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Research Note

Genetic variability studies in selected genotypes of khirni (*Manilkara hexandra)*

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Abstract
Khirni is one of the minor fruit crop of tropics. It is a well–known for medicinal and commercial importance. Totally, twenty six khirni genotypes were evaluated for yield, yield contributing characters and bio-chemical features during 2022-2023 at Department of Fruit Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, to estimate the extent of genotypic and phenotypic variability among them. The analysis of variance revealed highly significant differences among the genotypes for all studied traits. The magnitude of phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) for all the character. High PCV and GCV (>20%) were recorded for average yield per tree, number of flowers per fascicle, seed weight, number of leaves per seedling and seedling vigour. Low expression of phenotypic coefficient of variation (<10%) were observed for acidity followed by total sugars, vitamin C, reducing sugars and non reducing sugars. High heritability (>90%) estimates were obtained for average yield per tree, vitamin C, seedling vigour, length of seedling, total sugars, reducing sugars, germination percent, seed weight, non reducing sugars and fruit weight. The highest genetic advance was also observed for the average yield per tree, seed weight and seedling vigour. Based on yield and yield-contributing traits, as well as germination studies, the genotypes MGK-31 and MGK-60were identified as promising for future khirni breeding improvement programs.

Keywords: Khirni, *Manilkara hexandra*, genotypes, variability, yield

Khirni, scientifically known as *Manilkara hexandra* (Roxb.) Dubard, is a prominent tree species valued for its medicinal properties and commercial importance. It is widely used as an herbal remedy and serves as a source of livelihood for local tribal communities (Mishra and Pareek, 2014). This tree is cultivated across India for its fruit and as an ornamental tree for avenues. The ripe fruit is highly nutritious, comprising 68.61 g of water, 0.48 g of protein, 2.42 g of fat, 27.74 g of carbohydrates, 0.75% minerals, 83 mg of calcium, 17 mg of phosphorus, 0.92 mg of iron, 675 IU of carotene, 0.07 mg of thiamine, 0.077 mg of riboflavin, and 0.66 mg of niacin. The seeds contain 24.6% edible oil, which consists of 18.9% palmitic acid, 14.1% stearic acid, 63.2% oleic acid, and 2.7% linoleic acid (Shukla and Kumar, 2009). Khirni oil also has medicinal value. Khirni fruit and bark are used for numerous medicinal purposes, such as curing fever, flatulence, stomach disorder, leprosy, ulcers, opacity of the cornea, dyspepsia, urethrorrhea and bronchitis (Raju and Reddy 2005, Chanda and Parekh, 2020). In addition to its various medicinal and social applications, it is extensively utilized as a rootstock for sapota propagation.

In view of the increasing demand for khirni, there is a need for identification of promising genotypes by exploiting the variability. The existing khirni trees offer a wide range of variability for the selection of outstanding types. Genetic diversity pertaining to various features influences the breeding approach for genetic enhancement of fruit production. Before selecting and improving the base

population, it is necessary to evaluate the genetic variability parameters. Recently, an improved variety, Thar Ruturaj, with better fruit and yield characters has been developed by the Central Horticultural Experiment Station, Godhra, Gujarat (Singh *et al.,* 2015). Since research work on variability studies is limited to khirni, the present study was conducted to find out the variability in flowering, fruiting and fruit quality, as well as seed charactersamong selected genotypes of khirni so that, a suitable variety can be developed with better fruit yield and quality and better seedling vigour for exploitation as root stock for sapota.

The present study was conducted on a 37-year-old germplasm of Khirni, maintained at the experimental farm of the Department of Fruit Science, Dr. PDKV, Akola, during 2022-2023. Akola is located at an altitude of 307-457 meters above sea level, at a latitude of 20.420° N and a longitude of 72.020° E, characterized by a marginal tropical climate. The experiment involved 26 genotypes, laid out in Randomized Block Design with four replications. One plant from each selected genotype was marked as biological replication and monitored, and ten shoots in each direction were tagged for various observations. Ten fruits were randomly harvested from each replication for recording data. The fruit analysis was performed in the analytical laboratory of the Department of Fruit Science, Dr. PDKV, Akola.

For the germination studies, seeds were collected from each genotype and treated with 200 PPM GA3 for 24 hours. They were then sown in polybags filled with a media mixture of soil, silt, and farmyard manure (FYM) in a 2:1:1 ratio to record the germination percentage of the various genotypes. The mean of the observations were subjected to analysis of variance (Panse and Sukhatme, 1985). The genotypic and phenotypic variance were calculated as per the formulae of Burton and Devane, 1953. Heritability percentage in broad sense was calculated as per the formula proposed by Johnson *et al*. (1995).

Analysis of variance: An analysis of variance (ANOVA) was performed on various traits to assess differences among the khirni genotypes. The results, summarized in **Table 1**, indicate that the mean sum of squares for traits such as the number of flowers and fruits per fascicle, fruit length, weight, and diameter, seed weight, pulp-to-seed ratio, average yield per tree, total soluble solids (TSS), acidity, vitamin C content, reducing and non-reducing sugars, total sugars, germination percentage, days to germination, number of leaves, seedling length, and seedling vigor were all significant at both the 1% and 5% levels. This indicates the substantial genetic variability among the khirni genotypes studied. Machakanoor and Raut (2018) also reported that the number of flowers per fascicle, number of fruits per fascicle, leaf area (cm^2), fruit diameter (mm), fruit length (cm), fruit weight (g),

Table 1. Analysis of variance of means for different characters

S. No.	Character	Mean sum of square		
		Replication	Treatment	Error
	Degrees of freedom	3	25	75
1.	Number of Flower per fascicle	0.282	4.967**	0.629
2.	Number of fruit per fascicle	0.487	1.005**	0.347
3.	Fruit Weight (gm)	0.0123	0.803**	0.021
4.	Fruit Length (cm)	0.008	0.224**	0.007
5.	Fruit Diameter (mm)	0.412	7.07**	0.296
6.	Seed Weight (gm)	0.0003	0.014**	0.0002
7.	Pulp:Seed Ratio (%)	0.019	31.108**	2.087
8.	Average Yield/Tree (kg)	0.053	72.322**	0.041
9.	Fruit TSS (^{0}Brix)	1.964	25.953**	1.499
10.	Acidity %	0.0003	0.002**	0.0002
11.	Vitamin C (mg/100gm)	0.019	17.766**	0.027
12.	Total Sugar (%)	0.060	5.117**	0.018
13.	Reducing Sugar (%)	0.011	1.726**	0.007
14.	Non-Reducing Sugar (%)	0.109	1.969**	0.0047
15.	Germination percentage (%)	0.987	220.22**	1.534
16.	Days required for germination	6.949	40.442**	1.595
17.	Number of leaves	1.779	8.49**	0.952
18.	Length of seedling	0.018	5.815**	0.017
19.	Seedling vigour	116.44	78,047.8**	206.754

*-significant at 5% level **-significant at 5% and 1%level

seed weight (g), pulp: seed ratio, fruit yield per tree (kg), TSS (^{0}Brix), vitamin C (mg/100g), germination (%) and seedling vigour were highly significant in khirni. Suhasini *et al.* (2012) also reported that there was significant variation with respect to the number of flowers per shoot, fruit diameter, fruit length, fruit weight, TSS and Vitamin C in sapota.

Mean performance: The data presented in **Table 2** reveals that the number of flowers per fascicle across all genotypes ranged from 2.00 to 5.75. Genotype MGK-31 recorded the highest number of flowers per fascicle (5.75), followed by MGK-60 (5.50), while MGK-52 had the lowest (2.00). These findings are consistent with those reported by Anjali *et al.* (2018).For the number of fruits per fascicle, genotype MGK-31 achieved the highest value (3.25), closely followed by MGK-60 (3.00), whereas CRSK-1 had the lowest (1.25). The maximum fruit weight was observed in genotype MGK-63 (3.33 g), while CRSK-1 recorded the minimum (1.25 g), corroborating the results of Mahalle *et al.* (2023).Genotype MGK-59 exhibited the greatest fruit length (3.15 cm), while MGK-60 recorded the smallest (2.00 cm). The maximum fruit diameter (15.50 mm) was noted in MGK-63, while MGK-26 had the minimum (10.50 mm). Among the genotypes, CRSK-8 displayed the highest pulp-to-seed ratio (16.70%), whereas MGK-22 showed the lowest (8.27%).The average fruit yield per tree ranged from 5.55 to 22.50 kg, with genotype MGK-31 achieving the highest yield (22.50 kg/tree), followed by MGK-60 (20.60 kg/tree), while CRSK-1 had the lowest (5.50 kg). Additionally, MGK-63 reported the highest seed weight (0.35 g), whereas CRSK-1 and CRSK-8 recorded the lowest (0.12 g), similar to the findings of Singh *et al.* (2017).

Fruit Biochemical Parameters: Significant variation in TSS (Total Soluble Solids) was observed among the genotypes (**Table 2**). Genotype MGK-31 recorded the highest TSS (30.13 °Brix), while CSRK-7 had the lowest (21.75 °Brix). In terms of acidity, genotype CSRK-7 exhibited the highest value (0.38%), whereas MGK-31 (0.31%) recorded the lowest, consistent with the findings of Singh *et al.* (2017). Genotype MGK-31 also demonstrated the highest vitamin C content (28.84 mg/100g), while AHDSK-1 had the lowest (21.62 mg/100g), further aligning with the results reported by Singh *et al.* (2017). Additionally, MGK-31 showed the highest total sugars (17.65%), with AHDSK-1 recording the lowest (13.54%).For reducing sugars, MGK-31 again had the highest content (8.80%), while CRSK-8 recorded the lowest (6.17%), consistent with the findings of Lata *et al.* (2019). Non-reducing sugars across the khirni genotypes ranged from 6.36% to 8.85%, with MGK-31 having the highest (8.85%) and CRSK-2 the lowest (6.36%).

Germination study of khirni genotypes: The germination percentage of seeds collected from the various genotypes under study ranged from 61.75% to 87.50%. Genotype MGK-60 exhibited the highest germination percentage (87.50%), followed by MGK-30 (85.00%), while CRSK-7 recorded the lowest (61.75%). These results are consistent with the findings of Malay *et al.* (2015) and Rai *et al.* (2018).Genotype MGK-60 demonstrated the fastest germination, occurring within 14.50 days after sowing, whereas MGK-63 germinated the slowest, taking 26.75 days. The maximum seedling length (9.35 cm) was observed in MGK-60, while CRSK-7 recorded the shortest (5.16 cm). MGK-31 produced the highest number of leaves per seedling (7.75), followed by MGK-

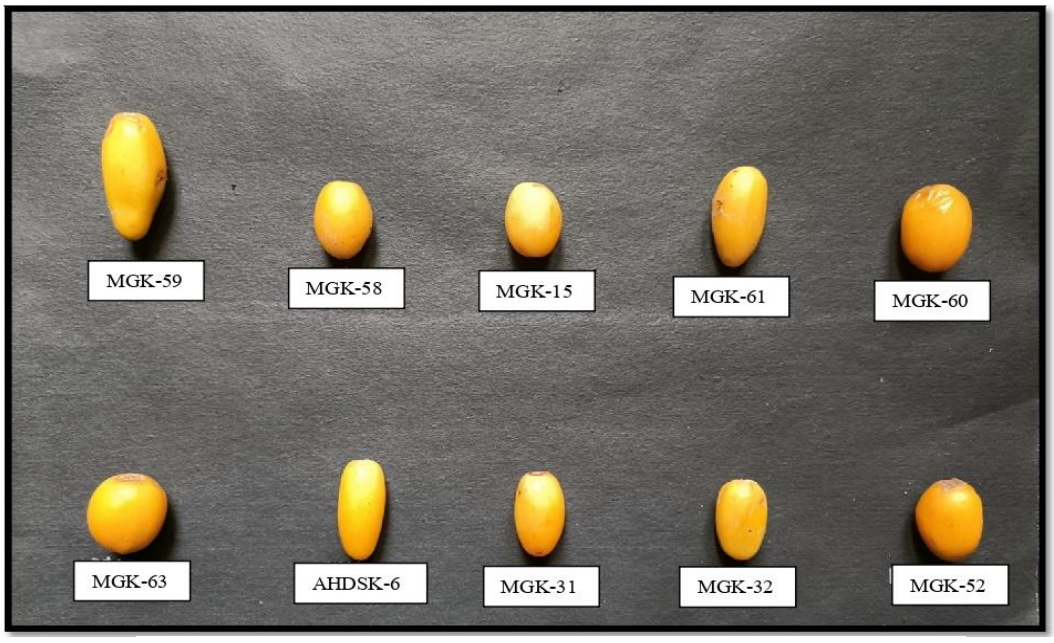

Fig 1. Variability in Fruits of khirni genotypes

Table 2. Mean performance of khirni genotypes based on fruit characters

Genotype	Number of flowers per fascicle	Number of fruits per fascicle	Fruit weight (g)	Fruit length (cm)	Fruit diameter (mm)	Pulp: seed ratio (%)	Avg yield/ tree (kg)	Seed weight (g)	Fruit TSS°Brix	Acidity (%)
MGK-14	3.25	1.75	2.48	2.45	13.13	13.38	12.40	0.17	24.75	0.36
MGK-15	3.75	2.00	2.29	2.10	13.75	8.55	14.51	0.24	26.50	0.34
MGK-16	3.25	2.00	1.61	2.18	11.13	8.95	13.43	0.16	28.00	0.33
MGK-22	2.50	1.50	2.06	2.05	11.38	8.27	11.35	0.22	24.00	0.36
MGK-26	2.75	1.50	2.20	2.18	10.50	15.35	9.43	0.14	26.00	0.34
MGK-31	**5.75**	**3.25**	2.33	2.33	12.50	13.69	**22.50**	0.16	**30.13**	**0.31**
MGK-32	4.00	2.25	2.03	2.20	12.00	10.92	16.90	0.18	29.75	0.32
MGK-52	2.00	1.50	2.82	2.10	15.00	11.22	8.35	0.23	29.75	0.32
MGK-58	5.00	2.75	2.46	2.13	13.50	9.49	17.20	0.24	30.00	0.31
MGk-59	4.50	2.25	3.24	**3.15**	13.63	10.23	14.53	0.29	26.75	0.34
MGK-60	5.50	3.00	3.00	2.00	15.25	10.26	20.60	0.27	25.25	0.35
MGK-61	4.25	2.75	2.36	2.64	12.63	10.66	17.53	0.20	27.75	0.32
MGK-63	3.50	1.75	**3.33**	2.23	**15.50**	8.68	12.50	**0.35**	26.25	0.34
CRSK-1	5.00	1.25	2.00	2.35	11.25	15.76	5.55	0.12	23.25	0.37
CRSK-2	2.50	1.75	2.23	2.28	12.38	15.87	12.35	0.13	24.00	0.36
CRSK-3	5.25	2.25	2.38	2.08	11.75	16.02	16.84	0.14	29.63	0.32
CRSK-4	3.00	2.00	2.10	2.40	11.75	15.86	11.40	0.13	25.50	0.36
CRSK-6	2.50	2.00	2.35	2.25	11.75	15.56	10.48	0.14	22.50	0.37
CRSK-7	2.25	2.50	2.13	2.18	11.13	14.58	6.56	0.14	21.75	0.38
CRSK-8	4.75	2.00	2.19	2.28	12.13	**16.70**	7.58	0.12	23.25	0.36
AHDSK-1	3.00	1.75	1.90	2.33	11.13	10.58	10.45	0.17	24.73	0.35
AHDSK-2	5.25	2.75	3.23	2.53	12.00	11.89	18.75	0.23	28.25	0.33
AHDSK-3	3.00	2.50	3.03	2.20	13.50	11.91	16.26	0.25	24.75	0.36
AHDSK-4	3.50	1.75	2.21	2.23	11.50	9.17	10.58	0.21	23.50	0.37
AHDSK-5	3.25	2.00	1.94	2.43	12.25	11.49	11.58	0.17	25.75	0.35
AHDSK-6	2.75	2.25	2.37	2.55	12.00	14.43	12.70	0.15	29.25	0.32
Range	**2.00-5.75**	**1.25-3.25**	**1.61-3.33**	**2.00-3.15**	**10.50-15.50**	**8.27-16.70**	**5.55-22.50**	**0.12-0.35**	**21.75-30.13**	**0.31-0.38**
Mean	**3.69**	**2.12**	**2.00**	**2.30**	**12.48**	**12.29**	**13.16**	**0.19**	**26.19**	**0.34**
SE(m)	**0.40**	**0.23**	**0.07**	**0.04**	**0.27**	**0.72**	**0.10**	**0.01**	**0.61**	**0.007**
C.D. (5%)	**1.11**	**0.83**	**0.20**	**0.12**	**0.77**	**2.04**	**0.29**	**0.02**	**1.73**	**0.019**

60 (7.25), whereas CRSK-1 had the lowest (2.75). Seedling vigor, measured 120 days after sowing, varied from 318.90 to 818.13 across the 26 genotypes. MGK-60 displayed the highest seedling vigor (818.13), followed by MGK-31 (777.85), while CRSK-7 recorded the lowest (318.90). These findings align with the observations of Machakanoor and Raut (2018) in khirni.

Genotypic and phenotypic coefficient of variation: It is crucial to evaluate the genetic components of variation within the total variation before utilizing it for genetic improvement. The additive genetic variance represents the inheritable portion of this total variation. In the present study, the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all traits (**Table 4**), suggesting that environmental factors significantly influenced the expression of all traits. These findings align with the observations of Rai *et al.* (1996), Malik *et al.* (2013) and Machakanoor and Raut (2018) in khirni, Rajasekhar (2009) in Sapota, Gupta and Kour (2019) in Barik *et al.* (2021) in Brinjal.

The highest PCV and GCV values were observed for average yield per tree (32.33 and 32.23), seed weight (31.49 and 30.46), number of flowers per fascicle (35.45 and 28.20), number of leaves per seedling (33.62 and

Table 3. Mean performance of khirni genotypes based on fruit biochemical parameters and germination study

Genotype	Vitamin C (mg/100gm)	Total sugars (%)	Reducing sugars (%)	Non reducing sugars (%)	Germination percent (%)	Days required for germination	Length of seedling (cm)	Number of leaves per seedling	Seedling vigour
MGK-14	23.18	15.20	7.15	8.05	81.25	20.25	8.55	6.25	694.60
MGK-15	25.34	15.33	7.69	7.64	77.75	21.25	8.10	5.75	629.85
MGK-16	24.28	15.08	6.62	8.46	75.50	21.75	7.80	5.50	588.93
MGK-22	23.35	14.15	6.48	7.68	69.50	23.25	6.58	4.75	456.88
MGK-26	23.13	13.75	7.20	6.55	72.00	24.25	7.20	4.25	518.45
MGK-31	28.84	17.65	8.80	8.85	85.00	16.50	9.15	7.75	777.85
MGK-32	27.31	16.29	7.88	8.42	83.25	18.50	8.30	6.50	691.00
MGK-52	23.35	14.78	7.51	7.27	63.00	22.50	7.28	3.00	458.28
MGK-58	26.31	15.58	7.24	8.34	78.75	17.75	8.18	5.75	643.85
MGk-59	25.32	15.28	6.96	8.32	77.00	21.25	7.70	3.50	592.95
MGK-60	28.51	16.91	8.40	8.51	87.50	14.50	9.35	7.25	818.13
MGK-61	27.60	16.28	7.85	8.43	84.25	18.25	8.93	6.75	751.95
MGK-63	26.30	15.65	7.70	7.95	70.50	26.75	7.05	4.75	497.15
CRSK-1	22.11	13.58	6.24	7.33	62.75	25.00	5.43	2.75	340.23
CRSK-2	25.20	14.22	7.86	6.36	72.00	24.50	7.38	3.50	530.95
CRSK-3	28.25	16.65	8.14	8.51	76.00	20.25	8.60	6.75	653.58
CRSK-4	24.20	14.43	6.94	7.48	72.75	24.00	6.43	3.75	467.48
CRSK-6	24.28	13.68	6.75	6.93	68.75	25.00	5.65	4.25	388.35
CRSK-7	23.20	14.28	7.08	7.20	61.75	25.25	5.16	3.25	318.90
CRSK-8	22.45	14.28	6.17	8.11	64.50	25.50	6.45	3.50	416.15
AHDSK-1	21.62	13.54	6.86	6.67	69.50	24.25	7.20	5.25	500.43
AHDSK-2	28.15	16.58	7.93	8.65	82.50	17.00	8.45	7.25	697.10
AHDSK-3	25.75	15.23	7.18	8.05	72.75	21.75	7.25	5.50	527.60
AHDSK-4	24.74	15.20	7.08	8.12	68.75	23.50	6.83	4.50	469.23
AHDSK-5	23.24	14.03	6.60	7.43	74.75	21.75	6.23	4.50	465.28
AHDSK-6	24.30	14.18	7.27	6.90	64.75	23.50	5.20	3.75	336.65
Range	**21.62-28.84**	**13.54-17.65**	**6.17-8.80**	**6.36-8.85**	**61.75-87.5**	**14.50-26.75**	**5.16-9.35**	**2.75-7.75**	**318.90-818.13**
Mean	**25.01**	**15.07**	**7.29**	**7.78**	**73.72**	**21.85**	**7.32**	**5.00**	**547.38**
SE(m)	**0.08**	**0.07**	**0.04**	**0.08**	**0.62**	**0.63**	**0.06**	**0.49**	**7.19**
C.D. (5%)	**0.23**	**0.19**	**0.12**	**0.24**	**1.74**	**1.78**	**0.18**	**1.38**	**20.31**

27.40), seedling vigour (25.62 and 25.49) and pulp: seed ratio (24.89 and 21.94), indicating significant variability and the potential for improvement through selection. These findings are consistent with the results of Machakanoor and Raut (2018) in khirni, as well as Arivazhagan (2019), Harshavardhan (2015), and Vikram (2020), who reported similar trends for seed weight, number of fruits per tree, and yield per tree in sapota.

Moderate estimates of PCV and GCV was observed for most of the characters, implying that selection for these characteristics should be approached with caution.

Heritability and Expected genetic advance (EGA): Genetic advance as percent over mean ranged from 10.39 % to 66.45 %. A high degree of genetic advance was found for the characters average yield per tree (66.45 %), seed weight (60.68 %), seedling vigour (52.22 %) and number of flowers per fascicle (46.22 %). Similar results demonstrating high expected genetic advance (EGA) for various traits have been reported in sapota. Rajasekhar (2009) observed high EGA for traits such as fruit weight, seed weight, pulp-to-seed ratio, total soluble solids (TSS), and ascorbic acid. Similarly, Harshavardhan (2015) reported high EGA for pulp-to-seed ratio, fruit weight, fruit yield per tree, and seed weight. These findings highlight the potential for genetic improvement in these traits through effective selection strategies.

The results presented in **Table 4** revealed high heritability for traits such as average yield per tree (99.77%), vitamin C (99.38%), seedling vigor (98.95%), total sugars

Table 4. Estimates of variability, heritability, genetic advance and expected genetic advances per cent of mean

S. No.	Character	Range	Mean	PCV (%)	GCV (%)	Heritability (%)	Expected Genetic advance as % over mean
1.	Number of flowers per fascicle	2.00-5.75	3.69	35.45	28.20	63.23	46.22
2.	Number of fruits per fascicle	1.25-3.25	2.12	33.81	19.17	32.13	22.38
3.	Fruit weight (gm)	1.61-3.33	2.00	19.43	18.47	90.42	36.19
4.	Fruit length (cm)	2.00-3.15	2.30	10.74	10.09	88.24	19.53
5.	Fruit diameter (mm)	10.5-15.50	12.48	11.31	10.43	85.12	19.83
6.	Seed weight (gm)	0.12-0.35	0.19	31.49	30.46	93.53	60.68
7.	Pulp:Seed ratio (%)	8.26-16.70	12.29	24.89	21.94	77.66	39.83
8.	Fruit TSS (^{0}Brix)	21.75-30.13	26.19	10.53	9.44	80.31	17.43
9.	Acidity (%)	0.31-0.38	0.34	7.19	6.04	70.05	10.39
10.	Vitamin C (mg/100g)	21.62-28.84	25.01	8.45	8.42	99.38	17.29
11.	Total sugars (%)	13.54-17.65	15.07	7.55	7.49	98.59	15.33
12.	Reducing sugars (%)	6.17-8.80	7.29	9.07	8.99	98.33	18.37
13.	Non-reducing sugars (%)	6.36-8.85	7.78	9.35	8.93	91.11	17.55
14.	Germination percent	61.75-87.50	73.72	10.16	10.02	97.27	20.37
15.	Days required for germination	14.5-26.75	21.85	15.39	14.27	85.89	27.23
16.	Number of leaves per seedling	2.75-7.75	5.01	33.62	27.40	66.43	46.01
17.	Length of seedling	5.16-9.35	7.32	16.54	16.44	98.81	33.67
18.	Seedling vigour	318.90 to 818.13	547.38	25.62	25.49	98.95	52.22
19.	Average yield/tree (kg)	5.50-22.50	13.12	32.33	32.23	99.77	66.45

(98.59%), non-reducing sugars (98.33%), seedling length (98.81%), germination percentage (97.27%), seed weight (93.53%), fruit weight (90.42%), fruit length (88.24%), days required for germination (85.89%), fruit diameter (85.12%), and fruit TSS (80.31%). These results for heritability corroborate the view of Machakanoor and Raut (2018) in khirni. High heritability, on the other hand, does not always imply high genetic gain, and heredity alone is insufficient to predict improvement by simple phenotypic selection. When a trait shows both high heritability and high genetic advance, it typically indicates that its inheritance is primarily controlled by additive gene effects. As a result, selecting for these traits in the current population would lead to more effective improvements.

In conclusion, the genotypes displayed a broad range of variation and heritability for phenotypic traits. The highest and lowest heritability, along with genetic advance for key traits, indicated the involvement of both additive and non-additive gene actions, which are critical in controlling these traits. This substantial variability offers opportunities to explore the material for further genetic improvement programs aimed at broadening the genetic background of various khirni genotypes. Based on their performance in terms of yield and yield-contributing characters and germination studies, genotypes MGK-31 and MGK-60 emerged as performing genotypes for improvement programs. Therefore, these genotypes could be considered for enhancing yield in khirni through targeted breeding efforts.

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