

SCREENING OF HORSE GRAM [*MACROTYLOMA UNIFLORUM* (LAM.) VERDC.] GENOTYPES FOR SEED YIELD AND QUALITY

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ABSTRACT

To assess genetic variability for seed yield and quality parameters, the present study was conducted on 95 horse gram genotypes. The experiment was aimed to evaluate the growth, seed yield, quality traits and disease resistance of horse gram genotypes. The analysis of variance revealed significant differences among genotypes for most traits, excluding 100-seed weight. Genotype IC-277630 demonstrated the highest (85.27cm) plant height, while IC-139554 recorded the maximum number (6.82) of primary branches per plant. Genotypes IC-26138 showed the highest (12.62g) seed yields, with a high genetic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and heritability for key traits. Disease screening identified genotypes immune or resistant to powdery mildew, leaf spot, and yellow vein mosaic disease. Growth habits varied, with 32 genotypes erect, 48 semi-erect, and 15 spreading. Seed quality assessments indicated variability in seed color, germination, vigour indices, and biochemical properties. Seed quality assessments showed IC-47461 with the highest seed germination (99.25%) and IC-55065 with the highest protein content (23.19%). Seedling vigour index-I and seedling vigour index-II was significantly higher in genotypes IC-15730 (3632) and IC-47461 (2829) respectively. Under controlled deterioration, genotypes IC-139333 and IC-47461 demonstrated higher seed vigour and reduced germination decline, indicating superior stress tolerance. Correlation analysis showed positive associations between seed germination and quality traits, with electrical conductivity negatively correlated with seed germination ($r = -0.550$). The study identified high-yielding, disease-resistant and quality-improving genotypes suitable for breeding programs aimed at enhancing horse gram productivity and adaptability.

KEYWORDS: Horse gram, genotypes, seed yield, seed quality

INTRODUCTION

Pulses play a crucial role in global agriculture due to their high nutritive value as a primary protein source and their contribution to sustainable and profitable farming practices. They are an affordable protein alternative to animal-based products, particularly in developing countries where large-scale animal protein production is economically challenging. Among pulses, horse gram (*Macrotyloma uniflorum* [Lam.] Verdc.) stands out for its exceptional nutritional quality and resilience to adverse environmental conditions. Native to the western ghats of India, horse gram is widely cultivated in Southeast Asia, Australia, and Africa, primarily for food and fodder. In India, it is predominantly grown in the southern states and the foothills of the Himalayas, covering an area of approximately 348 thousand hectares with a production of 226 thousand tons and a productivity of 650 kg/hectare (Anon., 2021).

Horse gram thrives in sub-humid to semi-arid climates, tolerating temperatures up to 40°C, and is well-suited to poor, marginal lands. Its ability to fix atmospheric nitrogen and improve soil fertility makes it a valuable crop for integrated fertility management and soil conservation. Recognized by the US National Academy of Sciences as a potential future food source (Shukla *et al.*, 2006), horse gram seeds are rich in protein (20-30%), carbohydrates (51.9-60.9%), and essential micronutrients like calcium (287 mg/100 g), making it an excellent dietary complement to cereals (Aditya *et al.*, 2019; Patangare *et al.*, 2019; Sharma *et al.*, 2019). Despite its benefits, horse gram cultivation has declined in recent decades due to social stigma, changing lifestyles, and lack of supportive policies. This underutilized crop faces challenges in price competitiveness, limiting its mainstream adoption. To harness its potential, it is essential to evaluate the available germplasm for improved seed yield and quality. This study aims to screen horse gram genotypes to achieve the following objectives: (1) assess growth and seed yield attributes, and (2) evaluate seed quality parameters. By addressing these objectives, this research seeks to promote the utilization and cultivation of horse gram, contributing to food security and sustainable agriculture.

MATERIALS AND METHODS

The studies on screening of horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] genotypes for seed yield for 95 genotypes were carried out in the field of AICRP on Arid Legumes, ZARS, GKVK, Bangalore during *rabi* 2023 and the laboratory experiments for 65 genotypes were conducted at Department of Seed Science and Technology, University of Agricultural Sciences, Bangalore. The seeds of 95 horse gram germplasm accessions were sown in ten blocks and two checks BGM-1 and PHG-9 were replicated once in each block in augmented design.

A total of 9 characters were recorded for growth and seed yield parameters which includes plant height (cm) at harvest, number of primary branches per plant at harvest, plant growth habit, days to maturity, pod length at harvest (cm), number of pods per plant at harvest, number of seeds per pod, seed yield per plant (g) and 100 seed weight (g) were recorded on five randomly selected plants in each entry. The statistical analysis and interpretation of experimental data for growth and seed yield attributes was done by using R software for augmented design and the structure of ANOVA of augmented design (Federer, 1956).

These genotypes were also screened for powdery mildew, yellow vein mosaic and leaf spot diseases. Seed quality parameters were assessed for 65 genotypes which includes seed colour of testa, seed germination (%), mean seedling length (cm), seedling vigour index-I, seedling vigour index-II, electrical conductivity of seed leachate ($\mu\text{S/m/g}$), total dehydrogenase activity (OD at A_{480}), total soluble proteins (%) and controlled deterioration test. The statistical analysis and interpretation of the experimental data for seed quality traits was done by adopting complete randomized design (CRD). Pearson correlation analysis was performed among seed quality parameters.

RESULTS AND DISCUSSION

Assessment of variability among genotypes for plant growth and seed yield parameters

Anova

The statistical analysis revealed significant variability among the genotypes for all the traits studied except for the 100 seed weight, indicating substantial diversity among the horse gram genotypes represented in Table 1. Furthermore, the interaction effect, specifically between accessions and checks, was found to be significant, indicating that the accessions differed significantly from the checks. Similar results were found by Neelima *et al.* (2020) and Thota *et al.* (2023).

Table 1: Analysis of Variance (ANOVA) for growth and seed yield attributes of horse gram genotypes

Source of variation	Df	Mean sum of square							
		PH at harvest	DM	PB	NOP	PL	SP	SY	100 SW
Block (Ignoring treatments)	9	348.39**	73.66**	0.94**	156.35**	0.56*	0.70**	9.15**	0.13
Treatment (Eliminating blocks)	96	307.34**	47.85**	0.61**	308.15**	0.68*	0.53**	9.50**	0.14
Treatment: Check	1	11.28	0.8	0.01	272.17**	0.01	0.01	1.07*	0.02
Treatment: Test and Test vs. Check	95	310.46**	48.35**	0.61**	308.52**	0.69*	0.54**	9.59**	0.14*
Residuals	9	6.96	1.91	0.07	5.78	0.16	0.07	0.19	0.05

^{ns} P > 0.05; * P ≤ 0.05; ** P ≤ 0.01, Df: Degree of freedom

PH at harvest: Plant height at harvest (cm), **DM:** Days to maturity, **PB:** No. of primary branches per plant, **NOP:** No. of pods per plant, **PL:** Pod length (cm), **SP:** No. of seeds per pod, **SY:** Seed yield per plant (g), **100 SW:** 100 seed weight (g)

Analysis of mean performance of genotypes

The germplasm material evaluated in the experiment was genetically diverse and exhibited a wide range of variation for all the quantitative characters studied.

The plant height exhibited a range of 23.05 cm (IC-139520) to 85.27 cm (IC-277630) with an average of 44.08 cm. The average duration for days to maturity trait was recorded 111.48 days and the range of variation for days to reach in maturity spans from 97.50 days (IC-321299 and IC-29029) to 127.00 days (IC-261321 and IC-90124). The range of variation for number of primary branches per plant extended from 3.72 (IC-27105 and IC-139521) to 6.82 (IC-139554), with a mean value of 5.04. The number of pods per plant ranged between 19.91 (IC-214807) to 88.61 (IC-26138) with a mean value of 40.86. Pod length ranged between 6.79 cm (IC-26125) to 3.33 cm (IC-56132) with a mean value of 4.73 cm. The mean value for number of seeds per pod was 5.30. The variation in this character ranged from 6.66 (IC-385835) to 4.06 (IC-561027). The average seed yield per plant was 6.61 g, with a range of 12.62 g (IC-26138) to 3.09 g (IC-139521). The 100 seed weight ranged from 4.59 g (IC-1230222) to 2.67 g (IC-15775) with a mean value of 3.42 g. The growth habit varied among different horse gram genotypes. Among 95 genotypes, growth habit was erect in thirty-two genotypes whereas it was observed semi-erect in forty-eight genotypes and it was observed spreading in fifteen genotypes. Similar results were found by by Prakash *et al.* (2010); Patel *et al.* (2019); Poornima and Sajjanar (2015); Gomashe *et al.* (2018); Mohiddin *et al.* (2022); Priyanka *et al.* (2019); Singh *et al.* (2004) and Vandana Joshi *et al.* (2006).

Analysis of genetic variability

The findings of the estimation of components of variation such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance as % of the mean are given in Table 2.

The magnitude of differences between the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) indicates the extent of influence of the environment on the phenotypic expression of a trait. Broad sense heritability (h^2_{bs}) gives an idea about the portion of observed variability attributable to genetic differences. In the present study, the estimates h^2_{bs} ranged from 65.13% to 97.04%. The estimates of heritability were high for all the traits indicating that selection for these characters will be suitable as they were least influenced by the environment. A relative comparison of heritability values and expected genetic gain expressed as per cent of mean (GAM) gives an idea about the nature of gene action governing for a particular character. In the present study, the estimates of high heritability coupled with high genetic advance as per cent of mean was observed for plant height at harvest (69.68%) followed by seed yield per plant (63.39), number of pods per plant (58.61%), no. of primary branches per plant (22.93%), number of seeds per pod (22.32%) and pod length (20.62%). Similar results were found by Gowsalya *et al.* (2016); Verma *et al.* (2019); Nimbale *et al.* (2021). These characters may be governed by additive-gene action and possess high selective value and thus, selection pressure could profitably be applied to these characters for their improvement.

Table 2: Descriptive statistics and genetic variability parameters for growth, yield traits in horse gram genotypes

Plant characters	Mean ± SE	Range		Coefficient of variability		H^2_{bs}	GAM
		Min	Max	GCV (%)	PCV (%)		
PH at harvest (cm)	44.08 ± 1.55	23.05	85.27	34.29	34.80	97.04	69.68
DM	111.48 ± 0.77	97.50	127.00	6.38	6.50	96.36	12.92
PB	5.04 ± 0.07	3.72	6.82	12.17	13.32	83.45	22.93
NOP	40.86 ± 1.32	19.91	88.61	28.99	29.58	96.04	58.61
PL (cm)	4.73 ± 0.07	3.33	6.79	12.19	14.86	67.23	20.62
SP	5.30 ± 0.07	4.06	6.66	11.81	12.88	83.99	22.32
SY (g)	6.61 ± 0.22	3.09	12.62	31.39	32.06	95.84	63.39
100 SW (g)	3.42 ± 0.04	2.67	4.59	9.05	11.22	65.13	15.07

SE: Standard Error, **PCV:** Phenotypic coefficient of variation, **GCV:** Genotypic coefficient of variation, **H^2_{bs} :** Heritability (Broad sense), **GAM:** Genetic advance as per cent of mean, **PH at harvest:** Plant height at harvest (cm), **DM:** Days to maturity, **PB:** No. of primary branches per plant, **NOP:** No. of pods per plant, **PL:** Pod length (cm), **SP:** No. of seeds per pod, **SY:** Seed yield per plant (g), **100 SW:** 100 seed weight (g)

2 Screening of genotypes for powdery mildew, yellow vein mosaic and leaf spot diseases

In the present study, ninety- five horse gram germplasm accessions were screened along with two checks viz., BGM-1 and PHG-9 under natural field conditions for powdery mildew, yellow mosaic virus and leaf spot (Plate 1). Classification of disease reactions for powdery mildew, yellow vein mosaic and leaf spot diseases based on per cent disease index are presented in Tables 3, 4 and 5 respectively. Similar results were found by Basandrai *et al.* (2011) and Bhaskar *et al.* (2017) for powdery mildew, Singh *et al.* (2000) and Bashir *et al.* (2006) and Iqbal *et al.* (2004) for leaf spot.

Table 3: Performance of horse gram genotypes against powdery mildew during rabi 2023

PDI (%)	Reaction	Number of genotypes	Frequency (%)	Genotypes
0	Immune	32	33.7	605761, IC-12141, IC-123033, IC-139333, IC-139347, IC-13938, IC-139412, IC-139521, IC-139522, IC-139554, IC-139557, IC-15775, IC-22759, IC-22794, IC-26125, IC-26132, IC-26140, IC-264704, IC-27105, IC-29029, IC-321300, IC-321861, IC-334273, IC-341261, IC-347892, IC-385918, IC-392329, IC-47461, IC-55065, IC-71771, IC-71841, IC-90124
0.1-10	Highly resistant	3	3.2	IC-139544, IC-49602, IC-27767
10.1-30	Resistant	9	9.5	IC-145300, IC-15730, IC-139520, IC-145256, IC-56130, IC-1230222, IC-214807, IC-23507, IC-56132
30.1-50	Moderately resistant	15	15.8	IC-221105, IC-277630, IC-89032, IC-89634, IC-139527, IC-45748, IC-15728, IC-26138, IC-96233, IC-121640, IC-139413, IC-22760, IC-55062, IC-13854, IC-71808
50.1-75	Moderately susceptible	27	28.4	IC-261321, IC-277687, IC-347182, IC-68554, IC-123030, IC-22785, IC-385835, IC-67011, IC-71815, IC-139453, IC-277671, IC-281059, IC-71775, IC-139556, IC-105785, IC-139339, IC-139548, IC-145268, IC-277668, IC-277670, IC-343105, IC-71802, IC-139518, IC-243564, IC-321299, IC-561027, IC-71809,
75.1-100	Highly susceptible	9	9.5	IC-15735, IC-22827, IC-406382, IC-139331, IC-283202, IC-45755, IC-47132, IC-71743, IC-806421

Table 4: Performance of horse gram genotypes against yellow mosaic during rabi 2023

Description	Infection category	Number of genotypes	Frequency (%)	Genotypes
All plants free of virus symptoms	Highly Resistant	14	14.7	605761, IC-139339, IC-139347, IC-145300, IC-23507, IC-26138, IC-27105, IC-277630, IC-27767, IC-277671, IC-321861, IC-334273, IC-341261, IC-561027
1-10% infection	Resistant	11	11.6	IC-139527, IC-71771, IC-243564, IC-29029, IC-47132, IC-71743, IC-15735, IC-105785, IC-139453, IC-55065, IC-90124
11-20% infection	Moderately resistant	13	13.7	IC-123033, IC-392329, IC-71815, IC-71841, IC-221105, IC-47461, IC-139554, IC-806421, IC-139518, IC-26125, IC-261321, IC-68554, IC-139331
21-30% infection	Moderately susceptible	10	10.5	IC-49602, IC-26140, IC-13938, IC-89634, IC-139333, IC-45755, IC-12141, IC-71802, IC-121640, IC-13854
31-50% infection	Susceptible	20	21.1	IC-139520, IC-22827, IC-283202, IC-56132, IC-96233, IC-123030, IC-67011, IC-15728, IC-71808, IC-22759, IC-406382, IC-22785, IC-321300, IC-71809, IC-139412, IC-139556, IC-139557, IC-15775, IC-56130, IC-139548
More than 50%	Highly susceptible	27	28.4	IC-139544, IC-264704, IC-277687, IC-385835, IC-145256, IC-347182, IC-343105, IC-145268, IC-347892, IC-89032, IC-139413, IC-385918, IC-1230222, IC-22760, IC-45748, IC-214807, IC-277668, IC-281059, IC-26132, IC-321299, IC-55062, IC-15730, IC-139521, IC-71775, IC-139522, IC-22794, IC-277670

Table 5: Performance of horse gram genotypes against leaf spot during rabi 2023

PDI (%)	Reaction	Number of genotypes	Frequency (%)	Genotypes
0	Immune	10	10.5	IC-139331, IC-139556, IC-214807, IC-22827, IC-277687, IC-347182, IC-406382, IC-71808, IC-71809, IC-89032
0.1-10	Highly resistant	26	27.4	IC-139333, IC-15735, IC-22760, IC-26138, IC-281059, IC-29029, IC-321299, IC-347892, IC-56130, IC-90124, IC-15730, IC-221105, IC-121640, IC-139544, IC-15728, IC-22794, IC-23507, IC-277670, IC-123033, IC-139548, IC-145268, IC-27767, IC-321300, IC-385918, IC-47132, IC-806421
10.1-30	Resistant	41	43.2	IC-13938, IC-26125, IC-334273, IC-145300, IC-27105, IC-71815, IC-139554, 605761, IC-13854, IC-139339, IC-139453, IC-139520, IC-139557, IC-15775, IC-261321, IC-341261, IC-392329, IC-45748, IC-45755, IC-68554, IC-71743, IC-139412 IC-139522, IC-321861, IC-139413, IC-145256, IC-22759, IC-22785, IC-26140, IC-47461, IC-71802, IC-71841, IC-105785, IC-385835, IC-49602, IC-71771, IC-123030, IC-139347, IC-277668, IC-55062, IC-71775
30.1-50	Moderately resistant	9	9.5	IC-277630, IC-561027, IC-139521, IC-243564, IC-26132, IC-89634, IC-1230222, IC-67011, IC-283202
50.1-75	Moderately susceptible	8	8.4	IC-139527, IC-55065, IC-264704, IC-96233, IC-12141, IC-139518, IC-277671, IC-343105
75.1-100	Highly susceptible	1	1.1	IC-56132

**Plate 1: Susceptible genotypes of horse gram of A) Powdery mildew B) yellow mosaic virus and C) Leaf spot****3. Seed Quality Parameters**

Germplasm lines were characterized by seed colour (Plate 2). Among 65 genotypes, the seed colour was cream in fifteen genotypes, brown in thirty-one genotypes, reddish brown in two genotypes (IC-47461 and IC-139333), grey in two genotypes (IC-22827 and IC-277630), light black in four genotypes (IC-15775, IC-105785, IC-139331 and IC-139453) and black in eleven genotypes. Similar results were found by Nisar *et al.* (2008) in pea and Rani *et al.* (2013) in horse gram.

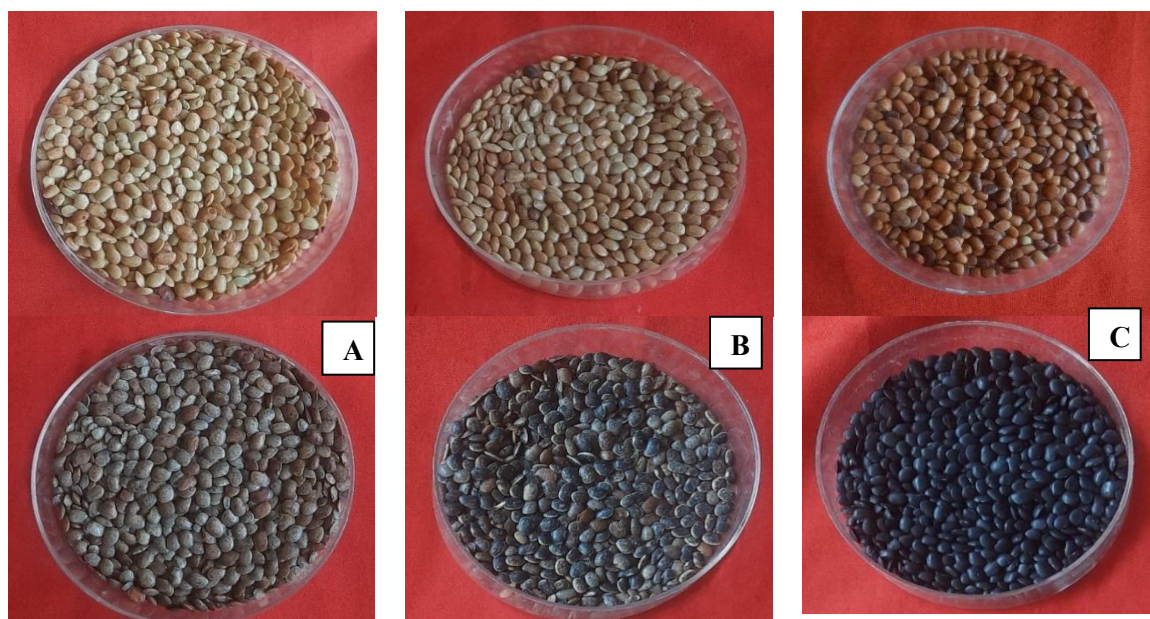


Plate 2: Variability related to colour of testa among germplasm accessions of horse gram A) Cream, B) Brown, C) Reddish brown, D) Grey E) Light black and F) Black

Among the quality attributes, horse gram genotypes differed significantly for germination percentage (Fig 1) and it ranged from 15.50 % (IC-90124) to 99.25 % (IC-47461) with a mean of 56.57 %. Similar results were found by Das *et al.* (2014). Horse gram genotypes differed significantly for mean seedling length and it ranged from 16.04 cm (IC-243564) to 29.62 cm (IC-55065) with a mean of 22.34 cm.

Horse gram genotypes differed significantly for seedling vigour index-I (Fig 2) and it was ranged from 373 (IC-90124) to 3632 (IC-15730) with a mean of 1717. Similar results were found by Das *et al.* 2014 and Mukherjee *et al.* (2024).

Horse gram genotypes differed significantly for seedling vigour index -II (Fig 2) and it was ranged from 207 (IC-385918) to 2829 (IC-47461) with a mean of 1020. Similar findings was reported by Durga and Keshavalu (2015) and Manjunatha *et al.*, (2019).

Horse gram genotypes differed significantly for electrical conductivity and it was ranged from 79.14 $\mu\text{S/m/g}$ (IC-47461) to 320.41 $\mu\text{S/m/g}$ (IC-71808) with a mean of 132.32 $\mu\text{S/m/g}$ (Table 6). Among checks, BGM-1 electrical conductivity was found 83.38 $\mu\text{S/m/g}$ whereas PHG-9 electrical conductivity was found 96.20 $\mu\text{S/m/g}$. Seed leachate electrical conductivity is a sensitive indicator of seed quality, showing a negative correlation with other quality traits (Hibbard and Miller, 1928., Puneeth Raj *et al.*, 2024).

Horse gram genotypes differed significantly for dehydrogenase activity and it was ranged from 1.208 (IC-56132) to 2.568 (IC-47461) with a mean of 1.796 (Table 6). Among checks, BGM-1 dehydrogenase activity was found 2.178 whereas PHG-9 dehydrogenase activity was found 2.236.

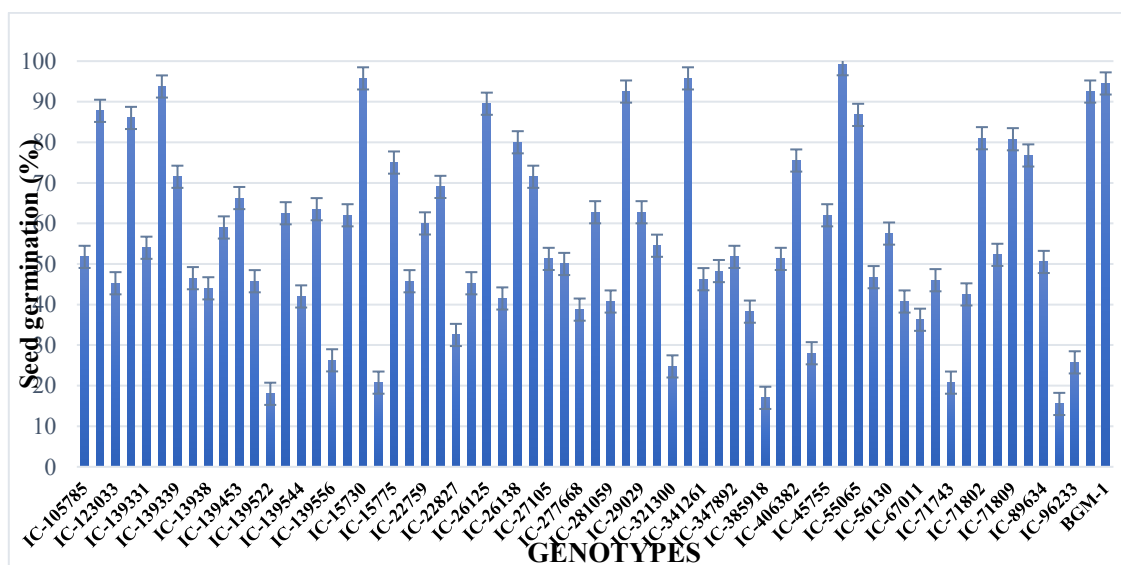


Fig. 1: Variation in seed germination (%) of among germplasm accessions of horse gram

Horse gram genotypes differed significantly for total soluble protein and it was ranged from 15.61 % (IC-22760) to 23.19 % (IC-55065) with a mean of 19.49 % . Among checks, BGM-1 total soluble protein was found 19.80 % whereas PHG-9 total soluble protein was found 18.53 %.

The significant variation in total seed protein content among genotypes and checks can be attributed to genetic differences, environmental factors, and the interaction between them. Additionally, environmental conditions, such as soil fertility, water availability, and nutrient uptake, can influence protein content in seeds. Variations in physiological processes, such as nitrogen metabolism and storage protein synthesis, may also contribute to these differences across genotypes and checks. Similar findings were reported by Guo *et al.* (2022) and Nimbal *et al.* (2024).

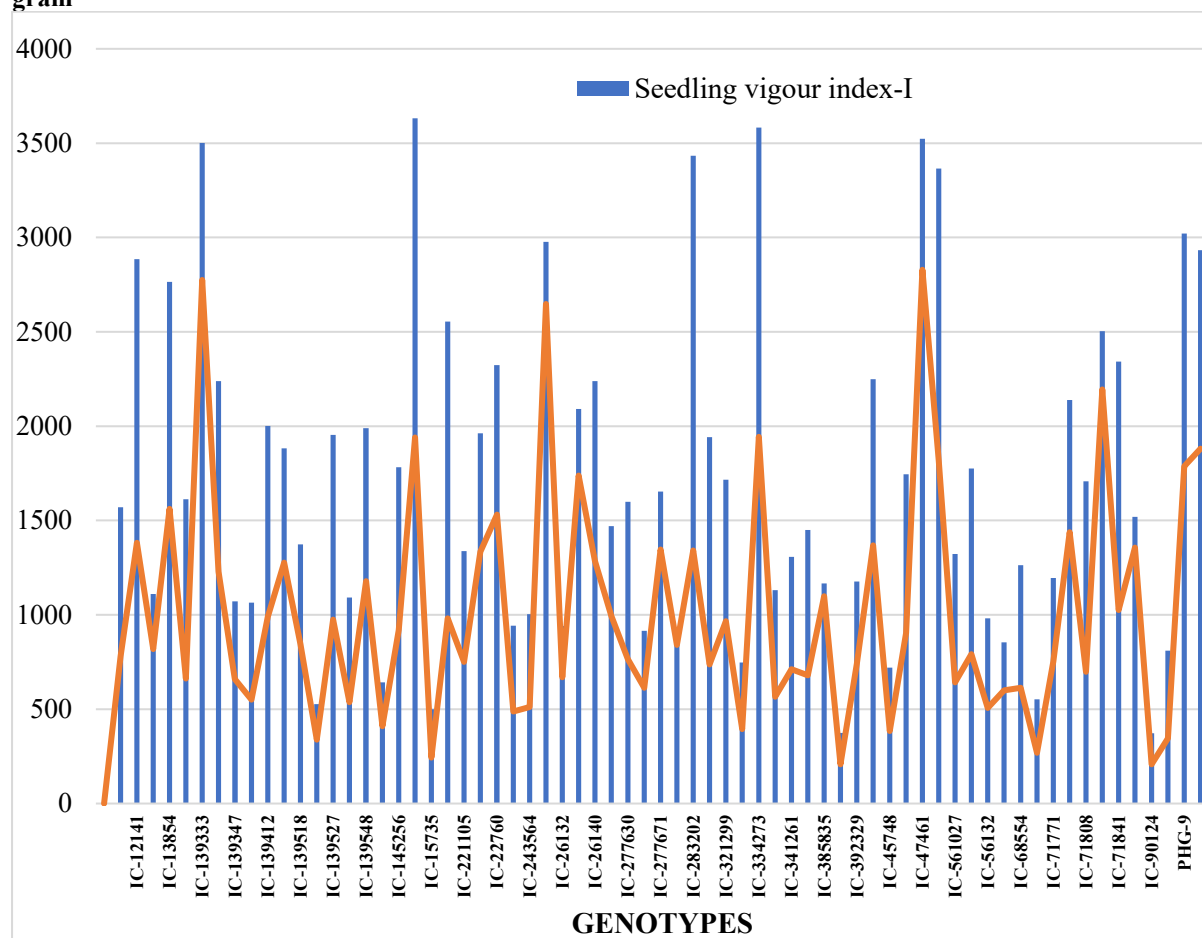
Genotypes such as PHG-9 (plate 3), BGM-1, IC-139333, and IC-47461 maintained high seed germination (>50%) even after 72 hours, indicating high vigor and better potential for storage and performance under stress (Fig 3). In contrast, IC-139331 and IC-26140 showed sharp declines (100% and 72.7%, respectively), suggesting poor tolerance to deterioration.

As seed moisture content increased to 20% and aging duration extended from 0 to 3 days, the percentage of normal seedlings declined across all genotypes. However, IC-139333 (42%) and IC-47461 (47%) showed relatively lower germination loss, likely due to their inherent genetic vigor. Similar trends have been observed in barley and okra (Mishra *et al.*, 2017 and Torres *et al.*, 2013).

Seed germination showed a significant positive correlation with all traits except electrical conductivity (Table 6), which had a significant negative correlation ($r = -0.550$). Strong correlations were observed between germination and seedling vigour index-I ($r = 0.972$), index-II ($r = 0.886$), and total dehydrogenase activity ($r = 0.790$). Electrical conductivity negatively correlated with all seed quality parameters.

Notably, seedling vigour index-I was strongly correlated with index-II ($r = 0.886$), seedling dry weight ($r = 0.806$), and total dehydrogenase activity ($r = 0.803$). These relationships help predict seed lot potential in advance, as also reported by Hemender *et al.* (2017, Anand *et al.*, 2023). Based on the results of this experiment, it can be concluded that these genotypes are well-suited to the current conditions and can be utilized as parents in breeding programs to achieve maximum yields and improved seed quality (Bhuker *et al.*, 2023., Manjula *et al.*, 2021)

Fig. 2: Variation in seed vigour index-I and seed vigour index-II among germplasm accessions of horse gram



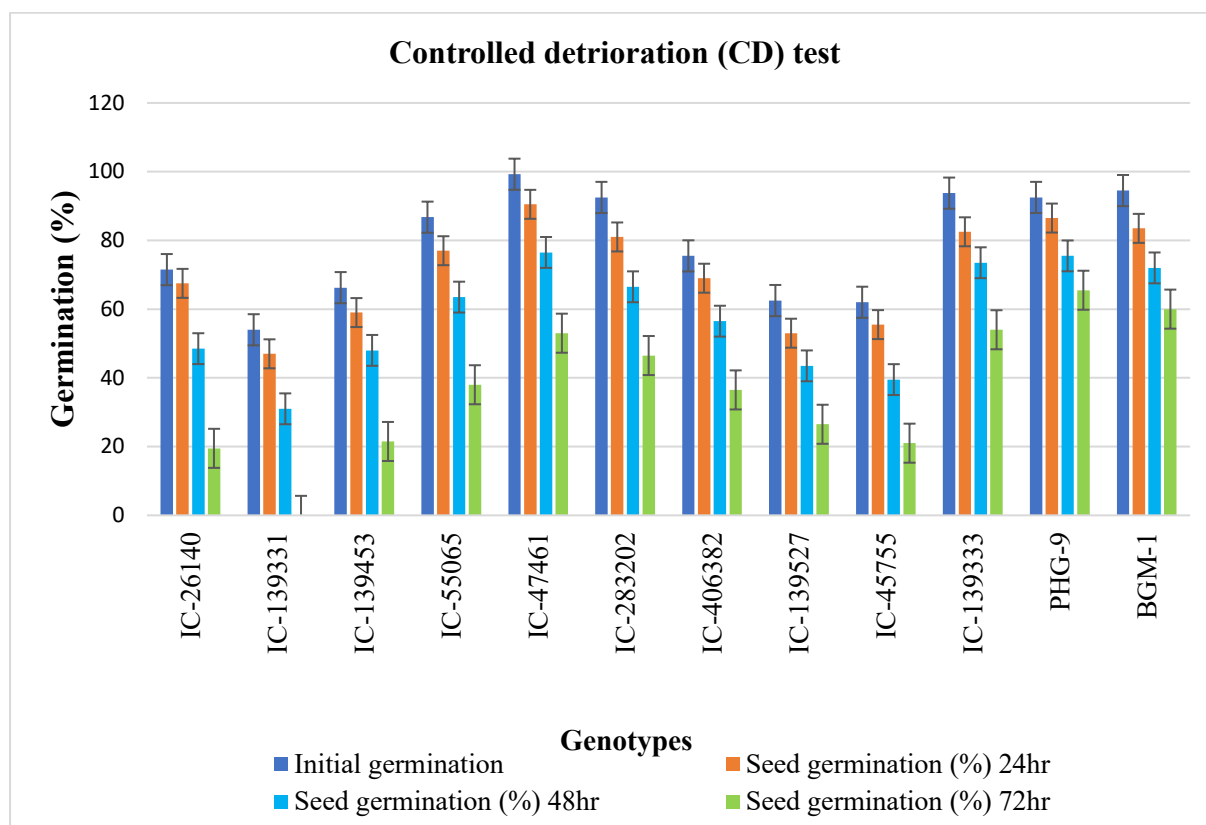


Fig. 3: Seed germination (%) of diverse genotypes of horse gram after controlled deterioration test

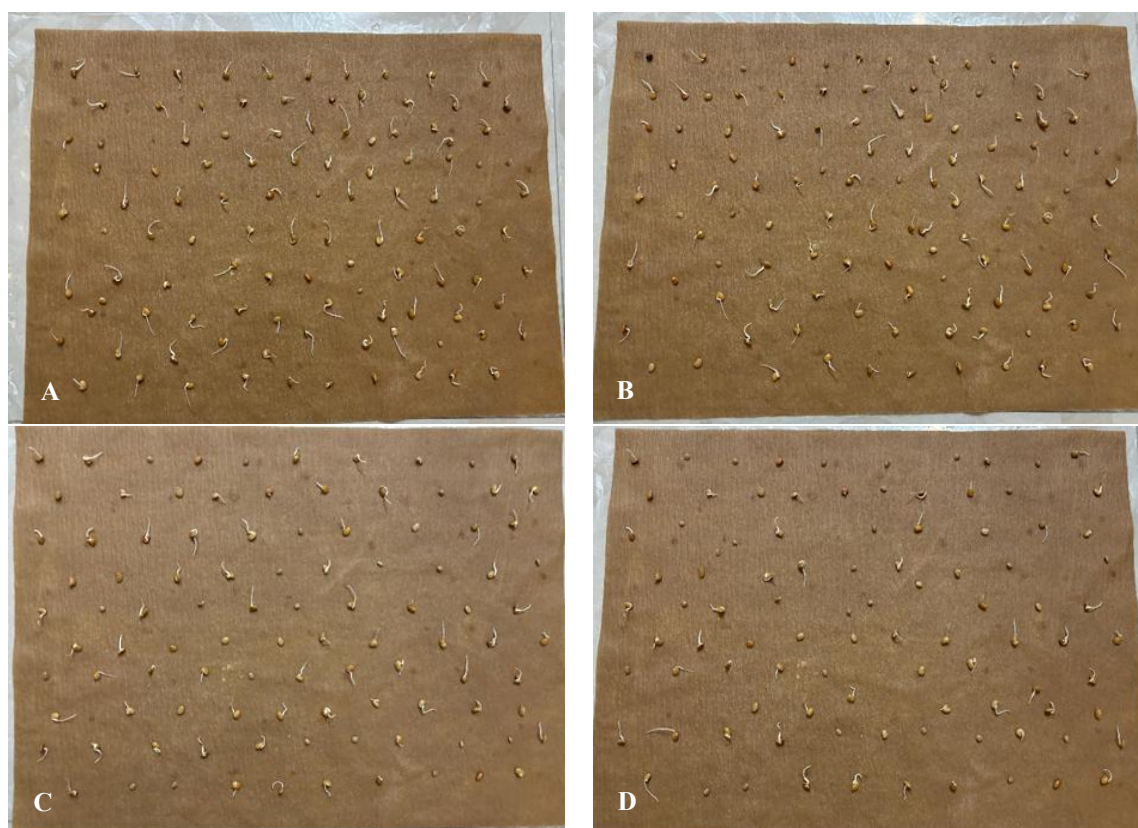


Plate 3: Effect of controlled deterioration on seed germination of PHG-9 genotype. A) Initial germination, B) Seed germination after 24hr, C) Seed germination after 48hr and D) Seed germination after 72hr

Table 6: Pearson correlation-coefficient among horse gram genotypes for seed quality parameters

	SG	SL	RL	MSL	SVI-I	SDW	SVI-II	EC	TDA	PC
SG	1									
SL	0.649**	1								
RL	0.516**	0.358**	1							
MSL	0.642**	0.621**	0.953**	1						
SVI-I	0.972**	0.727**	0.647**	0.777**	1					
SDW	0.486**	0.331**	0.401**	0.443**	0.507**	1				
SVI-II	0.886**	0.573**	0.528**	0.628**	0.886**	0.806**	1			
EC	-0.550**	-0.320**	-0.172 ^{NS}	-0.248*	-0.490**	-0.364**	-0.488**	1		
TDA	0.790**	0.537**	0.517**	0.601**	0.803**	0.472**	0.773**	-0.435**	1	
PC	0.469**	0.340**	0.316**	0.373**	0.481**	0.216 ^{NS}	0.444**	-0.238 ^{NS}	0.377**	1

Where, **SG**: seed germination, **SL**: Shoot length, **RL**: Root length, **MSL**: mean seedling length, **SDW**: seedling dry weight, **SVI**: seedling vigour index, **EC**: electrical conductivity, **TDA**: total dehydrogenase activity, **PC**: Protein content

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