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PHYSIOLOGICAL, BIOCHEMICAL AND MULTIVARIATE CHARACTERIZATION IN HORSE GRAM (*MACROTYLOMA UNIFLORUM* (LAM.) VERDC.) GENOTYPES FOR SEED LONGEVITY

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ABSTRACT
Seed longevity is crucial for maintaining seed viability and vigour during storage, but the extent of storage induced deterioration varies among genotypes. Identifying physiological and biochemical traits associated with better seed longevity is essential for characterizing superior horse gram genotypes for seed production and breeding programmes. Therefore, the present study was undertaken to evaluate physiological, lipid peroxidation and antioxidant changes associated with seed longevity in 20 horse gram genotypes. Seeds were evaluated for seed germination, seedling vigour indices, test weight, electrical conductivity of seed leachate, volatile aldehydes, malondialdehyde, catalase and superoxide dismutase activities before and after six months of storage under ambient conditions. Freshly harvested seeds exhibited higher seed quality, whereas progressive deterioration occurred with storage. After six months of storage, genotypes GPM-6, TCR-1635, TCR-140, Indira Kulthi-1 and CRHG-8 maintained higher seed germination, seedling vigour, superoxide dismutase and catalase activities, with lower electrical conductivity of seed leachate, volatile aldehyde and malondialdehyde production, indicating comparatively better seed longevity. In contrast, genotypes CRHG-17, CRIDA-18R, IC-100938, VLG-8, CRHG-22 and KGP-14-9 exhibited greater deterioration and poorer storage performance. Correlation analysis indicated positive associations of seed germination and seedling vigour with antioxidant activities, whereas deterioration through lipid peroxidation related parameters showed negative associations with seed quality attributes. Principal component analysis revealed substantial multivariate variation, with the first four principal components explaining 84.05% of the total variation. Hierarchical cluster analysis grouped the genotypes into four distinct clusters, with Cluster 4 showing comparatively superior storage performance and KGP-14-9 exhibiting the poorest. These findings demonstrate that antioxidant defence, membrane stability and oxidative damage related traits are important indicators of differential seed longevity in horse gram genotypes.

KEYWORDS: Horse gram; seed longevity; seed deterioration; antioxidant enzymes; multivariate analysis

INTRODUCTION
Horse gram is a drought tolerant crop (Bhardwaj *et al.,* 2013), withstands harsh environmental conditions such as salinity or metal stresses (Prasad and Singh, 2015) and it has been cultivated for food, traditional medicine and fodder but the main problem is seed longevity. Seed longevity is mostly a hereditary trait affected by seed maturation, pre-storage history and the environmental conditions experienced during both pre and post-harvest phases (Mahesha *et al.*, 2001). Seed germination rate with uniformity and vigour are important parameters in seed quality and thus, affect plant status, since during seed storage, reactive oxygen species (ROS) accumulate in ageing seed tissues and have a vital role in seedling vigour reduction (Pukacka and Ratajczak, 2005). Lipoxygenase activity leads to accumulation of volatile aldehydes (VA) during seed ageing, which are released upon hydration is an indicator of lipid peroxidation (Zhang *et al.,* 1993). Activity of active oxygen scavenging system (AOSS) *viz.*, catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) enzymes play an essential role during seed storage to maintain better seed longevity (Bailly *et al.*, 1996; Hosamani *et al.*, 2013a; Priyanka *et al.*, 2021). Therefore, finding of factors which are influencing seed deterioration in horse gram genotypes and characterizing genotypes with good seed longevity is necessary for the hybridization programme. Hence, in view of this the correlation studies was conducted to know the factors which are responsible for maintaining genotypes with good longevity and to assess characteristics which causes deterioration of the horse gram genotypes.

MATERIAL AND METHODS

Twenty horse gram genotypes were collected from horse gram breeder, Department of Genetics and Plant Breeding, College of Agriculture, Vijayapur. The seeds were stored in cloth bag under ambient conditions. The physiological and biochemical changes were recorded in the fresh and stored seeds. The vigour assessment was done through physiological parameters like seed germination (%) carried as per (ISTA, 2021), seedling vigour indices were calculated by adopting the formula given by Abdul-Baki and Anderson (1973) and expressed in number.

Test weight (g) was calculated by counting hundred seeds using seed counter machine from three replicates drawn randomly, the average of hundred seed weight was recorded in grams. Electrical conductivity (EC) of seed leachate was carried out as per Agrawal and Dadlani (1992) with minor modification. Lipid peroxidation assays were analyzed through the production of volatile aldehydes (VA) was carried as per the procedure of Wilson and McDonald (1986) with slight modifications, malondialdehyde (MDA) production was measured according to the standard procedure of Heath and Packer (1968), whereas antioxidant enzyme assays were carried through catalase activity and superoxide dismutase activity measured according to Aebi (1984) and Beauchamp and Fridovich (1971), respectively.

Statistical analysis

The data from laboratory experiments were analyzed using completely randomized design (Panse and Sukhatme, 1985) and correlation between physiological and biochemical changes were assessed through Pearson's correlation coefficient and hierarchical cluster analysis was performed by using R studio software package version R 4.3.2. Principal component analysis (PCA) using GRAPES (General R-shiny based analysis platform empowered by statistics), version 1.0.0, was used to assess multivariate variation and classify seed lots based on physiological, lipid peroxidation and antioxidant traits (Gopinath *et al.*, 2020). The PCA was performed using standardized variables and the principal components explaining the major proportion of total variation were considered for interpretation.

RESULTS AND DISCUSSION

Physiological and biochemical changes during seed storage were evaluated across horse gram genotypes and their interrelationships were assessed through correlation analysis to identify key factors associated with seed longevity and deterioration.

Seed germination (%)

In freshly harvested seeds, 16 genotypes exhibited more than 95 per cent seed germination, whereas four genotypes recorded lower germination. After six months of storage, only five genotypes maintained more than 90 per cent germination, while the remaining 15 genotypes recorded lower germination, indicating substantial genotypic variation in seed longevity. Similar genotypic differences in seed longevity and germination maintenance during storage have been reported in horse gram. Durga and Keshavulu (2015) identified distinct differences among horse gram genotypes in germination, seedling vigour and longevity. After six months of storage, GPM-6 maintained the highest seed germination (93.25%), whereas KGP-14-9 recorded the lowest (71.75%) (Table 1). Germination percentage declined progressively with increasing storage duration, with the magnitude of decline varying among genotypes. Similar genotypic differences in seed longevity and retention of germination during storage were reported in horse gram by Lokeshwari *et al.* (2024). The decline in germination during storage may be associated with progressive physiological deterioration, including membrane damage and oxidative processes, which adversely affect seed viability and vigour. Tyagi and Srinivasan (2002) reported that seed deterioration was associated with increased membrane leakiness and lipid peroxidation. In addition, storage insect infestation can accelerate deterioration in pulse seeds; Divya *et al.* (2017) reported substantial reductions in germination and seedling vigour in horse gram accessions following infestation by *Callosobruchus chinensis*.

Seedling vigour indices

In freshly harvested seeds, GPM-6 genotype recorded the highest seedling vigour index I **(**SVI) and SVI II of 2141 and 9877, while the KGP-14-9 genotype had the lowest of 1426 and 7445, respectively. After six months of storage, GPM-6 genotype maintained highest SVI I and SVI II of 1742 and 8883 respectively, whereas KGP-14-9 genotype recorded the lowest SVI I and II of 1085 and 5559, respectively (Table 1). As the storage period advanced, seedling vigour indices showed a substantial decline across all genotypes, indicating progressive deterioration in seed physiological quality during storage. This trend is consistent with the findings of Durga and Verma (2013), Durga and Keshavulu (2015) and Lokeshwari *et al.* (2024) in horse gram, Adebisi *et al.* (2008) in sesame and Kehinde *et al.* (2019) in pigeonpea, who attributed the reduction in vigour to progressive loss of seed viability and deterioration during storage. The preferential decline in seedling vigour during storage reflects the progressive ageing of seeds, with vigour generally declining before complete loss of viability (Walters, 2008)

Test weight (g)

In freshly harvested horse gram seeds, highest test weight was found in AK-12-7 (2.83 g), while the lowest in the KGP-14-9 genotype (2.06 g). Six months after storage, AK-12-7 (2.81 g) recorded the highest test weight, while KGP-14-9 (2.08 g) exhibited the lowest. The mean test weight for freshly harvested horse gram seeds was 2.54 g. This mean was decreased to 2.48 g after six months of storage (Table 1). Similar differences were also reported by Anantharaju and Muthiah (2008) in pigeonpea. The relatively small change in test weight suggests that storage had only a marginal effect on seed mass. The slight reduction in test weight during storage may be associated with

moisture fluctuations and progressive deterioration of stored seeds and also the decrease in test weight was mainly attributed to pulse beetle infestation as reported by Gadewar *et al.* (2016) and Deepak and Prasanta (2017), but there was no significant differences observed for test weight among genotypes.

Electrical conductivity of seed leachate (µS/cm/g seed fresh weight)

In freshly harvested horse gram seeds, GPM-6 (35.47 µS/cm/g) displayed the highest EC of seed leachate, while KGP-14-9 (13.96 µS/cm/g seed fresh weight) and AK-12-7 (13.38 µS/cm/g seed fresh weight) recorded the lower. Six months after storage, CRIDA-18R (72.90 µS/cm/g seed fresh weight) recorded the highest EC of seed leachate, while TCR-1635 (41.08 µS/cm/g seed fresh weight) had the lowest EC of seed leachate. The mean EC of seed leachate for freshly harvested horse gram genotypes was 25.11 µS/cm/g seed fresh weight, which was increased to 58.73 µS/cm/g seed fresh weight after six months of storage (Table 2). This surge 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 is often associated to declined seed viability and vigour in soybean [Panobianco and Vieira (2007); Hosamani *et al.*, 2013a], cotton cultivars (Goel *et al.*, 2003) and onion (Rao *et al.*, 2006).

Volatile aldehydes production (µg formaldehyde released/g seed fresh weight)

In freshly harvested horse gram seeds, CRHG-17 (0.33 µg formaldehyde released/g seed fresh weight) exhibited the highest production of VA, whereas HL-1 and Indira Kulthi-1 recorded the lower production of VA at 0.22 and 0.23 µg formaldehyde released/g seed fresh weight, respectively. Six months after storage, IC-100938 and CRHG-22 exhibited higher VA production at 0.55 and 0.53 µg formaldehyde released/g seed fresh weight, respectively. Conversely, GPM-4 and LONE-2 recorded the lowest production at 0.34 µg formaldehyde released/g seed fresh weight (Table 2). The mean VA production for freshly harvested seeds was 0.28 µg, which was increased to 0.43 µg formaldehyde released/g seed fresh weight after six months of storage. Notably, the amount of volatiles significantly increased after storage, accumulation of VA during seed ageing, which are released upon hydration, is an indicator of lipid peroxidation (Zhang *et al.,* 1993).

Malondialdehyde production (nmoles MDA produced/g seed fresh weight)

In freshly harvested seeds, IC-100938 recorded a highest MDA production of 4.43 nmoles MDA produced/g seed fresh weight, while the lowest noticed in CRHG-8 (2.46), but there is no clear distinction was noticed among genotypes. After six months of storage, MDA production was increased among all genotypes. The maximum MDA production was recorded in CRIDA-18R of 6.13 nmoles MDA produced/g seed fresh weight. In contrast, CRHG-8 showed the minimum MDA production of 3.07 nmoles MDA produced/g seed fresh weight. The mean MDA production in freshly harvested seeds was 3.33 nmoles MDA produced/g seed fresh weight, it was increased to 4.41 nmoles MDA produced/g seed fresh weight after six months of storage (Table 2). Significant variation was recorded in MDA production between fresh and stored seeds. The higher MDA content observed in stored seeds indicates enhanced lipid peroxidation and oxidative damage during storage, which may contribute to seed deterioration and reduced longevity. Further, higher hydroperoxide lyase activity during storage may promote the formation of volatile aldehydes from lipid hydroperoxides, thereby contributing to seed deterioration. Similar findings were reported by Kuchlan (2006), Freitas *et al.* (2006), Hosamani *et al.* (2013a) and Priyanka *et al.* (2020). More recent evidence also supports the association of oxidative damage and lipid peroxidation with seed ageing and loss of seed longevity (Naflath *et al.,* 2023).

Superoxide dismutase activity (Units/min/g seed fresh weight)

Freshly harvested seeds exhibited uniform SOD activity among the genotypes. The maximum SOD activity was recorded in genotypes GPM-6, TCR 140 and Indira kulthi-1 at 1.19 Units/min/g fresh weight. In contrast, minimum SOD activity was recorded in KBHG-1 at 1.04 Units/min/g seed fresh weight. Six months after storage, there was a decline in SOD activity across all genotypes recorded. Genotypes Indira Kulthi-1 and TCR-140 maintained higher SOD activity at 1.11 Units/min/g fresh weight. In contrast, CRHG-22 recorded the lowest activity at 0.81 Units/min/g fresh weight. The mean SOD activity in freshly harvested horse gram seeds was 1.13 Units/min/g fresh weight which was decreased to 1.01 Units/min/g fresh weight after six months of storage (Table 2). Superoxide dismutase helps in mitigating superoxide radicals (O•) generated during the electron transport process, thereby it plays a crucial roles in management of free radical reactions and is integral to the ageing process of seeds.

Catalase activity (µmoles H_2O_2 decomposed/min/g seed fresh weight)

In freshly harvested seeds, the genotype GPM-6 exhibited the highest CAT activity with 167.82 µmoles H_2O_2 decomposed/min/g seed fresh weight, while the lower activity was observed in the 11-SS and KBHG-1 genotypes at 147.63 and 147.65 µmoles H_2O_2 decomposed/min/g seed fresh weight, respectively. Six months after storage, CAT activity continued to decline across all genotypes. The genotype KGP-14-9 exhibited the lowest levels of CAT activity at 126.22 µmoles H_2O_2 decomposed/min/g seed fresh weight, while the highest levels recorded in GPM-6 at 162.18 µmoles H_2O_2 decomposed/min/g seed fresh weight. The mean CAT activity in fresh horse gram seeds was 157.89 µmoles H_2O_2 decomposed/min/g seed fresh weight and it was decreased to 146.30 µmoles H_2O_2 decomposed/min/g seed fresh weight after six months of storage (Table 2). Catalase enzyme primarily located in glyoxysomes and plays a crucial role in mitigating potentially harmful H_2O_2 generated under various stress conditions, thereby preventing oxidative stress-related damage. Hence, it has vital role in plants defense system. The decline in seed viability is linked to reduction in activities of crucial antioxidant enzymes *viz.,* SOD and CAT. These enzymes play a pivotal role in safeguarding seeds against the detrimental effects of highly reactive free

radicals during storage (Bailly *et al.*, 1996; Kuchlan, 2006; Hosamani *et al.*, 2013a and 2013b; Sooganna *et al.*, 2024). Consequently, this study underscores the significance of antioxidant enzymes in shaping the storage behaviour of horse gram genotypes under ambient conditions.

Correlation studies

Correlation studies examining physiological and biochemical changes during seed storage play a crucial role in understanding the factors influencing seed viability and longevity. Furthermore, provide valuable insights into the intricate relationships between different physiological and biochemical parameters, contributing to the development of sustainable seed storage practices for preservation of seed quality and also helps in selection of genotypes with better seed longevity in breeding programmes. The results of Pearson's correlation coefficient showed significant and non significant positive as well as negative trend association among various physiological and biochemical parameters during seed storage.

The results showed that seed germination showed significant and very high positive correlation with CAT (0.90***), significant and high positive correlation with SOD (0.89***) and SVI I (0.78***) (Fig. 1). The SVI I exhibited significant and high positive correlation with SOD (0.78***) and SVI II (0.77***), significant and moderate positive correlation with CAT (0.65**), significant and low positive correlation with test weight (0.45*), while SVI II showed significant and high positive correlation with seed germination (0.86***), CAT (0.84***) and SOD (0.77***) (Fig. 1). Test weight showed significant and moderate positive correlation with SVI II (0.64**), CAT (0.48*), while non significant and low positive correlation with SOD and seed germination (Fig. 1).

Electrical conductivity of seed leachate showed significant and very high positive correlation with VA (0.70***), significant and moderate positive correlation with MDA (0.58**), whereas significant and high negative correlation with seed germination (-0.74***), significant and moderate negative correlation with SOD (-0.66**) and CAT (-0.60*) and significant and low negative correlation with SVI II (-0.47*) and SVI I (-0.55*), but it also exhibited non significant and negligible negative correlation with test weight (Fig. 1).

Volatile aldehydes production exhibited significant and high positive correlation with MDA (0.72***), whereas it showed significant and very high negative correlation with seed germination (-0.81***), CAT (-0.76***), significant and moderate negative correlation with SVI II (0.63**), SVI I (0.61**), but it also exhibited non significant and negligible negative correlation with test weight (Fig. 1). The MDA showed significant and very high negative correlation with CAT (-0.90***), seed germination (-0.84***), SVI II (-0.76***), SOD (-0.74***), significant and moderate negative correlation with SVI I (-0.60**), non significant and low negative correlation with test weight (Fig. 1). A sharp increase in VA and MDA leads to reduction in seed germination under ambient conditions of storage and role of lipid peroxidation in seed longevity behavior means it leads to poor longevity of seeds (Hosamani *et al.,* 2013a). The CAT exhibited significant and high positive correlation with SOD (0.81***) and significant and moderate positive correlation with SVI I (0.65**). Antioxidants CAT and SOD play a significant role in providing protection against highly reactive free radicals under storage conditions (Bailly *et al.*, 1996 and Kuchlan, 2006), which helps in maintaining seed longevity.

Principal component analysis (PCA)

Principal component analysis revealed substantial multivariate variation among the 20 genotypes, with the first four principal components accounting for 84.05% of the total variation. The PC1 alone explained 51.68%, followed by PC2 (15.72%), PC3 (8.82%) and PC4 (7.82%) (Fig.2; Supplementary table 1, 2 and 3). The component PC1 was mainly associated with germination and vigour retention after storage together with antioxidant related traits, whereas electrical conductivity of seed leachate, volatile aldehydes and malondialdehyde contributed contrastingly to the major axis. The PC2 was influenced particularly by fresh seeds electrical conductivity of seed leachate, test weight and vigour related attributes, while PC3 was strongly associated with volatile aldehyde and MDA production. Thus, the major variation among seed lots was explained by the combined effects of seed viability and vigour, membrane integrity, oxidative damage and antioxidant defence. Such relationships are consistent with the established mechanism of seed ageing, in which increasing reactive oxygen species and declining antioxidant protection contribute to membrane damage, lipid peroxidation and progressive loss of seed quality (Bailly, 2004; Ebone *et al.*, 2019).

Hierarchical cluster analysis

Hierarchical cluster analysis further differentiated the 20 horse gram genotypes into four distinct clusters, supporting the grouping observed in the PCA. The Cluster 4, comprising ten genotypes, exhibited the most favourable overall storage quality profile with the highest seed germination mean value after six months of storage (88.28%), highest SVI-I (1524.80) and SVI-II (7913.10), comparatively lower electrical conductivity of seed leachate (53.54 µS/cm/g seed fresh weight) and MDA (3.98 nmoles MDA produced/g seed fresh weight) after storage and greater retention of catalase (154.82 µmoles H_2O_2 decomposed/min/g seed fresh weight) and SOD (1.063 Units/min/g seed fresh weight) activity. In contrast, Cluster 2 represented by genotype KGP-14-9, showed the lowest seed germination after six months of storage (71.75 %) and vigour, together with the highest stored seed electrical conductivity of seed leachate (68.99 µS/cm/g seed fresh weight) and lower CAT (126.22 µmoles H_2O_2 decomposed/min/g seed fresh weight) and SOD (0.830 Units/min/g seed fresh weight) activities. The Cluster 1 also exhibited pronounced deterioration, with seed germination declining from 94.69 to 76.69%, after six months of storage, while EC of seed leachate increased from 28.54 to 68.39 µS/cm/g seed fresh weight and MDA from

4.04 to 5.94 nmoles MDA produced/g seed fresh weight (Supplementary Table 4 and 5). The increase in electrolyte leakage and MDA is consistent with deterioration associated membrane disruption and lipid peroxidation, while reduced antioxidant protection may enhance susceptibility to oxidative damage during storage (Bailly, 2004; Ebone *et al.*, 2019).

The comparatively lower volatile aldehyde accumulation in Cluster 4 (0.38 µg formaldehyde released/g seed fresh weight) than in Cluster 1 (0.53 µg formaldehyde released/g seed fresh weight) and Cluster 2 (0.52 µg formaldehyde released/g seed fresh weight) after six months of storage (Supplementary Table 4 and 5) provides additional evidence of differences in oxidative deterioration among genotypes. Volatile compounds released during seed ageing have been associated with lipid peroxidation, alcoholic fermentation and Maillard reactions, and their accumulation can increase with storage induced deterioration (Colville *et al.*, 2012). Collectively, the PCA and cluster analysis indicate that Cluster 4 possessed comparatively greater seed longevity tolerance, characterized by better retention of germination and vigour, lower membrane leakage and oxidative damage, and stronger maintenance of antioxidant activity. Conversely, KGP-14-9 represented the most distinct and comparatively storage sensitive genotypes. These findings demonstrated that seed longevity was associated with the integrated behavior of physiological quality, membrane stability, oxidative damage and antioxidant defence rather than with seed germination alone.

CONCLUSION

Seed storage resulted decline in seed germination, seedling vigour and viability, accompanied by increased electrical conductivity of seed leachate, volatile aldehydes and malondialdehyde production and reduced antioxidant enzyme activities. The PCA revealed substantial variation among genotypes, with the first four principal components explaining 84.05% of the total variation, while hierarchical cluster analysis effectively differentiated the genotypes based on their physiological, lipid peroxidation and antioxidant responses. Genotypes GPM-6, TCR-1635, TCR-140, Indira Kulthi-1 and CRHG-8 exhibited comparatively better longevity through higher seed germination and vigour, better antioxidant activity and lower deterioration indicators. In contrast, CRHG-17, CRIDA-18R, IC-100938, VLG-8, CRHG-22 and KGP-14-9 showed comparatively poor longevity. Overall, the combined PCA and cluster analyses effectively identified variation in seed longevity and can be useful for characterizing horse gram genotypes for improved seed storage and breeding programmes. The observed changes are consistent with the established role of oxidative damage, membrane deterioration and antioxidant imbalance during seed ageing.

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Table 1. Physiological changes in freshly harvested and six months stored seeds of horse gram genotypes

Genotypes	SG		SVI I		SVI II		TW	
	Fresh seeds	6 MAS	Fresh seeds	6 MAS	Fresh seeds	6 MAS	Fresh seeds	6 MAS
GPM-6	99.50 (87.13)	93.25 (75.01)	2141	1742	9877	8883	2.63	2.64
TCR-1635	98.50 (84.04)	91.75 (73.34)	1636	1496	8323	7545	2.33	2.35
TCR-140	96.00 (78.91)	91.25 (72.85)	1897	1385	8548	7940	2.46	2.47
Indira Kulthi-1	99.25 (86.53)	90.75 (72.44)	1921	1622	9155	7926	2.51	2.48
CRHG-8	98.50 (84.04)	90.00 (71.68)	1842	1589	9214	8416	2.48	2.46
TRC-1503	95.50 (78.10)	87.00 (68.90)	1838	1454	8783	7960	2.56	2.43
GPM-4	97.00 (80.42)	86.50 (68.49)	1878	1529	9334	8001	2.60	2.49
PHG-2a	98.50 (84.04)	86.25 (68.26)	1864	1590	9208	7830	2.54	2.60
LONE-2	95.50 (77.85)	85.25 (67.46)	1607	1461	8643	7519	2.61	2.53
11-SS	97.00 (80.32)	85.00 (67.22)	1791	1593	8560	7224	2.44	2.39
AK-12-7	96.25 (78.90)	84.50 (66.83)	1560	1350	9313	7751	2.83	2.81
HL-1	96.50 (79.36)	84.50 (66.83)	1919	1481	9312	7926	2.69	2.60
KBHG-1	93.25 (75.07)	82.75 (65.47)	1876	1446	9351	7820	2.72	2.58
TCR-1690b	94.25 (76.16)	81.75 (64.74)	1895	1559	8292	7096	2.63	2.58
CRHG-17	95.50 (78.10)	78.25 (62.21)	1903	1351	8313	6513	2.45	2.28
CRIDA-18R	95.25 (77.56)	77.75 (61.86)	1762	1303	8788	7016	2.49	2.40
IC-100938	95.00 (77.36)	76.25 (60.84)	1637	1370	8527	6898	2.64	2.45
VLG-8	95.50 (77.85)	75.50 (60.34)	1847	1412	9476	7190	2.61	2.63
CRHG-22	93.00 (74.68)	74.50 (59.68)	1568	1123	8834	6817	2.50	2.45
KGP-14-9	92.50 (74.22)	71.75 (57.90)	1426	1085	7445	5559	2.06	2.08
Mean	**96.11 (79.53)**	**83.73 (66.61)**	**1786**	**1461**	**8900**	**7573**	**2.54**	**2.48**
S.Em (±)	**0.81 (1.50)**	**0.83 (0.70)**	**34.54**	**23.04**	**203.29**	**163.3**	**0.04**	**0.04**
CD @ 1%	**3.03 (5.66)**	**3.11 (2.63)**	**129.96**	**86.68**	**764.8**	**614.36**	**0.16**	**0.14**

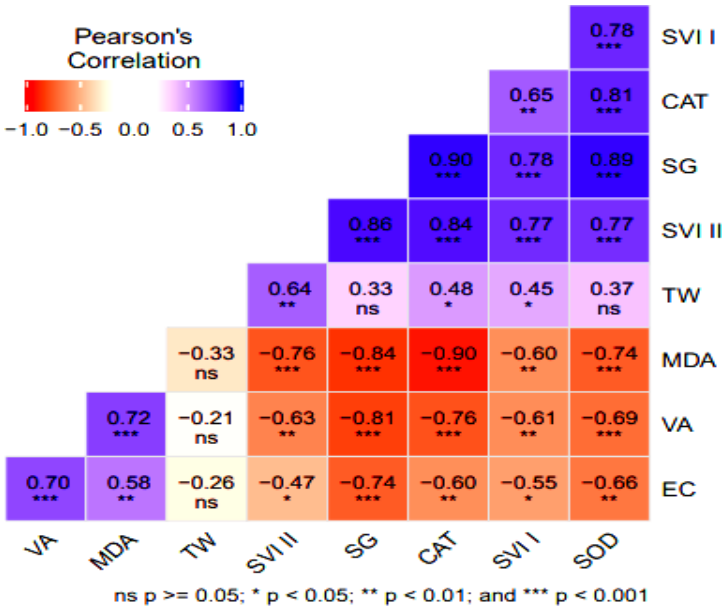
Note: MAS = Months After Storage, SG = Seed Germination, SVI = Seedling vigour Index, TW = Test Weight,

Table 2. Biochemical changes in freshly harvested and six months stored seeds of horse gram genotypes

Genotypes	EC		VA		MDA		SOD		CAT	
	Fresh seeds	6 MAS	Fresh seeds	6 MAS	Fresh seeds	6 MAS	Fresh seeds	6 MAS	Fresh seeds	6 MAS
GPM-6	35.47	51.68	0.31	0.39	3.26	3.84	1.19	1.10	167.82	162.18
TCR-1635	16.94	41.08	0.28	0.36	3.30	3.91	1.18	1.07	159.68	153.63
TCR-140	23.60	54.88	0.31	0.39	3.04	3.81	1.19	1.11	167.22	161.55
Indira Kulthi-1	28.54	52.72	0.23	0.37	3.33	3.93	1.19	1.11	155.64	151.39
CRHG-8	24.51	53.76	0.28	0.39	2.46	3.07	1.16	1.07	164.22	158.40
TRC-1503	26.02	67.62	0.29	0.50	3.26	3.83	1.15	1.05	159.72	153.67
GPM-4	22.42	61.74	0.24	0.34	3.15	4.16	1.14	1.04	157.43	151.27
PHG-2a	25.98	56.03	0.32	0.43	3.19	4.54	1.12	1.04	158.24	152.12
LONE-2	20.75	57.85	0.25	0.34	3.04	4.15	1.10	1.01	159.89	153.85
11-SS	18.39	49.37	0.29	0.39	4.02	5.03	1.16	1.06	147.63	140.97
AK-12-7	13.38	47.45	0.32	0.44	2.93	4.21	1.17	1.04	163.77	153.93
HL-1	29.80	64.88	0.22	0.38	2.54	3.70	1.12	1.02	166.68	160.99
KBHG-1	34.16	66.05	0.30	0.49	3.38	4.28	1.04	0.98	147.65	140.99
TCR-1690b	28.34	59.20	0.31	0.51	3.36	4.39	1.09	0.99	156.64	148.44
CRHG-17	26.46	62.89	0.33	0.52	3.94	5.76	1.08	0.90	149.10	130.16
CRIDA-18R	34.07	72.90	0.25	0.51	3.77	6.13	1.15	0.85	153.78	132.93
IC-100938	20.44	71.05	0.31	0.55	4.43	6.11	1.11	1.03	156.92	131.82
VLG-8	25.73	66.08	0.30	0.52	3.50	5.48	1.09	0.89	154.20	127.87
CRHG-22	33.18	66.72	0.26	0.53	4.01	5.75	1.07	0.81	158.36	133.54
KGP-14-9	13.96	68.99	0.26	0.52	3.72	5.24	1.05	0.83	153.20	126.22
Mean	**25.11**	**58.73**	**0.28**	**0.43**	**3.33**	**4.41**	**1.13**	**1.01**	**157.89**	**146.30**
S.Em (±)	**0.71**	**1.39**	**0.01**	**0.01**	**0.04**	**0.09**	**0.02**	**0.01**	**4.20**	**3.36**
CD @ 1%	**2.73**	**5.33**	**0.02**	**0.04**	**0.17**	**0.34**	**0.07**	**0.05**	**16.06**	**12.86**

Note: MAS = Months After Storage, EC = Electrical Conductivity of seed leachate, VA = Volatile Aldehydes Production, MDA = Malondialdehyde Production, SOD = Superoxide Dismutase Activity, CAT = Catalase Activity

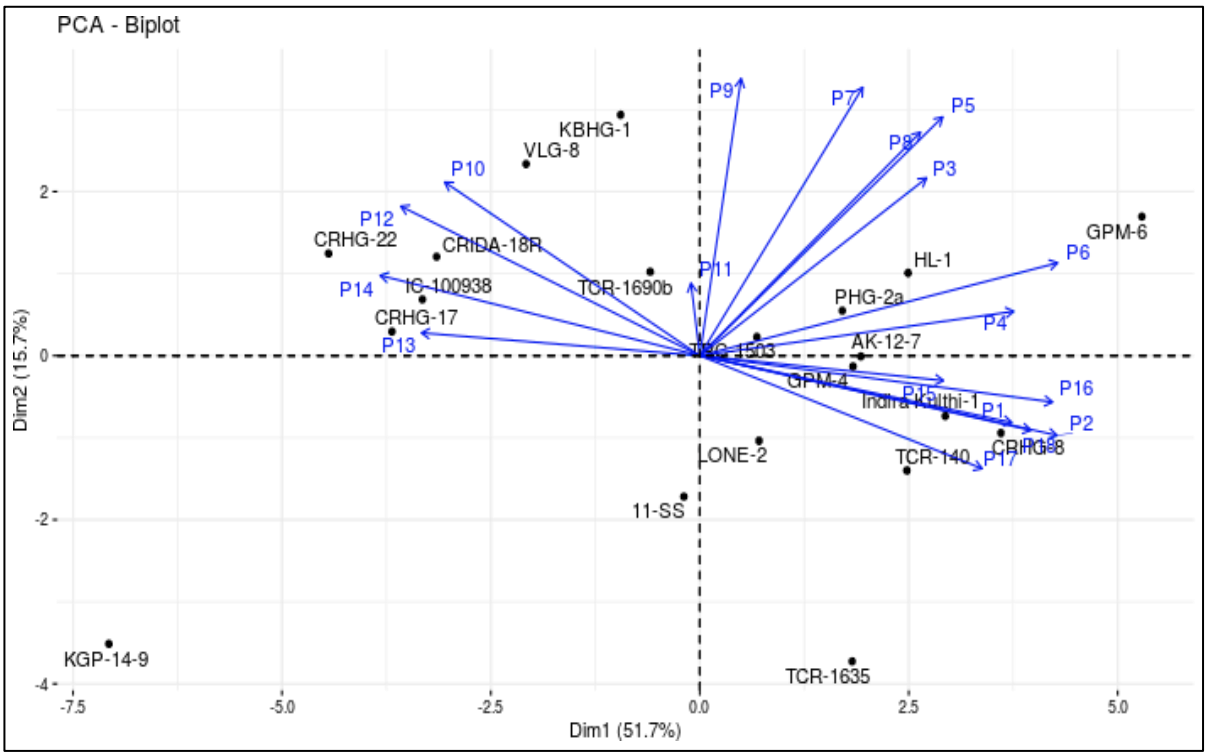
Figure 1: Pearson's correlation heat map between physiological and biochemical changes during seed storage of horse gram genotypes



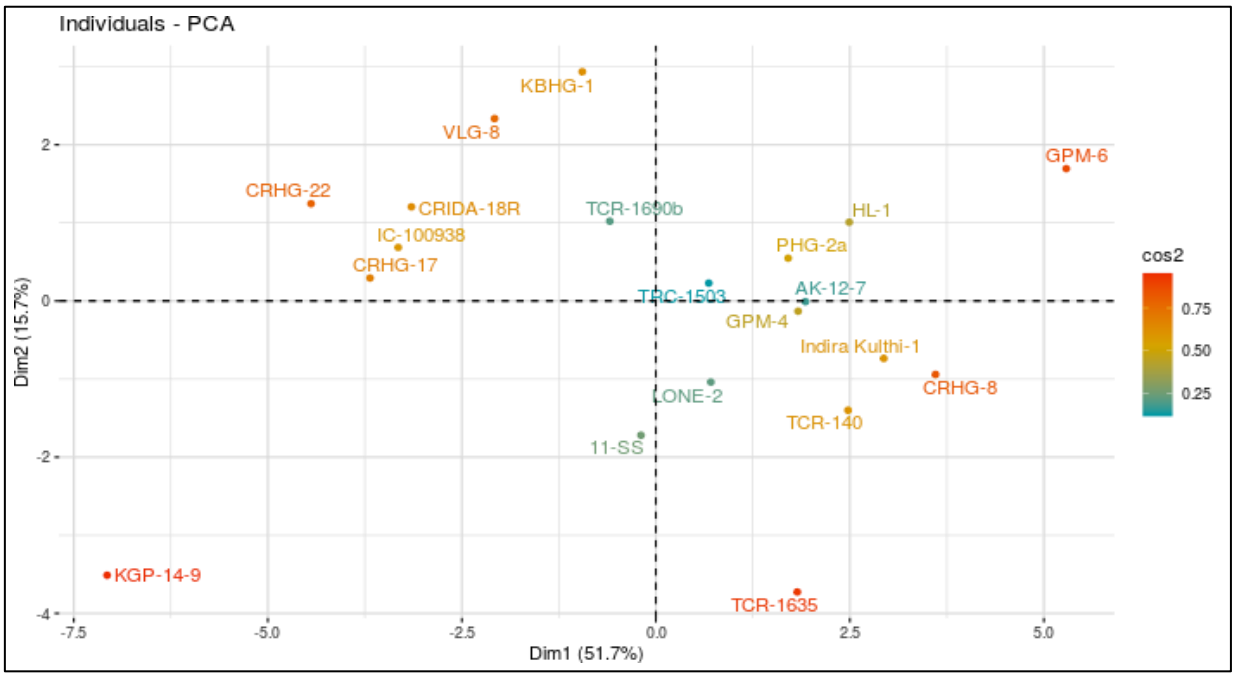
Note: SG = Seed Germination, SVI = Seedling vigour Index, TW = Test Weight, EC = Electrical Conductivity of seed leachate, VA = Volatile Aldehydes Production, MDA = Malondialdehyde Production, SOD = Superoxide

Dismutase Activity, CAT = Catalase Activity

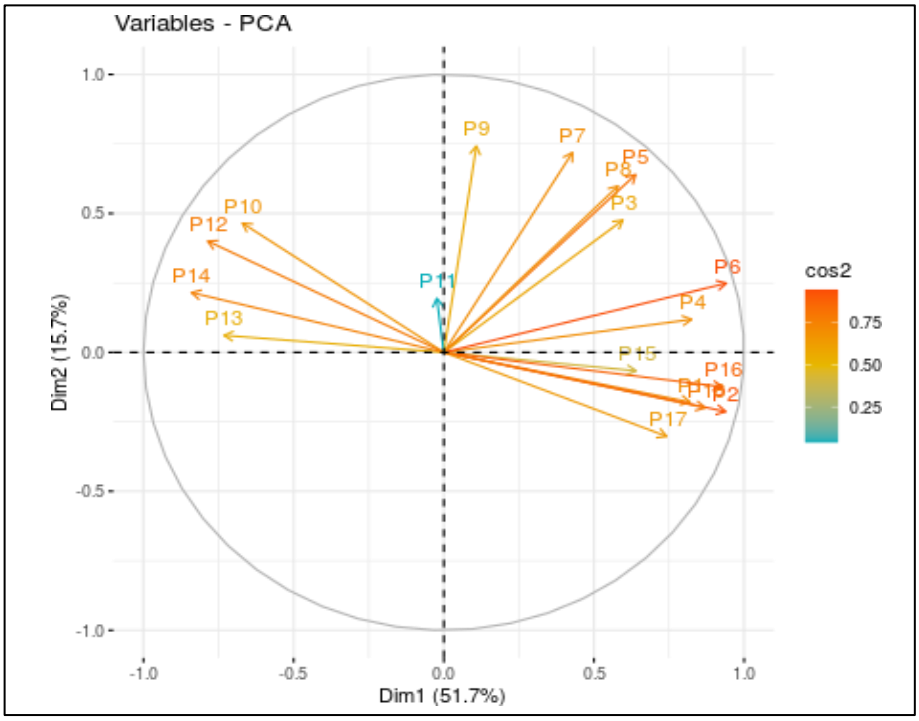
Figure 2: Principal component analysis biplot, individual PCA and variable PCA showing the association among physiological and biochemical traits of horse gram genotypes during storage



(a) PCA biplot



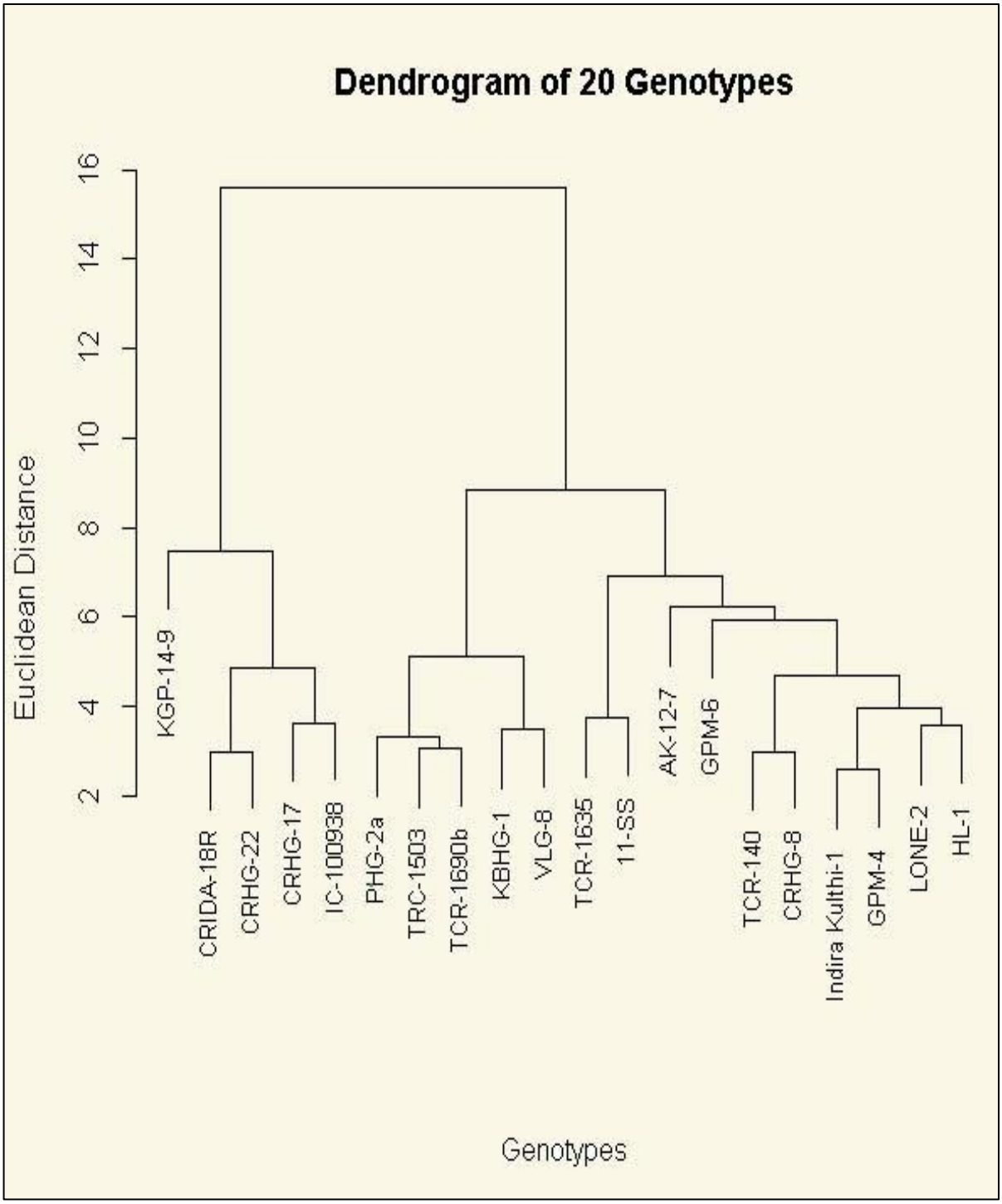
(b) Individuals PCA



(c) Variables PCA

P1 - Seed germination (%) of fresh seeds, P2 - Seed germination (%) after six months of storage of seeds, P3 - Seedling vigour index – I of fresh seeds, P4 - Seedling vigour index – I after six months of storage of seeds, P5 - Seedling vigour index – II of fresh seeds, P6 - Seedling vigour index – II after six months of storage of seeds, P7 - Test weight of fresh seeds, P8 - Test weight of seeds after six months of storage, P9 - Electrical conductivity of seed leachate of fresh seeds, P10 - Electrical conductivity of seed leachate after six months of storage of seeds, P11 - Volatile aldehydes production of fresh seeds, P12 - Volatile aldehydes production after six months of storage of seeds, P13 - Malondialdehyde production of fresh seeds, P14 - Malondialdehyde production after six months of storage of seeds, P15 - Catalase activity of fresh seeds, P16 - Catalase activity after six months of storage of seeds, P17 - Superoxide dismutase activity of fresh seeds, P18 - Superoxide dismutase activity after six months of storage of seeds

Figure 3. Hierarchical cluster analysis of horse gram genotypes based on physiological and biochemical traits during seed storage



Supplementary Table 1. Eigenvalues, percentage variance and cumulative percentage variance of principal components of horse gram genotypes

Principal component	PCA eigenvalue	Variance (%)	Cumulative variance (%)
PC1	9.30	51.68	51.68
PC2	2.83	15.72	67.40
PC3	1.59	8.82	76.22
PC4	1.41	7.82	84.04
PC5	0.81	4.48	88.52
PC6	0.71	3.95	92.47
PC7	0.40	2.25	94.72
PC8	0.29	1.59	96.31
PC9	0.20	1.13	97.44
PC10	0.16	0.87	98.31
PC11	0.13	0.72	99.03
PC12	0.07	0.39	99.42
PC13	0.05	0.26	99.68
PC14	0.03	0.18	99.86
PC15	0.02	0.08	99.94
PC16	0.01	0.04	99.98
PC17	0.00	0.01	99.99
PC18	0.00	0.00	100.00

P1 - Seed germination (%) of fresh seeds, P2 - Seed germination (%) after six months of storage of seeds, P3 - Seedling vigour index – I of fresh seeds, P4 - Seedling vigour index – I after six months of storage of seeds, P5 - Seedling vigour index – II of fresh seeds, P6 - Seedling vigour index – II after six months of storage of seeds, P7 - Test weight of fresh seeds, P8 - Test weight of seeds after six months of storage, P9 - Electrical conductivity of seed leachate of fresh seeds, P10 - Electrical conductivity of seed leachate after six months of storage of seeds, P11 - Volatile aldehydes production of fresh seeds, P12 - Volatile aldehydes production after six months of storage of seeds, P13 - Malondialdehyde production of fresh seeds, P14 - Malondialdehyde production after six months of storage of seeds, P15 - Catalase activity of fresh seeds, P16 - Catalase activity after six months of storage of seeds, P17 - Superoxide dismutase activity of fresh seeds, P18 - Superoxide dismutase activity after six months of storage of seeds

Supplementary Table 2. Principal component scores of the 20 horse gram genotypes based on PC1–PC5 and cluster category

Genotype	PC1	PC2	PC3	PC4	PC5	Cluster
GPM-6	5.29	1.70	1.19	-0.81	0.62	4
TCR-1635	1.82	-3.73	0.74	0.14	-0.22	4
TCR-140	2.48	-1.40	-0.12	0.47	1.67	4
Indira Kulthi-1	2.94	-0.74	0.44	-1.91	-1.25	4
CRHG-8	3.60	-0.94	-0.75	-0.33	0.78	4
TRC-1503	0.68	0.23	-0.28	-0.15	1.26	3
GPM-4	1.83	-0.13	-0.81	-0.88	-1.15	4
PHG-2a	1.71	0.55	1.06	0.37	0.23	3
LONE-2	0.71	-1.04	-1.67	0.57	-0.75	4
11-SS	-0.19	-1.72	2.46	-0.39	-1.27	4
AK-12-7	1.93	-0.01	-0.49	4.03	-0.94	4
HL-1	2.49	1.01	-2.85	-0.76	-0.09	4
KBHG-1	-0.95	2.94	-0.05	-0.09	0.45	3
TCR-1690b	-0.59	1.02	0.47	0.59	1.31	3
CRHG-17	-3.68	0.29	2.02	-0.54	0.84	1
CRIDA-18R	-3.15	1.21	-0.34	-1.65	-0.77	1
IC-100938	-3.32	0.69	1.33	1.21	-0.50	1
VLG-8	-2.08	2.34	0.36	0.53	-0.70	3
CRHG-22	-4.44	1.25	-1.45	-0.08	-0.32	1
KGP-14-9	-7.07	-3.51	-1.26	-0.32	0.80	2

PC: principal component, P1 - Seed germination (%) of fresh seeds, P2 - Seed germination (%) after six months of storage of seeds, P3 - Seedling vigour index – I of fresh seeds, P4 - Seedling vigour index – I after six months of storage of seeds, P5 - Seedling vigour index – II of fresh seeds, P6 - Seedling vigour index – II after six months of storage of seeds, P7 - Test weight of fresh seeds, P8 - Test weight of seeds after six months of storage, P9 - Electrical conductivity of seed leachate of fresh seeds, P10 - Electrical conductivity of seed leachate after six months of storage of seeds, P11 - Volatile aldehydes production of fresh seeds, P12 - Volatile aldehydes production after six months of storage of seeds, P13 - Malondialdehyde production of fresh seeds, P14 - Malondialdehyde production after six months of storage of seeds, P15 - Catalase activity of fresh seeds, P16 - Catalase activity after six months of storage of seeds, P17 - Superoxide dismutase activity of fresh seeds, P18 - Superoxide dismutase activity after six months of storage of seeds

Supplementary Table 3. **Variable loadings and percentage contribution of the 18 seed quality, deterioration and antioxidant traits to PC1–PC4**

Variables	PC1 loading	PC1 contribution (%)	PC2 loading	PC2 contribution (%)	PC3 loading	PC3 contribution (%)	PC4 loading	PC4 contribution (%)
P1	-0.27	7.23	-0.11	1.13	0.24	5.80	0.16	2.53
P2	-0.31	9.48	-0.13	1.61	0.09	0.85	0.09	0.74
P3	-0.20	3.82	0.28	8.00	0.24	5.66	0.37	13.48
P4	-0.27	7.32	0.07	0.50	0.29	8.58	0.17	2.85
P5	-0.21	4.38	0.38	14.45	-0.05	0.29	0.01	0.01
P6	-0.31	9.51	0.15	2.19	-0.03	0.07	0.04	0.15
P7	-0.14	1.97	0.43	18.26	-0.06	0.37	-0.34	11.64
P8	-0.19	3.62	0.36	12.66	-0.07	0.50	-0.40	15.73
P9	-0.04	0.13	0.44	19.47	-0.04	0.17	0.46	20.87
P10	0.22	4.84	0.28	7.64	-0.24	5.60	0.21	4.20
P11	0.01	0.01	0.12	1.33	0.53	27.67	-0.46	21.11
P12	0.26	6.64	0.24	5.66	0.12	1.50	-0.13	1.71
P13	0.24	5.76	0.04	0.13	0.42	17.36	0.07	0.47
P14	0.28	7.59	0.13	1.63	0.22	4.99	0.01	0.00
P15	-0.21	4.41	-0.04	0.16	-0.34	11.55	-0.19	3.54
P16	-0.30	9.25	-0.07	0.55	-0.17	2.96	-0.05	0.26
P17	-0.24	5.92	-0.18	3.23	0.16	2.63	0.04	0.15
P18	-0.29	8.15	-0.12	1.41	0.19	3.46	-0.08	0.56

PC: principal component, P1 - Seed germination (%) of fresh seeds, P2 - Seed germination (%) after six months of storage of seeds, P3 - Seedling vigour index – I of fresh seeds, P4 - Seedling vigour index – I after six months of storage of seeds, P5 - Seedling vigour index – II of fresh seeds, P6 - Seedling vigour index – II after six months of storage of seeds, P7 - Test weight of fresh seeds, P8 - Test weight of seeds after six months of storage, P9 - Electrical conductivity of seed leachate of fresh seeds, P10 - Electrical conductivity of seed leachate after six months of storage of seeds, P11 - Volatile aldehydes production of fresh seeds, P12 - Volatile aldehydes production after six months of storage of seeds, P13 - Malondialdehyde production of fresh seeds, P14 - Malondialdehyde production after six months of storage of seeds, P15 - Catalase activity of fresh seeds, P16 - Catalase activity after six months of storage of seeds, P17 - Superoxide dismutase activity of fresh seeds, P18 - Superoxide dismutase activity after six months of storage of seeds

Supplementary Table 4. Cluster membership of the 20 horse gram genotypes obtained through hierarchical cluster analysis

Cluster	Seed lot	No. of genotypes
Cluster 1	CRHG-17	4
	CRIDA-18R	
	IC-100938	
	CRHG-22	
Cluster 2	KGP-14-9	1
Cluster 3	TRC-1503	5
	PHG-2a	
	KBHG-1	
	TCR-1690b	
	VLG-8	
Cluster 4	GPM-6	10
	TCR-1635	
	TCR-140	
	Indira Kulthi-1	
	CRHG-8	
	GPM-4	
	LONE-2	
	11-SS	
	AK-12-7	
	HL-1	

Supplementary Table 5. Cluster-wise mean values of physiological and biochemical traits of the horse gram genotypes

Cluster	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
1	94.69	76.69	1717.50	1286.75	8615.50	6811.00	2.52	2.40	28.54	68.39	0.29	0.53	4.04	5.94	154.54	132.11	1.10	0.90
2	92.50	71.75	1426.00	1085.00	7445.00	5559.00	2.06	2.08	13.96	68.99	0.26	0.52	3.72	5.24	153.20	126.22	1.05	0.83
3	95.40	82.65	1864.00	1492.20	9022.00	7579.20	2.61	2.56	28.05	63.00	0.30	0.49	3.34	4.50	155.29	144.62	1.10	0.99
4	97.40	88.28	1819.20	1524.80	9027.90	7913.10	2.56	2.52	23.38	53.54	0.27	0.38	3.11	3.98	161.00	154.82	1.16	1.06

P1 - Seed germination (%) of fresh seeds, P2 - Seed germination (%) after six months of storage of seeds, P3 - Seedling vigour index – I of fresh seeds, P4 - Seedling vigour index – I after six months of storage of seeds, P5 - Seedling vigour index – II of fresh seeds, P6 - Seedling vigour index – II after six months of storage of seeds, P7 - Test weight of fresh seeds, P8 - Test weight of seeds after six months of storage, P9 - Electrical conductivity of seed leachate of fresh seeds, P10 - Electrical conductivity of seed leachate after six months of storage of seeds, P11 - Volatile aldehydes production of fresh seeds, P12 - Volatile aldehydes production after six months of storage of seeds, P13 - Malondialdehyde production of fresh seeds, P14 - Malondialdehyde production after six months of storage of seeds, P15 - Catalase activity of fresh seeds, P16 - Catalase activity after six months of storage of seeds, P17 - Superoxide dismutase activity of fresh seeds, P18 - Superoxide dismutase activity after six months of storage of seed