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Estimation of Heterosis in Hybrid Seedlings of Drumstick (*Moringa oleifera*)

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ABSTRACT: Two hybrid seedlings of drumstick (*Moringa oleifera*) derived from two crosses between Jaffna and PKM1 (MF_1H1) and IC632344 and PKM1 (MF_1H2) were evaluated for juvenile growth and nutrient content to study the heterosis (hybrid vigor) at College of Agriculture, Kerala agricultural University, Trissur during 2022-2023. Positive heterosis was evident in both the hybrids for seedling height, root collar girth, number of live leaves, stem volume index, dry matter of leaf and beta-carotene. Also they marked superiority over parents and check variety in case of short intermodal length. Specifically noted the positive heterosis of MF_1H1 for Seedling vigour index as well as iron content and MF_1H2 for protein content. Regarding the seed germination and vit. C content, both hybrids noted for inferior to the parents and check variety. The heterotic advantages emphasize the potential of harnessing hybrid vigor through hybridisation for the genetic improvement of drumstick.

Keywords: *Moringa oleifera*, hybrid, heterosis, germination, growth, biochemical.

INTRODUCTION

Moringa oleifera, commonly known as drumstick or horseradish tree, is a multi-purpose plant that is regarded as one of the most important and beneficial trees. It originated in African countries and typically thrives in semi-dry, desert, or tropical soils. However, it has been successfully cultivated all around the world. The plant is highly valued for its medicinal and nutritional properties and its leaves of contain an impressive array of nutrients (Patil *et al.*, 2022; Shivangini *et al*., 2022). The fresh leaves and dried leaf powder are abundant in vitamin A (beta carotene), calcium, iron, vitamin C, protein, potassium etc. (Fuglie, 1999; Prabhakar and Hebbar 2008; Saini *et al.*, 2013).

The development of hybrids in moringa is of utmost importance in the realm of plant breeding and crop improvement to fully explore and harness its potential. The importance of hybrids in *Moringa oleifera* lies in their ability to optimize productivity, increase disease resistance, enhance nutritional value, improve adaptability, and promote overall crop quality. By harnessing the advantages of hybridization, growers can achieve higher yields, resilient crops, and a more sustainable and profitable moringa cultivation industry.

In spite of the numerous on-going investigations in drumstick research, a substantial void persists in fully exploiting the complete potential of the plant through hybridization and targeted crop improvement strategies. Limited efforts, such as the development of the annual moringa hybrid derivative PKM 2 at Horticultural College and Research Institute, Tamil Nadu Agricultural University, have been reported to harness the heterosis of moringa (Ponnuswami, 2012).

Admitting this conspicuous research gap, the present study was undertaken to create new drumstick hybrids and a comprehensive assessment of their performance in comparison to their parent plants at College of Agriculture, Velanikkara, Kerala Agricultural University. This pioneering research seeks to bridge the prevailing knowledge gap and establish new insights within this field.

MATERIALS AND METHODS

A. Plant material and its Morphological characterization

In this study, a total of five plant materials were used, including two F_1Moringa hybrids and their respective parents. The first cross involved using the Jaffna type as the female parent and the PKM 1 variety as the male parent. For the second cross, Acc. 2 was used as the female parent, while PKM1 served as the male parent to obtain the F_1 population. The pollination procedure outlined in Mathew (2002) was employed for the hybridization of *Moringa oleifera* in the study. Pollination was performed between 7-9 am on the first day of flower opening. The hermaphrodite flower bud was emasculated on the previous day of flower opening. The freshly dehisced pollen grains were collected on the first day of anthesis and brushed onto the stigma of the emasculated flower on the second day of anthesis when the stigma had reached its most receptive stage. The pollinated inflorescences were bagged with butter paper for the next three days. Mature dried pods were harvested, and the seeds were sown within one week. Throughout the study, the seedlings were watered and fertilized uniformly as needed. During the study, data on germination, growth, and biochemical parameters were collected from all the seedlings.

B. Germination parameters

Seed germination percentage (%): Seedling emergence was recorded daily when the first leaf appeared and the percentage of seedling emergence was determined using the formula, Germination % = (No. of seeds germinated / No. of seeds sown) × 100.

Seedling Vigour Index: Seedling vigour index is calculated by multiplying germination (%) and seedling length on 5th day of germination (Kumar *et al*., 2012).

C. Growth parameters

The growth parameters of parents and hybrids *viz*., seedling height, root collar girth, fresh and dry weight of leaf, and the number of live leaves were recorded during 120-130 days of seedlings. Mean internode length was determined by dividing stem height by the number of internodes. Pant volume index was calculated for each seedling by multiplying stem height by the square of the root collar girth and dividing by 100 (Li *et al*., 1998).

D. Biochemical parameters

The biochemical parameters like protein, Iron, Calcium, Vitamin C (Sadasivam and Manickam 1996) and β carotene (Srivastava and Kumar 2014) of dried leaf powder of seedlings of parents and hybrids were estimated according to the suggested procedure.

E. Data analysis

The data of biochemical components and dry leaf recovery were recorded in completely randomised design (CRD) five replications and computed the analysis of variance (ANOVA) according to Scheffe (1999). After statistical analysis, average heterosis (Tang and Xiao 2013), heterobeltiosis (Fehr, 1987) and economic heterosis (Meredith and Bridge 1972) were computed for F_1 hybrids with respect to the mean value of each characters using the given formula:

(a) Average heterosis = (F_1- Mid parent) × 100 ÷ Mid parent

It describes the superiority of the F_1 compared to the average value of both the parents.

(b) Heterobeltiosis = (F_1– Better parent) × 100÷ Better parent

It is the superiority of F_1 over the better parent.

(c) Economic heterosis or useful heterosis = (F_1 – Check variety) × 100 ÷ Check variety

It describes the superiority of the F_1 compared to the high yielding commercial variety or check variety in a particular crop.

RESULT AND DISCUSSION

A. Morphological and biochemical characterization

Two successful crosses were made between Jaffna and PKM1 and IC632344 and PKM1. Mature seeds were harvested from the crosses and their respective selfed parents. The average values of growth and biochemical parameters of the moringa hybrids and their parents are shown in Tables 1 and 2. Analysis of variance revealed significant differences among both the hybrids and their parents for dry matter of leaf, iron, calcium, protein and vit. C content. PKM1 exhibited the highest seed germination rate as 92%, while both hybrids showed lower rates compared to their respective parents. However, the hybrid seedling of Jaffna × PKM1 displayed the maximum vigor index. After 120 days old, the height and girth of both hybrid seedlings were recorded as superior to those of their parents. The highest dry matter percentage of drumstick leaf was observed in MF_1H1 (30.13%). Although the beta carotene content did not significantly differ between the hybrids and parents, both hybrids exhibited higher content compared to their parents. The maximum iron content was found in MF_1H1 (22 mg/100g), followed by PKM1 (21.1 mg/100g). Jaffna had the highest calcium (702.75 mg/100g) and protein content (28.8 g/100g). PKM1 had the highest vitamin C content (119.68 mg/100g), followed by MF_1H1 (117.31 mg/100g).

B. Estimation of heterosis

Heterosis, also referred to as hybrid vigor, quantifies the extent to which F1 hybrids outperform the average of their parent plants or the superior parent, often represented by a standard commercial variety. The assessment of heterosis plays a pivotal role in hybrid development as it validates the potential of the hybrid. For the success of a hybrid as commercially viable, it must distinctly exhibit substantial heterosis. Therefore the superiority of drumstick hybrids were estimated through various approaches, including comparisons with the mid-parent (relative heterosis), the superior parent (heterobeltiosis), and a standard variety like PKM1 (standard or economic heterosis), and detailed in Table 3.

Relative heterosis measures the deviation of the F1 generation from the mid-parental value; however, it has

limitations due to its reliance on the mid-parental benchmark (Grakh and Choudhary 1985). On the other hand, heterobeltiosis gauges hybrid vigor in relation to the superior parent. Recognizing the significance of calculating standard heterosis for effective hybrid utilization, experts such as Bobby and Natarajan (1994) have emphasized its importance

C. Germination parameters

In both the hybrids the seed germination was down regulated compared to the mid parent, better parent and also the check variety PKM1, in which MF_1H2 (IC632344 × PKM1) showed high negative heterosis (-11.49% to -6.30%). Chen (1994); Jiang *et al*. (1996); Zhang *et al.* (1998); Lu (2000) also reported lower germination rates for hybrid rice seeds than the conventional. Reduced hybrid seed germination can stem from genetic incompatibility between parent lines, improper embryo development, dormancy traits inherited from parents (Nunez-Gastelum *et al.*, 2023). However, the seedling vigour index was superior to mid parent as well as better parent for MF_1H1 (Jaffna ×PKM1) and it ranged from 1.82% to 3.72%. MF_1H2 showed positive heterosis over the mid parent, the seedling showed less vigour compared to the better parent. This explains that, the expression of heterosis in plant vigour relies heavily on the specific genotypes employed in the crosses. As reported by Reed *et al*. (2022), the contradictory situation of MF_1H1 hybrid to have reduced seed germination and increased seedling vigour index can occur due to the complex interactions between genetic traits, environmental conditions, and developmental processes in hybrid plants.

D. Growth parameters

Both the hybrids exhibited positive value for all the three heterosis aspects with respect to the seedling height, root collar girth and number of live leaves, which indicates the superioty of the hybrid seedlings over the mid parent, better parent and standard check variety. MF_1H2 showed maximum standard heterosis of 16.92% for seedling height. MF_1H1 and MF_1H2 showed high standard heterosis as 8.33% in case of root collar girth. Increased seedling height, root collar girth, and number of live leaves in hybrid seedlings signify robust growth potential. They will lead to better light capture, efficient resource uptake, stress resilience, and enhanced photosynthesis. These traits collectively drive higher crop yields, contributing to improved agricultural productivity and stability (Novikova *et al*., 2023).

Negative heterotic values for intermodal length was noted for both the hybrids. This suggest the advantage of the hybrids over the mid parent, better parent and standard variety in producing dwarf plants and it can be considered as positive heterosis. The heterosis value of MF_1H1 ranged from -12.37% to -12.88% and MF_1H2 from -4.04% to -19.49%. Reduced internodal length and a higher number of leaves in plants indicate increased foliage, compact growth and can often lead to better structural stability. The drumstick tree benefits from shorter internodes, ensuring sturdier growth that can counteracts wind breakage, a common vulnerability. However, the broader implications of these traits require consideration based on specific cultivation goals. Growth, measured as seedling volume index, recorded positive heterosis for MF_1H1 ranging from 23.09% to 43.74% and MF_1H2 as 31% to 83.45%. A larger seedling volume index indicates accelerated early growth, allowing the plant to establish itself faster and allocate more resources towards yield development (Li *et al*., 1998).

Both the drumstick hybrids showed positive mid-parent heterosis, heterobeltiosis and economic heterosis for leaf dry matter of leaf. In contrast, MF_1H2 exhibits a lower standard heterosis at 2.72%. This indicates a less pronounced improvement over PKM1 variety. However, despite its lower standard heterosis, MF_1H2 still demonstrates an advantage over the mid-parent (average of the parents) by 3.07%. This suggests that while the improvement isn't as substantial as in the case of MF_1H1, MF_1H2 still performs better than expected based on the average of its parents' performance.

E. Biochemical parameters

MF_1H2 posess high negative heterosis with respect to iron content (-16.82%). MF_1H2 was not as vigorous as the mid parent or better parent as indicated by the negative values (–11.49% to –16.30%). Although both the hybrids showed negative relative heterosis (-1.77 to -1.92 in MF_1H1 and -10.36 to -29.9 in MF_1H2) and heterobeltiosis (-32.5%) for calcium content, they showed positive value for economic heterosis, since the check variety PKM1 exhibited a lower amount of calcium. High negative heterobeltiosis was shown by MF_1H1 for calcium content However the economic heterosis for calcium content is highest positive (39.1%) compared with the check variety PKM1.

Both hybrids, showed an increase in beta carotene content compared to the mid-parent, better parent, and standard variety. MF_1H1 exhibited a positive heterosis ranging from 4.43% to 10.60%, and MF_1H2 displaying an increase ranging from 1.88% to 8.03%.MF_1H2 demonstrated a notable positive effect in terms of protein content. Specifically, it exhibited a significant increase of 16.4% over the mid-parent value. While there were positive values observed for heterobeltiosis and economic heterosis, the actual increase in protein content for MF_1H2 was relatively modest, measuring at only 0.86%. In contrast, MF_1H1 exhibited negative heterosis across all measured aspects, resulting in reductions in protein content. The extent of these reductions ranged from -13.72% to -23.61%. This indicating that this hybrid did not perform as well as the parent plants in terms of protein production.

In terms of vitamin C content, both hybrids showed negative heterosis when compared to the mid-parent, better parent, and standard variety. This suggests that the hybridization process led to a reduction in vitamin C content, which could be less desirable. The degree of negative heterosis was more pronounced in MF_1H2, ranging from -10.36% to -29.9%. This indicates a significant decrease in vitamin C content in MF_1H2 compared to both its parent plants and the standard variety. MF_1H1 also exhibited negative heterosis,

though to a lesser extent, with a range of -1.77% to -1.92%. The reduction in vitamin C content for MF_1H1 was relatively less severe than that observed in MF_1H2.

Table 1: Germination and growth parameters of seedlings of *Moringa oleifera* hybrids and parents.

Character	Seed germination (%)	Seedling vigour index	Height of seedling (cm)	Root collar girth (cm)	Number of live leaves	Inter-nodal length	Seedling stem volume index
PKM1	92	822	65	6	11	5.9	23.4
Jaffna	89	792	70	5.2	12	5.83	18.93
IC632344	82	690	52	4.7	13	4	11.49
MF_1H1 (Jaffna × PKM1)	87	837	72	6.5	14	5.14	30.42
MF_1H2 (IC632344 × PKM1)	77	707	76	6.5	16	4.75	32

Table 2: Biochemical parameters of seedlings of *Moringa oleifera* hybrids and parents.

Characteristics	Dry matter of leaf (%)	Iron (mg/100g)	Calcium (mg/100g)	Beta carotene (mg/100 g)	Protein (g/100 g)	Vit C (mg/100g)
PKM1	25.47^{b}	21.10^{ab}	341^{e}	15.55^{ab}	25.50^{ab}	119.62^{a}
Jaffna	27.63^{ab}	12.12^{d}	702.75^{a}	16.47^{bc}	28.80^{a}	117.28^{a}
IC632344	25.33^{b}	15.72^{cd}	417.00^{c}	16.49^{c}	18.69^{c}	67.47^{c}
MF_1H1 (Jaffna × PKM1)	30.13^{a}	22.00^{a}	474.33^{b}	17.20^{a}	22.00^{bc}	117.31^{a}
MF_1H2 (IC632344 × PKM1)	26.18^{b}	17.55^{bc}	388.50^{d}	16.80^{a}	25.72^{ab}	83.85^{b}
Coefficient of Variation (CV)	5.152	16.337	3.872	-	15.19	5.59
CD(0.01)	3.60	6.029	37.499	-	-	14.59
CD(0.05)	2.52	4.359	27.116	-	5.550	10.26

Table 3: Estimation of heterosis for drumstick hybrids.

Characteristics	MF_1H1 (Jaffna × PKM1)			MF_1H2 (IC632344 × PKM1)		
	Relative heterosis	Hetero-beltiosis	Economic heterosis	Relative heterosis	Hetero-beltiosis	Economic heterosis
Seed germination	-3.8	-5.40	-5.40	-11.49	-16.30	-16.30
Seedling vigour index	3.72	1.82	1.82	6.48	-13.99	-13.99
Height of seedling	6.67	2.85	10.76	29.91	16.92	16.92
Root collar girth	16.07	8.33	8.33	21.49	8.33	8.33
Number of live leaves	21.74	16.67	27.27	33.33	45	23
Inter-nodal length	-12.37	-12.88	-12.88	-4.04	-19.49	-19.49
Seedling stem volume index	43.74	23.09	23.09	83.45	31	31
Dry matter of leaf	13.48	9.01	18.31	3.07	2.72	2.72
Iron	32.45	4.20	4.20	-4.67	-16.82	-16.82
Calcium	-9.11	-32.50	39.10	25.15	-6.83	13.92
Beta carotene	7.43	4.43	10.60	4.86	1.88	8.03
Protein	-18.9	-23.61	-13.72	16.4	0.86	0.86
Vit C	-0.95	-1.93	-1.93	-9.69	-29.90	-29.90

CONCLUSIONS

The evaluation of two hybrid drumstick seedlings, resulting from crosses between Jaffna and PKM1 (MF_1H1) and IC632344 and PKM1 (MF_1H2) revealed significant insights into the phenomenon of heterosis. In the analysis of initial growth and biochemical parameters, the hybrids expressed superiority over parents for majority of the characters like seedling height, root collar girth, live leaf count, stem volume index, dry leaf recovery, and beta-carotene content. Specifically, the hybrid MF_1H1 displayed noteworthy heterosis in terms of Seedling Vigor Index and iron content, while MF_1H2 exhibited such enhancement in protein content. However, with respect to seed germination and vitamin C content, both hybrids displayed inferior performance compared to their parent plants and the check variety. These findings collectively emphasize the potential of harnessing hybrid vigor through heterosis breeding for the genetic improvement

of drumstick varieties, as well as the necessity for further research to fully comprehend the underlying mechanisms driving these observed variations.

FUTURE SCOPE

The positive heterosis observed in this work provides a promising foundation for further exploration and application in agricultural and nutritional contexts. These results could spearhead the genetic improvement of drumstick varieties through selective breeding, potentially leading to commercially cultivated strains with enhanced growth traits and increased nutritional content. Molecular analyses could unveil the genetic underpinnings of these traits. The implications like micropropagation of the hybrids for mass multiplication and optimization of crop management practices might drive holistic approaches for tackling agricultural and nutritional challenges on a broader scale.

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Conflict of Interest. None.

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