.034^{NS}	-0.386*	-0.196^{NS}	-0.190^{NS}	-0.198^{NS}	0.111^{NS}	0.252^{NS}	-0.104^{NS}		
TCH	0.213^{NS}	-0.302^{NS}	0.237^{NS}	0.284^{NS}	0.554**	0.534**	-0.050^{NS}	-0.066^{NS}	0.124^{NS}	0.088^{NS}	0.124^{NS}	-0.030^{NS}	-0.144^{NS}	0.155^{NS}	-0.021^{NS}	-0.083^{NS}	-0.030^{NS}	-0.123^{NS}	0.015^{NS}	-0.373*	
CART	0.711**	-0.071^{NS}	0.317*	0.307*	0.494**	0.355*	0.298^{NS}	-0.047^{NS}	0.339*	0.356*	0.360*	0.234^{NS}	-0.450**	0.092^{NS}	-0.146^{NS}	0.300^{NS}	0.342*	-0.087^{NS}	-0.275^{NS}	-0.324*	0.542**

Note 1: Genotypic correction coefficient is at left lower side and phenotypic correction coefficient is at right upper side of table (bold red digits)
Note 2: *. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).
Note 3: SW- Seed Weight (g/100seeds); FG- First Germination (%); LG- Last Germination (%); GP- Germination Percentage (%); SP- Survival Percentage (%); PP- Plant Percentage (%); SH- Seedling Height (cm); CD- Collar Diameter (mm); LL- Leaf Length (cm); LW- Leaf Width (cm); PL- Petiole Length (cm); NOP- Number of Pinnae; DM- Dry Matter (g/100g); CP- Crude Protein (g/100g DM); CFt-Crude Fat (g/100g DM); Ash- Ash Content (g/100g DM); CFb- Crude Fiber (g/100g DM); TCb- Total Chlorophyll (mg/g FW); TEV- Total Energy Value (Mcal/kg); TSS- Total Soluble Sugars (g/100g DM); TCH- Total Carbohydrates (g/100g DM): CART- Total Carotenoids (g/100g DM).

Table 3: Phenotypic Correlations among seed germination, seedling growth and nutritional characteristics of open-pollinated progenies and F_1 hybrids of *M. oleifera* Lam.

	SW	*FG*	*LG*	*GP*	*SP*	*PP*	*SH*	*CD*	*LL*	*LW*	*PL*	*NoP*	*DM*	*CP*	*CFt*	*Ash*	*CFb*	*TCb*	*TEV*	*TSS*	*TCH*
FG	0.055NS																				
LG	-0.105NS	0.035NS																			
GP	0.471**	-0.050NS	-0.260NS																		
SP	0.579**	-0.244NS	-0.186NS	0.755**																	
PP	0.321*	-0.148NS	-0.113NS	0.534**	0.600**																
SH	0.479**	0.158NS	0.037NS	0.181NS	0.014NS	-0.155NS															
CD	-0.150NS	0.281NS	0.350*	-0.079NS	-0.356*	-0.285NS	0.518**														
LL	0.478**	0.130NS	0.037NS	0.066NS	0.116NS	-0.221NS	0.648**	0.250NS													
LW	0.484**	0.292NS	-0.171NS	0.075NS	0.181NS	-0.273NS	0.541**	0.003NS	0.769**												
PL	0.434**	-0.070NS	-0.153NS	0.002NS	0.178NS	-0.201NS	0.614**	0.169NS	0.664**	0.605**											
NoP	0.500**	-0.120NS	-0.263NS	0.481**	0.473**	0.009NS	0.492**	0.048NS	0.458**	0.465**	0.495**										
DM	-0.442**	-0.072NS	0.028NS	0.341*	0.027NS	-0.016NS	-0.298NS	0.096NS	-0.411**	-0.386*	-0.536**	-0.072NS									
CP	-0.054NS	0.087NS	0.183NS	-0.001NS	-0.212NS	0.218NS	0.482**	0.544**	0.096NS	-0.083NS	0.049NS	-0.106NS	0.030NS								
CFt	0.198NS	0.256NS	-0.133NS	0.358*	0.297NS	0.222NS	0.136NS	-0.067NS	0.127NS	0.235NS	0.020NS	0.194NS	0.120NS	-0.029NS							
Ash	0.300NS	-0.009NS	-0.019NS	0.387*	0.091NS	0.299NS	0.155NS	0.022NS	-0.080NS	-0.084NS	-0.291NS	0.042NS	0.307*	0.343*	0.201NS						
CFb	0.257NS	-0.137NS	0.033NS	0.126NS	0.193NS	0.248NS	-0.045NS	-0.170NS	0.106NS	-0.042NS	0.140NS	0.041NS	-0.396**	-0.233NS	-0.173NS	-0.220NS					
TCb	-0.047NS	-0.143NS	-0.128NS	-0.149NS	0.102NS	-0.298NS	-0.494**	-0.478**	-0.107NS	0.027NS	-0.005NS	0.040NS	-0.104NS	-0.947**	-0.261NS	-0.512**	0.288NS				
TEV	0.057NS	0.256NS	-0.123NS	0.175NS	0.251NS	0.082NS	0.063NS	-0.076NS	0.162NS	0.270NS	0.153NS	0.172NS	-0.022NS	-0.186NS	0.893**	-0.260NS	-0.070NS	-0.023NS			
TSS	0.058NS	0.112NS	-0.029NS	-0.373*	-0.400**	-0.131NS	-0.023NS	-0.032NS	-0.021NS	-0.122NS	-0.121NS	-0.021NS	-0.353*	-0.193NS	-0.175NS	-0.149NS	0.132NS	0.242NS	-0.104NS		
TCH	0.195NS	-0.092NS	0.095NS	0.264NS	0.517**	0.443**	-0.041NS	0.011NS	0.097NS	0.045NS	0.133NS	-0.016NS	-0.136NS	0.150NS	0.006NS	-0.048NS	-0.023NS	-0.130NS	0.028NS	-0.328*	
CART	0.658**	-0.016NS	0.031NS	0.303NS	0.468**	0.342*	0.269NS	-0.038NS	0.305*	0.296NS	0.345*	0.165NS	-0.439**	0.096NS	-0.131NS	0.263NS	0.284NS	-0.092NS	-0.250NS	-0.305*	0.517**

Note 1: *. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

Note 2: SW- Seed Weight (g/100seeds); FG- First Germination (%); LG- Last Germination (%); GP- Germination Percentage (%); SP- Survival Percentage (%); PP- Plant Percentage (%); SH- Seedling Height (cm); CD- Collar Diameter (mm); LL- Leaf Length (cm); LW- Leaf Width (cm); PL- Petiole Length (cm); NOP- Number of Pinnae; DM- Dry Matter (g/100g); CP- Crude Protein (g/100g DM); CFt-Crude Fat (g/100g DM); Ash- Ash Content (g/100g DM); CFb- Crude Fiber (g/100g DM); TCb- Total Chlorophyll (mg/g FW); TEV- Total Energy Value (Mcal/kg); TSS- Total Soluble Sugars (g/100g DM); TCH- Total Carbohydrates (g/100g DM): CART- Total Carotenoids (g/100g DM).

Table 4: Percentage of variance explained by different principal components among open-pollinated progenies and F_1 hybrids of *M. oleifera* Lam.

Characteristics	*Principal Components*	
	1	*2*
SW	0.000	-1.000
FG	0.915	0.402
LG	-0.915	-0.402
GP	-0.693	0.721
SP	0.994	0.000
PP	0.780	-0.626
SH	0.787	0.617
CD	0.594	0.804
LL	0.000	-0.995
LW	0.857	-0.516
PL	-0.971	0.000
NoP	0.696	0.718
DM	0.911	-0.412
CP	0.959	0.000
CFt	-0.568	0.823
Ash	-0.985	0.000
CFb	0.420	-0.907
TCb	-0.915	-0.402
TEV	0.448	0.894
TSS	0.888	-0.459
TCH	0.000	0.959
CART	0.900	0.436
Eigen value	12.834	9.166
Percent of variability	58.330	41.664
Cumulative percent of variability	58.330	~100

Note 2: SW- Seed Weight (g/100seeds); FG- First Germination (%); LG- Last Germination (%); GP- Germination Percentage (%); SP- Survival Percentage (%); PP- Plant Percentage (%); SH- Seedling Height (cm); CD- Collar Diameter (mm); LL- Leaf Length (cm); LW- Leaf Width (cm); PL- Petiole Length (cm); NOP- Number of Pinnae; DM- Dry Matter (g/100g); CP- Crude Protein (g/100g DM); CFt-Crude Fat (g/100g DM); Ash- Ash Content (g/100g DM); CFb- Crude Fiber (g/100g DM); TCb- Total Chlorophyll (mg/g FW); TEV- Total Energy Value (Mcal/kg); TSS- Total Soluble Sugars (g/100g DM); TCH- Total Carbohydrates (g/100g DM): CART- Total Carotenoids (g/100g DM).

phenotypic correlation coefficient. Similarly, number of seeds per fruit pod was significantly correlated with fruit/pod length, while 100 seed weight was significantly correlated with fruit/pod length, pod weight, number of seed per locule and number of seed per pod and significant relationship between days to 50% flowering and days to 50% maturity is indicative that there is a strong relationship between the stage of plant growth at which flowering is initiated and the time taken to complete the crop's life cycle (Akinyele and Osekita, 2006; Aremu, 2011).

Principal component analysis

Principal component analysis is a statistical data reduction technique to reduces a large data set with a number of correlated variables into a significantly lower set of new variables that comprises most of the variation present in the traits, so that the interpretation of results becomes feasible. The principle components of the correlation matrix having less than one Eigen value should be dropped as these components are less informative (Kaiser, 1958). It provides an insight into complex relationship existing between different parameters and reflects their contribution to the every character towards total variation (Kumari *et al.,* 2025).

Since, it is difficult to conceive 22 different characters, principle component analysis was performed and the summarized in Table 4. It was observed that two out of 22 components showed eigenvalues greater than unity and elucidated a significant amount of variation, *i.e.,* ~100 % of the total variation. The scree plot of eigenvalues is furnished in Figure 4.1. The first principal component (λ1= 12.834) explained 58.336 % variation of the total variation, with a maximum loading recorded for survival percent (0.994), crude protein (0.959), first germination (0.915) and dry matter (0.911). The second principal component (λ2= 9.166) explains 41.664 % of the total variation, with the highest loading for total carbohydrates (0.959) followed by total energy value (0.894), crude fat (0.823), and collar diameter (0.804). The principal component analysis had indicated some of the important components associated with reproductive and crossability traits in *M. oleifera,* which could be utilized for optimizing the productivity of elite *M. oleifera* progenies. Thus, maximum weightage should be given to survival percentage with maximum variable loading (0.994) followed by crude protein and total carbohydrates (0.959), first germination (0.861) and length (0.853), number of leaflets (0.832), leaf width (0.915) and dry matter (0.911), since they showed the highest contribution towards total genetic variability present in the hybrid and open pollinated progenies of *M. oleifera*. Similarly, Zeru and Hassen (2022), while evaluating provenance variation in Moringa, reported that principal component analysis revealed distinct population differentiation associated with variability for plant height and seedling survival rate.

Heterosis studies

In the present study, the results obtained with respect to the magnitude of heterosis have been presented in Tables 5 to 8. Hybrid H_1 exhibited maximum magnitude of heterosis for seedling height, leaf width, and chlorophyll content, carbohydrates, crude protein and crude fibre. Hybrid H_2 exhibited maximum magnitude of heterosis for seedling

Table 5: Standard heterosis (%) of progeny of *PKM-1* × *Bhagya* (H_1) of *M. oleifera* Lam. over their parents and check varieties for growth and nutritional characteristics

Characters	*Mean*	*% over Bhagya*	*% over PKM-1*	*% over PAU Local*
Growth characteristics				
Seedling height (cm)	62.44	16.92**	51.55**	-11.57**
Collar diameter (mm)	7.15	19.96ns	50.74**	-8.80ns
Leaf length (cm)	22.05	16.55ns	55.26**	8.60ns
Leaf width (cm)	13.60	31.78*	44.63**	16.24ns
Petiole length (cm)	7.38	10.15ns	38.81**	-4.98ns
Number of pinnae	6.76	-6.45ns	-6.45ns	-29.20**
Nutritional characteristics				
Dry matter (g/100g)	24.38	-0.73 ns	1.99 ns	13.02**
Ash content (g/100g DM)	10.93	5.13*	7.89**	1.86 ns
Crude protein (g/100g DM)	37.29	-4.02**	12.04**	-3.19**
Crude fibre (g/100g DM)	20.48	-4.07**	-16.29**	-4.12**
Crude fat (g/100g DM)	5.43	120.27**	7.24ns	3.82 ns
Carbohydrates (g/100g DM)	13.06	-5.79**	3.68*	7.96**
Total soluble sugars (g/100g DM)	0.04	-7.76 ns	-19.55**	7.00 ns
Total energy value (Mcal/kg)	383.43	3.43**	-0.36 ns	0.05 ns
Chlorophyll content (mg/g FW)	46.35	-4.01**	-16.39**	1.82*
Total carotenoids (g/100g DM)	42.50	-0.65 ns	19.96**	13.59**

Table 6: Standard heterosis (%) of progeny of *PKM-1*× *PKM-2* (H_2) of *M. oleifera* Lam. over their parents and check varieties for growth and nutritional characteristics

Characters	*Mean*	*% over PKM-1*	*% over PKM-2*	*% over PAU Local*
Growth characteristics				
Seedling height (cm)	63.92	55.14**	28.60**	-9.47**
Collar diameter (mm)	7.32	54.25**	34.42**	-6.68 ns
Leaf length (cm)	20.67	45.54**	11.53 ns	1.81 ns
Leaf width (cm)	9.00	-4.25 ns	-23.70 ns	-23.05 ns
Petiole length (cm)	6.80	27.90**	9.68 ns	-12.45 ns
Number of pinnae	6.85	-5.17 ns	-5.21 ns	-28.26**
Nutritional characteristics				
Dry matter (g/100g)	22.98	-3.88**	-18.75**	-18.03**
Ash content (g/100g DM)	10.23	0.99 ns	-3.46 ns	-4.66*
Crude protein (g/100g DM)	40.63	22.10**	38.87**	5.50**
Crude fibre (g/100g DM)	23.37	9.43**	-4.51**	9.38**
Crude fat (g/100g DM)	4.60	-9.21*	32.69**	-12.10**
Carbohydrates (g/100g DM)	12.07	-4.21*	7.19**	-0.25 ns
Total soluble sugars (g/100g DM)	0.05	9.77**	12.31**	46.00**
Total energy value (Mcal/kg)	382.07	-0.71**	1.90**	-0.30 ns
Chlorophyll content (mg/g FW)	44.53	-19.66**	-19.69**	-2.17*
Total carotenoids (g/100g DM)	39.65	11.90**	2.35 ns	5.96**

Table 7: Standard heterosis (%) of progeny of *PKM-2* × *ODC-3* (H_3) of *M. oleifera* Lam. over their parents and check varieties for growth and nutritional characteristics

Characters	*Mean*	*% over PKM-1*	*% over PKM-2*	*% over ODC-3*	*% over PAU Local*
Growth characteristics					
Seedling height (cm)	51.50	25.00**	3.62 ns	-14.02**	-27.06**
Collar diameter (mm)	7.09	49.54**	30.31**	20.23 ns	-9.52 ns
Leaf length (cm)	17.17	20.92 ns	-7.34 ns	-10.79 ns	-15.42 ns
Leaf width (cm)	9.00	-4.25 ns	-23.70 ns	-19.23 ns	-23.05 ns
Petiole length (cm)	5.57	4.76 ns	-10.16 ns	-17.07 ns	-28.28
Number of pinnae	6.00	-16.97 ns	-17.01ns	-28.03**	-37.19**
Nutritional characteristics					
Dry matter (g/100g)	25.74	7.65**	-9.00**	0.55 ns	-8.19**
Ash content (g/100g DM)	10.33	1.97 ns	-2.52 ns	-8.82**	-3.73 ns
Crude protein (g/100g DM)	39.45	34.32**	29.40**	11.81**	2.42*
Crude fibre (g/100g DM)	22.47	5.21**	-8.19**	-5.03**	5.16**
Crude fat (g/100g DM)	5.03	-0.66 ns	45.19**	13.53**	-3.82 ns
Carbohydrates (g/100g DM)	12.32	-2.20 ns	9.44**	-3.47*	1.85 ns
Total soluble sugars (g/100g DM)	0.05	3.01 ns	5.38 ns	0.74 ns	37.00**
Total energy value (Mcal/kg)	383.83	-0.25 ns	2.37**	1.86**	0.16 ns
Chlorophyll content (mg/g FW)	45.19	-18.48**	18.51**	-7.69**	-0.73 ns
Total carotenoids (g/100g DM)	33.39	-5.77**	-13.81**	-26.63**	-10.77**

Table 8: Standard heterosis (%) of progeny of *PKM-2* × Mandya, Karnataka (H_4) of *M. oleifera* Lam. over their parents and check varieties for growth and nutritional characteristics

Characters	*Mean*	*% over PKM-1*	*% over PKM-2*	*% over Mandya Local*	*% over PAU Local*
Growth characteristics					
Seedling height (cm)	47.00	14.08**	-5.43ns	-21.54**	-33.43**
Collar diameter (mm)	7.61	60.44**	39.80**	28.98*	-2.93ns
Leaf length (cm)	16.33	15.02ns	-11.85ns	-15.14ns	-19.54ns
Leaf width (cm)	9.33	-0.74ns	-20.90ns	-16.27ns	-20.23ns
Petiole length (cm)	5.83	9.72ns	-5.91ns	-13.15ns	-24.89**
Number of pinnae	6.33	-12.36ns	-12.40ns	-24.03**	-33.71**
Nutritional characteristics					
Dry matter (g/100g)	25.47	6.53**	-9.96**	11.50**	-9.16**
Ash content (g/100g DM)	9.47	-6.58**	-10.69**	-16.47**	-11.80**
Crude protein (g/100g DM)	32.17	-3.33**	9.56**	-8.81**	-16.46**
Crude fibre (g/100g DM)	22.6	5.99**	-7.51**	-4.33**	5.94**
Crude fat (g/100g DM)	3.33	-34.21**	-3.85 ns	-24.81**	-36.31**
Carbohydrates (g/100g DM)	12.73	1.06 ns	13.08**	-0.26 ns	5.23 **
Total soluble sugars (g/100g DM)	0.05	1.50 ns	3.85 ns	-0.74 ns	35.00**
Total energy value (Mcal/kg)	378.80	-1.56**	1.03**	0.52 ns	-1.16**
Chlorophyll content (mg/g FW)	55.03	-0.73 ns	-0.76 ns	12.41**	20.88**
Total carotenoids (g/100g DM)	38.81	9.53**	0.18 ns	-14.72**	3.71*

height, collar diameter, chlorophyll content, crude protein, crude fibre and crude fat. Hybrid H_3 exhibited maximum magnitude of heterosis for seedling height, chlorophyll content, crude protein and crude fibre, and total carotenoids. Hybrid H_4 exhibited maximum magnitude of heterosis for seedling height, collar diameter, chlorophyll content, dry matter, ash content, crude protein and crude fibre, total energy value and total carotenoids.

The importance of intra- and inter-specific hybridization in the genetic improvement of Willows has been demonstrated by significant and stable heterosis/hybrid vigour expressed in F1 hybrids. The vigour in terms of growth of hybrids depends on the selection of parental species/genotype and filial generation of hybrids (Zsuffa, 1984). There were indications of heterosis in hybrids already produced between *S. matsudana* and *S. alba* which had showed 100 per cent superiority in diameter growth and 30 per cent in height growth over the growth of either parents (Hathaway, 1979). Superiority of intra-specific hybrids of has been already demonstrated by earlier workers (Hathaway and Kraayenoord, 1979; Dongsen *et al.,* 1992; Li and Wu, 1997; Ronnberg and Gulberg, 1999; Ozel *et al.,* 2010 in Poplars. Earlier Stott (1984) reported better productivity and higher adaptability of *Salix alba* × *Salix alba* hybrids as compared to hybrids between species (*S. alba* × *S. fragilis*)

In conclusion, all the studied parameters of reproductive, crossability, pod, germination, seedling growth, nutritional, and physiological showed a significant amount of variation present in the seed sources. First and last germination traits were found to be highly influenced with the environment and others are highly heritable traits for which we can select the hybrids for further evaluation. Significant correlations were recorded for the traits studies. The majority of nursery and seedling traits had a positive significant correlation, and nutritional and physiological traits had a negative significant correlation. However, some have a significant negative correlation with total carotenoids. Two out of 22 components showed eigenvalues greater than unity and elucidated a significant amount of variation, i.e., ~100 % of the total variation existed among the progenies. Only two hybrid progenies, i.e., H_1 and H_2, had the maximum heterosis for the majority of traits. Hybrid H_1 exhibited maximum magnitude of heterosis for seedling height, leaf width, and chlorophyll content, carbohydrates, crude protein and crude fibre. Hybrid H_2 exhibited maximum magnitude of heterosis for seedling height, collar diameter, chlorophyll content, crude protein, crude fibre and crude fat.

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