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# EVALUATION OF PHYSIOLOGICAL TRAITS OF HORSE GRAM [*MACROTYLOMA UNIFLORUM* (LAM.) VERDC.] GENOTYPES FOR SEED LONGEVITY POTENTIAL

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**ABSTRACT**

A study was conducted to evaluate seed longevity of 20 horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] genotypes, including GPM-6, CRIDA-18R, CRHG-22, KGP-14-9, Indira kulthi-1, TCR-1635, 11-SS, IC-100938, LONE-2, AK-12-7, TCR-1690b, TRC-1503, TCR-140, CRHG-17, CRHG-8, GPM-4, HL-1, KBHG-1, PHG-2a and VLG-8, which were stored under ambient conditions for six months and categorized as good, medium and poor storers based on the observed physiological changes in seeds during the six months storage period. The results showed that as the storage period progressed, there was a noticeable decline in seed quality parameters, with freshly harvested seeds exhibiting higher quality. After six months of storage, good storers (GPM-6, TCR-1635, TCR-140, Indira kulthi-1 and CRHG-8) showed higher germination rate, seedling vigour indices and lower electrical conductivity of seed leachate compared to poor storers (CRHG-17, CRIDA-18R, IC-100938, VLG-8, CRHG-22 and KGP-14-9). Additionally, an increase in seed moisture content and electrical conductivity of seed leachate was observed, while the test weight of seeds decreased, possibly due to food reserve depletion and pulse beetle infestation, thereby contributing to the decline in vigour and viability of horse gram genotypes.
***Keywords* :** Physiological, Seed germination, Electrical conductivity, Seed viability, Seedling vigour, Seed longevity.

## Introduction

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] belongs to the family fabaceae with diploid (2n) chromosome number 2n = 2x = 20 and a genome size estimated to be 400 Mbp, commonly known as kulthi bean, hurali, gahat and madras gram. It is drought tolerant crop (Bhardwaj *et al.*, 2013) and also withstands harsh environmental conditions such as salinity or metal stresses. It is a legume native to tropical southern Asia. It has been cultivated for food, traditional medicine and fodder mainly by rural and tribal communities since prehistoric times in Asian and African countries and now it is broadly cultivated in India, Malaysia, Sri Lanka and the West Indies. Additionally, the crop is grown for fodder and green manure in tropical countries in southeastern Asia and in northern Australia (Martin Brink, 2006).

At physiological maturity, seed shall have maximum seed viability and vigour is primarily a genotypic character influenced by environmental factors (Hosamani *et al.*, 2012 and Mahesha *et al.*, 2001a). Storability of seeds is mainly a genetic character. It is influenced by seed maturation, pre-storage history and the environmental conditions experienced during both pre and postharvest stages (Mahesha *et al*., 2001b and Siddaraj *et al*., 2019). Seed deterioration that occurs during storage causes decrease of seedling establishment and ultimately plant yield in the field (McDonald, 1999). Since seedstorage is associated with reduction of seed longevity, conditions must be optimal for conserving genetic and seed commercial storage (Hosamani *et al*., 2013a; Hosamani *et al*., 2013b; Rajjou *et al*., 2007).

Seed germination rate with uniformity and vigour are important parameters in seed quality and thus, affect plant status. The evaluation of seeds physiological potential is an essential tool in seed industry, since it permits to obtain consistent and reproducible results for the detection and solution of problems during the productive process (Fessel *et al*., 2010 and Manjunath *et al*., 2019). Improper storage conditions leads to poor germination and less vigour. It is very important to store the horse gram seeds with good germinability and vigour for longer periods. Hence, there is a need to identify the horse gram genotypes with good seed longevity for use in hybridization programme. In view of the above, a study has been conducted for physiological phenotyping of horse gram genotypes for seed longevity.

## Materials and Methods

A laboratory experiment was conducted at Department of Seed Science And Technology, College of Agriculture, Vijayapur to study about physiological phenotyping of 20 horse gram genotypes for seed longevity, including GPM-6, CRIDA-18R, CRHG-22, KGP-14-9, Indira kulthi-1, TCR-1635, 11-SS, IC-100938, LONE-2, AK-12-7, TCR-1690b, TRC-1503, TCR-140, CRHG-17, CRHG-8, GPM-4, HL-1, KBHG-1, PHG-2a and VLG-8, which were collected from horse gram breeder, Department of Genetics and Plant Breeding, College of Agriculture, Vijayapur and seeds are used for the study after one cycle of multiplication. Harvested seeds were stored in cloth bag under ambient conditions and physiological changes were recorded in the fresh seeds, three and six months after storage period.

Vigour assessment was done through physiological parameters like seed germination (%) as per (ISTA, 2021) and seedling vigour indices were calculated by adopting the formula given by Abdul-Baki and Anderson (1973) and expressed in number. Electrical conductivity (EC) (µS/cm/g seed fresh weight) of seed leachate was carried out as per Agrawal and Dadlani (1992) with minor modifications, test weight (g) was calculated by counting hundred seeds using seed counter machine from the three replicates drawn randomly, the average of hundred seed weight was recorded in grams. Seed moisture content (%) was estimated using hot air oven drying method, for horse gram seeds coarse grinding is necessary, the seed moisture content as percentage by weight was calculated to one decimal place (ISTA, 2021).

## Results and Discussion

Among the 20 different horse gram genotypes in freshly harvested, three and six months after stored seeds, genotypic variability was significantly noticed for seed germination, EC of seed leachate and seedling vigour indices (I & II). In freshly harvested seeds, 16 out of 20 genotypes exhibited more than 95 per cent seed germination, while four recorded less than 95 per cent. After six months of seed storage, only five genotypes recorded more than 90 per cent germination. Genotype GPM-6 maintained a high seed germination rate of 93.25 per cent, followed by TCR-1635 (91.75%), TCR-140 (91.25%), Indira kulthi-1(90.75%) and CRHG-8 (90.00%), while KGP-14-9 genotype recorded the lowest seed germination of 71.75 per cent, followed by CRHG-22 (74.50%), VLG-8 (75.50%), IC-100938 (76.25%), CRIDA-18R (76.25%) and CRHG-17 (90.00%) (Table 1). After six months of storage, genotypes were categorized into three groups *viz.,* more than 90 per cent germination (good storers), 80-90 per cent (medium storers) and less than 80 per cent (poor storers). Poor storers did not meet Indian Minimum Seed Certification Standards of 80 per cent for horse gram (IMSCS, 2013). Notably, genotypes stored under ambient conditions exhibited a decline in seed germination after three and six months of storage compared to their freshly harvested seeds. These findings corroborate previous research by Durga and Verma (2013) and, Durga and Keshavulu (2015), who highlighted a decrease in seed germination over the storage period due to the depletion of seed reserves and losses attributed to storage pests. These results align with the findings reported by Deepak and Prasanta (2017).

In freshly harvested seeds, GPM-6 genotype recorded the highest seedling vigour index I (SVI – I) of 2141, while KGP-14-9 showed the lowest (1426). After three months of seed storage, GPM-6 still recorded the highest (1974), while KGP-14-9 showed the minimum (1238). After six months of seed storage, GPM-6 maintained the highest (1743), KGP-14-9 recorded the lowest (1085). The mean SVI - I decreased from 1786 to 1653 and 1461 at three and six months of storage, respectively. In freshly harvested seeds, GPM-6 recorded the highest seedling vigour index II (SVI – II) of 9877, while KGP-14-9 showed the lowest (7445). After six months of storage, GPM-6 genotype maintained the highest SVI - II of 8883, while KGP-14-9 retained the lowest (5559) (Table 1). Over a time, a consistent decline in seed germination and seedling vigour indices was observed, aligning with previous research by Adebisi *et al*. (2008) in sesame, Durga and Verma (2013), Durga and Keshavulu (2015) and Kehinde *et al.* (2019) in horse gram and pigeonpea, respectively. This decline was primarily due to physical deterioration and reduced seed quality, as indicated by Balesevic-Tubic *et al.* (2011) regarding insect damage impacting germination rates.

Throughout storage period, higher test weight was recorded in AK-12-7, while lower in KGP-14-9. In fresh seeds, after three and six months of storage, test weight was varied within the range of 2.83 to 2.06 g, 2.77 to 2.08 g and 2.81 to 2.08 g, respectively. The results indicated that the mean test weight was decreased to 2.48 g as compared to freshly harvested seeds (2.54) (Table 2). Similar findings were reported by Anantharaju and Muthiah (2008). The decrease in test weight was mainly attributed to pulse beetle infestation, which aligns with the reports of Gadewar *et al.* (2016) and, Deepak and Prasanta (2017).

Seed moisture content exhibited variability among genotypes, spanning from 8.24 to 10.71, 8.03 to 10.14 and 9.61 to 11.83 per cent in fresh seeds, three months and six months stored seeds, respectively. A noticeable increase in seed moisture content was observed in seeds stored for three and six months, with mean levels reaching 9.07 and 10.92 per cent, respectively (Table 2). These figures were in contrast to the moisture content of freshly harvested seeds, which was at 8.94 per cent. This observed variability in seed moisture content assumes significance when considering ambient storage conditions, as it may potentially impact seed viability during storage. These findings align with the conclusions drawn by Maruthi (2006) and Heena Kouser *et al*. (2020).

The EC of seed leachate exhibited an increased trend as the storage period progressed. The mean EC of seed leachate for freshly harvested horse gram genotypes was 25.11 µS/cm/g, which was increased to 41.01 µS/cm/g after three months of storage and further to 58.73 µS/cm/g after six months of storage (Table 2). This rise in EC of seed leachate can be attributed to the leakage of ions and solutes from deteriorating membranes and cellular structures. Such an increase in EC of seed leachate and seed moisture content is often associated with reduced seed viability and vigour. Similar results have been reported in other crops, including soybean (Hosamani *et al*., 2013a; Hosamani *et al*., 2013b; Hosamani *et al*., 2020; Maruthi, 2006; Panobianco and Vieira, 2007), cotton cultivars (Goel *et al*., 2003) and onion (Rao *et al*., 2006).

During seed storage, poor storers (CRHG-17, CRIDA-18R, IC-100938, VLG-8, CRHG-22 and KGP-14-9) have shown significantly reduced seed germination, SVI I and SVI II, and increased EC of seed leachate as compared to good storers (GPM-6, TCR-1635, TCR- 140, Indira kulthi-1 and CRHG-8) which have maintained higher seed germination, seedling vigour indices and lower EC of seed leachate, whereas medium storers *i.e*., TRC-1503, GPM-4, PHG-2a, LONE-2, 11-SS, AK-12-7, HL-1, KBHG-1 and TCR-1690b which have maintained seed germination, seedling vigour indices and released medium level of EC of seed leachate. The mean test weight of genotypes decreased after storage due the pulse beetle attack. By these observations after six months of seed storage under ambient conditions, viability and vigour assessment can be done to clearly categorizing the genotypes into good storers, medium storers and poor storers.

## Conclusion

The storage of seeds leads to a decrease in seed germination and seedling vigour. This decline is attributed to a combination of extrinsic and intrinsic factors. Additionally, an increase in EC of seed leachate was observed. These findings hold practical importance in the characterization of horse gram genotypes for seed longevity, emphasizing the need for proper storage conditions and management practices to maintain seed quality and ensure sustainable crop production.

**Table 1 :** Seed germination and seedling vigour indices of horse gram genotypes stored under ambient conditions

| Genotypes | Seed germination (%) | | | Seedling vigour index - I | | | Seedling vigour index - II | | |
|---|---|---|---|---|---|---|---|---|---|
| | Freshseeds | 3 MAS | 6 MAS | Freshseeds | 3 MAS | 6 MAS | Freshseeds | 3 MAS | 6 MAS |
| **Good storers** | | | | | | | | | |
| GPM-6 | 99.50 (87.13)* | 96.25 (78.90)* | 93.25 (75.01)* | 2141 | 1974 | 1743 | 9877 | 9505 | 8883 |
| TCR-1635 | 98.50 (84.04) | 94.50 (76.58) | 91.75 (73.34) | 1636 | 1569 | 1496 | 8323 | 7849 | 7546 |
| TCR-140 | 96.00 (78.91) | 93.25 (75.01) | 91.25 (72.85) | 1897 | 1843 | 1385 | 8548 | 8161 | 7941 |
| Indira Kulthi-1 | 99.25 (86.53) | 94.75 (76.82) | 90.75 (72.44) | 1921 | 1835 | 1622 | 9155 | 8598 | 7927 |
| CRHG-8 | 98.50 (84.04) | 94.25 (76.16) | 90.00 (71.68) | 1842 | 1762 | 1589 | 9214 | 8673 | 8416 |
| **Medium storers** | | | | | | | | | |
| TRC-1503 | 95.50 (78.10) | 89.75 (71.34) | 87.00 (68.90) | 1838 | 1728 | 1454 | 8783 | 8119 | 7960 |
| GPM-4 | 97.00 (80.42) | 92.5 (74.22) | 86.50 (68.49) | 1878 | 1791 | 1529 | 9334 | 8767 | 8001 |
| PHG-2a | 98.50 (84.04) | 88.50 (70.20) | 86.25 (68.26) | 1864 | 1675 | 1590 | 9208 | 8144 | 7831 |
| LONE-2 | 95.50 (77.85) | 87.25 (69.09) | 85.25 (67.46) | 1607 | 1468 | 1462 | 8643 | 7767 | 7520 |
| 11-SS | 97.00 (80.32) | 89.25 (70.87) | 85.00 (67.22) | 1791 | 1648 | 1594 | 8560 | 7742 | 7225 |
| AK-12-7 | 96.25 (78.90) | 86.50 (68.49) | 84.50 (66.83) | 1560 | 1402 | 1350 | 9313 | 8235 | 7751 |
| HL-1 | 96.50 (79.36) | 91.50 (73.08) | 84.50 (66.83) | 1919 | 1820 | 1482 | 9312 | 8692 | 7926 |
| KBHG-1 | 93.25 (75.07) | 85.75 (67.85) | 82.75 (65.47) | 1876 | 1725 | 1447 | 9351 | 8470 | 7820 |
| TCR-1690b | 94.25 (76.16) | 86.50 (68.49) | 81.75 (64.74) | 1895 | 1740 | 1560 | 8292 | 7476 | 7096 |
| **Poor storers** | | | | | | | | | |
| CRHG-17 | 95.50 (78.10) | 87.75 (69.58) | 78.25 (62.21) | 1903 | 1750 | 1352 | 8313 | 7496 | 6513 |
| CRIDA-18R | 95.25 (77.56) | 87.50 (69.34) | 77.75 (61.86) | 1762 | 1619 | 1303 | 8788 | 7931 | 7017 |
| IC-100938 | 95.00 (77.36) | 86.50 (68.62) | 76.25 (60.84) | 1637 | 1491 | 1370 | 8527 | 7647 | 6898 |
| VLG-8 | 95.50 (77.85) | 84.75 (67.07) | 75.50 (60.34) | 1847 | 1639 | 1413 | 9476 | 8281 | 7190 |
| CRHG-22 | 93.00 (74.68) | 83.50 (66.10) | 74.50 (59.68) | 1568 | 1408 | 1123 | 8834 | 7811 | 6817 |
| KGP-14-9 | 92.50 (74.22) | 80.25 (63.77) | 71.75 (57.90) | 1426 | 1238 | 1085 | 7445 | 6328 | 5559 |
| **Mean** | **96.11 (79.53)** | **89.04 (71.08)** | **83.73 (66.61)** | **1786** | **1653** | **1461** | **8900** | **8126** | **7573** |
| **S.Em (±)** | **0.81 (1.50)** | **1.31 (0.70)** | **0.83 (0.70)** | **34.54** | **35.85** | **23.04** | **203.29** | **187.69** | **163.30** |
| **CD @ 1%** | **3.03 (5.66)** | **4.26 (3.68)** | **3.11 (2.63)** | **129.96** | **134.89** | **86.68** | **764.80** | **706.13** | **614.36** |
| **Significance** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** |

**Note:** MAS - Months after storage, S – Significance, *Values in the parentheses are arc sin transformations

**Table 2 :** Test weight, seed moisture content and electrical conductivity of seed leachate of horse gram genotypes stored under ambient conditions

| Genotypes | Test weight (g) | | | Seed moisture content (%) | | | Electrical conductivity (µS/cm/g seed fresh weight) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Freshseeds | 3 MAS | 6 MAS | Freshseeds | 3 MAS | 6 MAS | Freshseeds | 3 MAS | 6 MAS |
| **Good storers** | | | | | | | | | |
| GPM-6 | 2.63 | 2.63 | 2.64 | 8.37 (16.81)* | 8.38 (16.82)* | 11.83 (20.12)* | 35.47 | 41.50 | 51.68 |
| TCR-1635 | 2.33 | 2.32 | 2.35 | 8.69 (19.08) | 9.71 (18.15) | 11.82 (20.11) | 16.94 | 31.35 | 41.08 |
| TCR-140 | 2.46 | 2.40 | 2.47 | 8.36 (16.80) | 8.03 (16.46) | 10.21 (18.63) | 23.60 | 45.32 | 54.88 |
| Indira Kulthi-1 | 2.51 | 2.42 | 2.48 | 10.71 (17.24) | 8.78 (17.23) | 11.35 (19.68) | 28.54 | 42.98 | 52.72 |
| CRHG-8 | 2.48 | 2.42 | 2.46 | 9.04 (17.49) | 10.14 (18.57) | 10.02 (18.45) | 24.51 | 49.81 | 53.76 |
| **Medium storers** | | | | | | | | | |
| TRC-1503 | 2.56 | 2.52 | 2.43 | 8.24 (16.69) | 9.74 (18.18) | 9.72 (18.14) | 26.02 | 48.11 | 67.62 |
| GPM-4 | 2.60 | 2.52 | 2.49 | 8.78 (17.23) | 8.96 (17.37) | 11.25 (19.57) | 22.42 | 40.42 | 61.74 |
| PHG-2a | 2.54 | 2.50 | 2.60 | 8.96 (17.37) | 8.56 (17.01) | 10.60 (19.00) | 25.98 | 43.91 | 56.03 |
| LONE-2 | 2.61 | 2.56 | 2.53 | 8.70 (17.11) | 8.61 (17.06) | 10.59 (18.99) | 20.75 | 31.50 | 57.85 |
| 11-SS | 2.44 | 2.40 | 2.39 | 9.34 (17.15) | 9.11 (17.57) | 10.42 (18.83) | 18.39 | 36.06 | 49.37 |
| AK-12-7 | 2.83 | 2.77 | 2.81 | 8.70 (17.16) | 9.60 (18.04) | 10.72 (19.12) | 13.38 | 27.27 | 47.45 |
| HL-1 | 2.69 | 2.63 | 2.60 | 8.86 (17.32) | 9.05 (17.50) | 10.97 (19.33) | 29.80 | 45.22 | 64.88 |
| KBHG-1 | 2.72 | 2.62 | 2.58 | 9.02 (17.48) | 10.09 (18.52) | 11.78 (20.06) | 34.16 | 45.62 | 66.05 |
| TCR-1690b | 2.63 | 2.61 | 2.58 | 9.83 (18.28) | 9.80 (18.24) | 10.77 (19.16) | 28.34 | 39.29 | 59.20 |
| **Poor storers** | | | | | | | | | |
| CRHG-17 | 2.45 | 2.34 | 2.28 | 9.11 (17.57) | 9.04 (17.49) | 11.54 (19.86) | 26.46 | 48.23 | 62.89 |
| CRIDA-18R | 2.49 | 2.44 | 2.40 | 8.72 (17.17) | 8.74 (17.19) | 11.16 (19.53) | 34.07 | 42.42 | 72.90 |
| IC-100938 | 2.64 | 2.58 | 2.45 | 8.66 (17.79) | 8.99 (17.43) | 9.61 (18.05) | 20.44 | 35.66 | 71.05 |
| VLG-8 | 2.61 | 2.61 | 2.63 | 8.87 (17.32) | 9.15 (17.06) | 11.37 (19.70) | 25.73 | 43.20 | 66.08 |
| CRHG-22 | 2.50 | 2.48 | 2.45 | 9.05 (17.51) | 8.65 (17.10) | 11.10 (19.46) | 33.18 | 47.89 | 66.72 |
| KGP-14-9 | 2.06 | 2.08 | 2.08 | 8.79 (16.79) | 8.39 (16.83) | 11.62 (19.93) | 13.96 | 34.46 | 68.99 |
| **Mean** | **2.54** | **2.49** | **2.48** | **8.94 (17.37)** | **9.07 (17.52)** | **10.92 (19.29)** | **25.11** | **41.01** | **58.73** |
| **S.Em (+)** | **0.04** | **0.04** | **0.04** | **0.29 (0.27)** | **0.28 (0.29)** | **0.34 (0.32)** | **0.71** | **1.01** | **1.39** |
| **CD @ 1%** | **0.16** | **0.14** | **0.14** | **1.16 (1.10)** | **1.17 (1.16)** | **1.38 (1.30)** | **2.73** | **3.87** | **5.33** |
| **Significance** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** |

**Note:** MAS - Months after storage, S – Significance, *Values in the parentheses are arc sin transformation

## Author's contribution

Conceptualization and designing of the research work (LN and JH); Execution of field/lab experiments and data collection (LN, JH, RJB, BW and SG); Analysis of data and interpretation (LN and JH); Preparation of manuscript (LN and JH).

## Declaration

The authors do not have any conflict of interest.

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