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Impact of physio-biochemical responses on amelioration of short-term storage and management of post-harvest seed quality of greengram (*Vigna radiata*)

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ABSTRACT

The present study was carried out during winter (*rabi*) and rainy (*kharif*) seasons of 2021 and 2022 at Assam Agricultural University, Jorhat, Assam to address the significance of maintaining post-harvest seed quality and its management throughout the nation, and to promote the knowledge and create awareness among the farming communities and pulse growers at the national level in terms of the area with high rainfall and high relative humidity. The greengram [*Vigna radiata* (L.) R. Wilczek] variety (SGC-16) seeds were stored in 4 different types of packaging materials, viz. jute bag, polylined jute bags; HDPE (High Density Polyethylene) interwoven bags; and 700-gauze polythene bags and treated with treatment combination of emamectin benzoate (40 mg/kg) (chemical based); 5 ml/kg neem oil (plant based); 3 g/kg black pepper (recommended practise for the state); and 3 g/kg silica gel (desiccants). With an increase in storage time, a gradual decline in seed quality parameters such as viability, germination, seedling length, seedling dry weight, Seed vigour index-I, Seed vigour index-II, germination index value, field emergence, and chlorophyll content was observed. Variations for biochemical indicators of seed quality were also recorded. The seed germination above Indian Minimum Seed Certification Standard (IMSCS) level was maintained by all treatments up to 120 days of storage. However, emamectin benzoate (40 mg/kg) along with 3 g/kg of silica gel in HDPE interwoven bags found to be best in keeping seed quality standards with lowest lipid peroxidation activity (0.36 g/gm) and highest in seeds stored in jute bag (0.68 g/gm).

Keywords: α-amylase, Dehydrogenase activity, Emamectin benzoate, HDPE interwoven bags, Lipid peroxidation, Seed storage

Pulses are referred as the poor man's meat since these are among the most affordable plant-based sources of protein (23–24%), vitamins, complex carbohydrates (54–56%) and fibre (Hou *et al.* 2019). The world's total acreage planted to pulses is approximately 93.18 (Mha), yielding 89.82 (Mt) at a yield level of 964 kg/ha and India is the world's largest producer of pulses, with a cultivated area of around 28 million hectares (Anonymous 2022). As per report (Anonymous 2022), Madhya Pradesh (3.84 Lha), Andhra Pradesh and Punjab (0.30 Lha), Gujarat (0.19 Lha), Tamil Nadu (0.12 Lha) and Uttar Pradesh (0.11 Lha) have reported higher areas than the same period of the previous year, 2021. On the other hand, as per the statistical data, the production volume of pulses in the north-eastern Indian states, especially Assam, increased from the 2009 financial year to approximately 108 thousand metric tonnes in the 2021 financial year (Minhas 2022).

The leguminous plant species known as greengram [*Vigna radiata* (L.) R. Wilczek] is a member of the Fabaceae family, also referred as moong or mungbean. As a seasonal crop, greengram seeds undergo ageing from the time of harvest to the following planting season. The north-eastern region, including Assam, is known for its poor storability because of its hot and humid climate in addition to high rainfall as per the meteorological data. In India, limited technology, ignorance, and improper storage conditions causes a large amount of produce to be wasted during the post-harvest process. A number of variables, including moisture content, relative humidity in the area around seed storage, temperature during seed drying, kinds of packaging materials and length of storage, are directly related to this condition (Khatun *et al.* 2009).

Therefore, the current study was carried out using various packaging materials for different storage periods of greengram seeds in order to determine comparative effect of chemical and plant-based seed treatments on physiological and biochemical quality of seeds during storage.

MATERIALS AND METHODS

Experimental materials and storage conditions: Present study was carried out during winter (*rabi*) and rainy (*kharif*) seasons of 2021 and 2022 at Assam Agricultural University, Jorhat, Assam. Greengram variety (SGC-16) seeds containing 5 kg/bag were acquired from Regional

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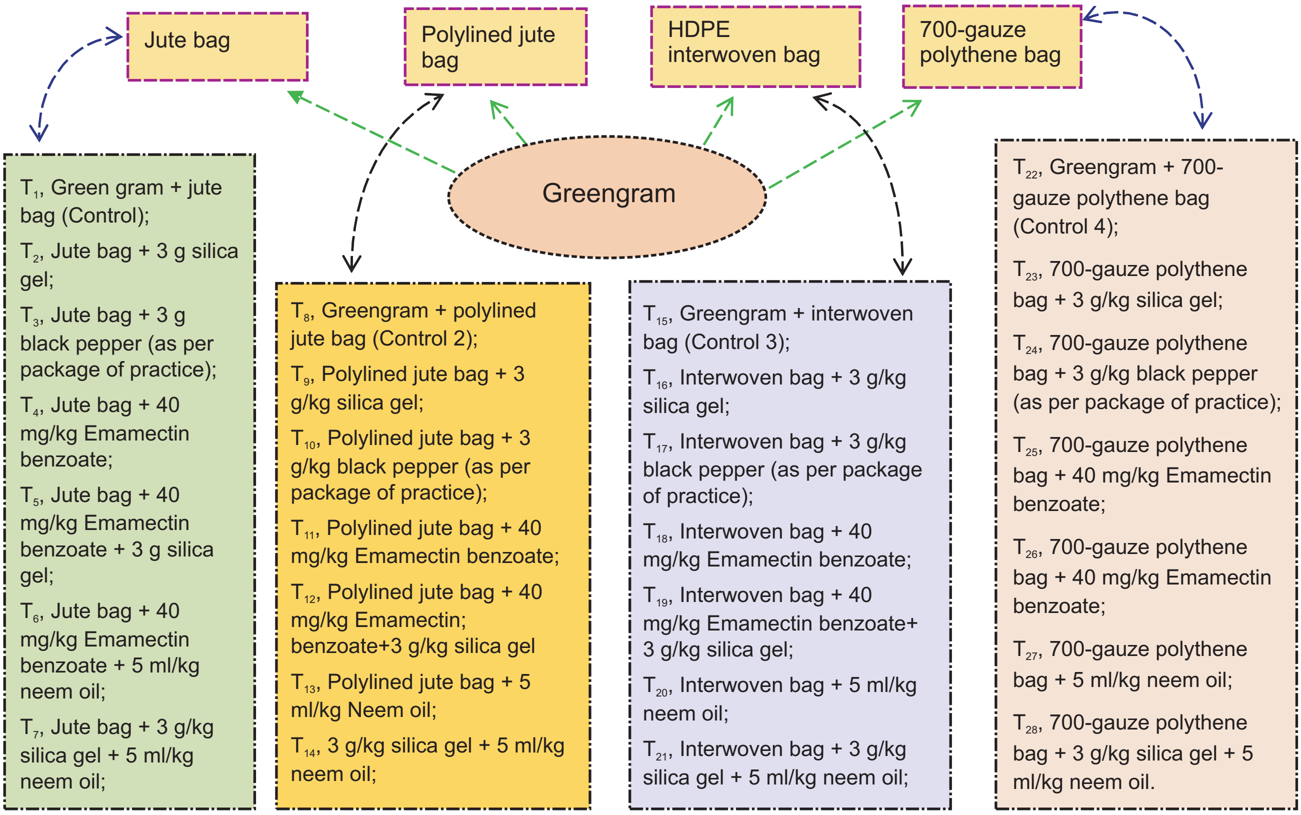

Fig. 1 Twenty-eight (28) treatments employed in the study.

Agricultural Research Station (Assam Agricultural University), Shillongoni, Nagaon, Assam. Based on the rainfall pattern and cropping season, the study was conducted two times. Four distinct kinds of packaging materials were used in the storage investigation. The representative samples were taken from the freshly harvested seed lots and were pooled using sleeve type triers for estimation. The meteorological weather report for the year 2021 and 2022 were collected from Assam Agricultural University, Jorhat, Assam. Packing materials for experiments mostly consisted of 700-gauze polythene bags of size 0.175 mm, HDPE (High Density Polyethylene) interwoven bags of width 15 cm × 33 cm; jute bags of size 32 cm × 18 cm; and polylined jute bags of size 33 cm × 14 cm. In addition to the packing materials, treatments included 40 mg/kg of chemical-based emamectin benzoate 5 ml/kg of entirely plant-based neem oil; 3 g/kg of black pepper (recommended for the state); and 3 g/kg of silica gel (40–63 µm particle size) in the form of desiccants. A total of 28 treatments were employed in the study (Fig. 1).

Seed viability percentage was determined by measuring the staining pattern's intensity using Tetrazolium test (Lakon 1942) and the germination percentage was determined by following protocol ISTA (2004). Total of 10 seedlings were taken and oven dried to calculate the mean length of the seedlings, which was then measured in milligram using an electronic scale. Calculation of the seed vigour index I and II followed the guidelines provided by Agarwal (1976) and Copeland and McDonald (1985). The Association of Official Seed Analysts' (1983) method was used to determine the germination index (GI). The Arnon (1949) method was used to determine the total chlorophyll concentration using spectrophotometer. To compute the field emergence percentage, observations recorded in the experimental plot were used as a basis for calculations up to a 15-day period. Lipid peroxidation value was expressed in µg/g (Heath and Packer 1968). Dehydrogenase activity was calculated and reported in terms of optical density, or OD values at 480 nm, using Kittock and Law's (1968) methodology with a few minor adjustments. The alpha amylase enzyme activity was measured in mg/maltose released/min (Khan and Faust 1967). Using a digital conductivity meter, the electrical conductivity of the seed leachate was measured and estimated in µS/cm/g using the ISTA (2019) technique.

Statistical analysis: Based on the physiological and biochemical studies, recorded data were subjected to statistical analysis using MS Excel 2016 following completely randomized block design (CRBD) with 3 replications. The mean values were compared by least significant difference based on ANOVA (Gomez and Gomez 1984). The correlation coefficient between different parameters was also worked out using OP STAT software with the help of using Pearson's correlation formula. Ranking of each mean values for different treatments were done at CD (*P*=0.05%) level.

RESULTS AND DISCUSSION

Seed physiological quality parameters: Throughout the whole storage period (270 days after storage) T_{19} HDPE bags Emamectin benzoate (40 mg/kg) + silica gel at 3 g/kg was found to be a better storage treatment with the increase of least amount of moisture content (9.56%). Our results corroborate with the findings of other researchers (Odokonyero *et al.* 2021) who confirmed that jute bags are vapour and moisture permeable, which may allow the seeds inside to absorb more moisture from the air as the temperature changes. The length of seed storage and the treatments had a major impact on the viability of the seeds (Table 1, Fig. 2a). Seeds stored under T_{19} HDPE interwoven bags with Emamectin benzoate [40 mg/kg+silica gel (3 gm/kg)] demonstrated the highest viability percentage among the treatments (93.1%), however, T_1 (jute bag) demonstrated the lowest percentage with a value of 64.0%, respectively after storage. The best treatment within jute bags was confirmed to be T_5 (75.00%) as per the need of Indian Minimum Seed Certification standard (IMSCS). However, viability of the greengram seeds decreased with increasing storage time, i.e. at 270 days of storage, which is consistent with the findings of Sanjay *et al.* (2023) in greengram. The initial germination was recorded to be 86.33% and at 240 days after storage, the seeds kept in jute bag, both with and without fungicide, exhibited germination below the IMSCS limit. After 270 days after storage, seeds treated with T_{19} [HDPE interwoven bags with Emamectin benzoate (40 mg/kg) and silica gel (3 gm/kg)] had the highest germination percentage (81.9%), while T_1 (jute bag) had the lowest (52.33%) value (Fig. 2b). Our findings were in agreement with Satasiya *et al.* (2021) who opined that seeds packaged in 700-gauze polythene bags may retain a high germination percentage. The seedlings' initial length was measured to be 28.28 cm and when treated with T_{19} [HDPE interwoven bags containing 40 mg/kg of Emmemactin benzoate and 3 g/kg of silica gel] had the highest average seedling length (18.80 cm) at 270 days after storage, while T_1 (jute bag) had the lowest value (9.20 cm). During the whole storage period (270 DAS), T_{19} was found to have the highest SVI-I (1539.72) (Table 1) and T_1 had the lowest SVI-I (481.44). A decreasing trend in the value of seedling vigour Index-I was observed in T_1 (481.44), that involved jute bags, T_8 (indicating polylined jute bags, 667.28), T_{16} (HDPE interwoven bags) (1026.68), and T_{22} (754.80) stored in 700-gauze polythene bags. The HDPE interwoven bags with 40 mg/kg of Emamectin benzoate combined with 3 gm/kg of silica gel treatments (T_{19}) had the highest average shoot (14.40 cm) and root length (4.40 cm) of all the treatment combinations. T_1 (jute bag) had the shortest shoot (9.77 cm) and root (3.08 cm) length at the end of the storage period (Table 1). The initial drying weight was recorded to be 210 mg and the treatment T_{19} fortified with HDPE interwoven bags along with 40 mg/kg of Emamectin benzoate and silica pouch had the highest seedling dry weight (185.64 mg), whereas T_4 (jute bag) had the lowest value (173.88 mg) (Table 1). At the end of the 9-month storage period, T_{19}'s SVI-II, which had a value of 15203.9 (HDPE bags combined with seed treatment with 40 mg/kg of Emamectin benzoate and 3 gm/kg of silica gel), was significantly greater than the other treatments. It was remarkably observed that the maximum value of SVI-II was found to be present in the seeds stored in jute bags combined with 40 mg/kg Emamectin benzoate along with 3 gm silica gel (T_5) (13164.9) and lowest value (6084.41) was found in treatment T_1 (jute bags). In terms of germination index, the maximum value (35) was found in T_{19} fortified with HDPE interwoven bags added to Emamectin benzoate (40 mg/kg) along with silica gel (3 gm/kg) (Table 1) and T_1, had the lowest values (12.90). Seeds treated with a comparable procedure (T_{19}), particularly those packaged in HDPE interwoven bags with 40 mg/kg of Emamectin benzoate and 3 g/kg of silica gel, had the greatest value (35.11) of chlorophyll content at a 270-day interval. The lowest value (4.08) was obtained from the seeds stored in jute bags without any treatments (T_1) (Table 1). Regarding

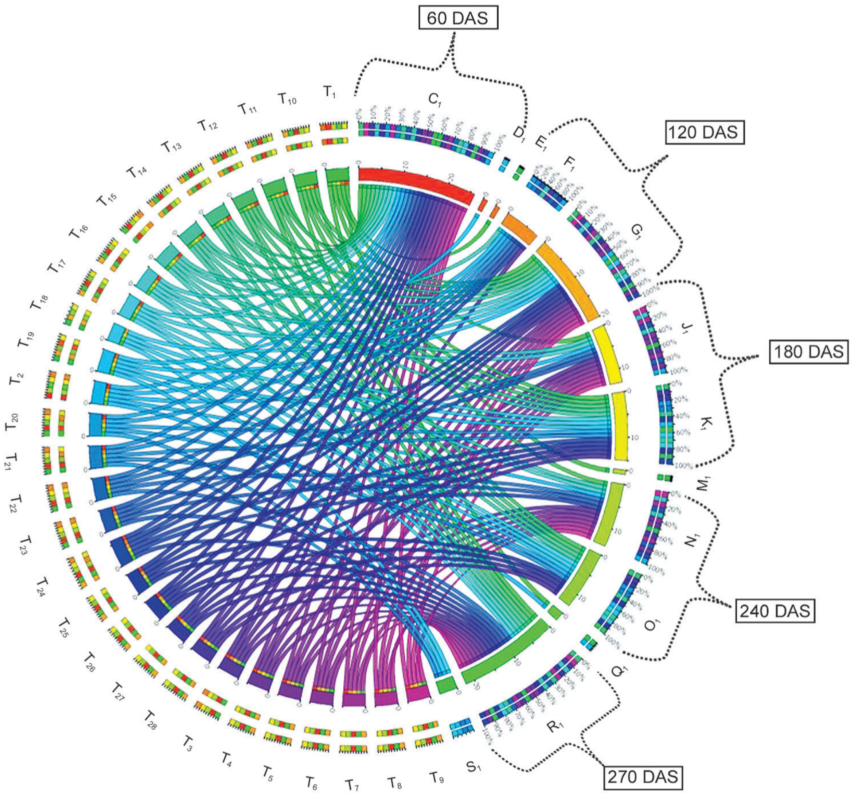

Fig. 2a Circos plot representation on effect of greengram seeds on viability percentage at 60, 120, 180, 240 and 270 DAS (days after storage).
Treatment details are given under Materials and Methods. C_1, D_1, E_1, F_1, G_1, J_1, K_1, M_1, N_1, O_1, Q_1, R_1 and S_1 classifies viability percentage obtained individually from the treatments T_1–T_{28} kept at different storage periods (60, 120, 180, 240 and 270 DAS).

Table 1 Best and least effective treatments among the packaging materials for morpho-physiological and biochemical characters throughout the storage period

Parameter	Highest value							Lowest value						
	Treatment	Initial value	60 DAS	120 DAS	180 DAS	240 DAS	270 DAS	Treatment	Initial value	60 DAS	120 DAS	180 DAS	240 DAS	270 DAS
Moisture content	T_1	9.5	11.0	11.8	13.0	14.6	15.0	T_{19}	9.5	9.6	9.8	10.0	12.2	13.4
Viability test (%)	T_{19}	93.3	93.0	90.0	88.0	85.5	83.6	T_1	93.3	83.4	80.2	72.7	68.3	64.5
Germination (%)	T_{19}	86.3	86.0	84.5	83.7	82.4	81.9	T_1	86.3	75.2	68.0	58.7	55.3	52.3
Seedling length (cm)	T_{19}	28.3	27.5	26.0	24.9	22.4	18.8	T_1	28.3	16.9	14.5	14.0	12.9	9.2
Seed vigour index-I	T_{19}	2441.4	2363.3	2164.9	2086.2	1849.1	1539.7	T_1	2441.4	1268.6	982.6	820.2	711.0	481.4
Shoot length (cm)	T_{19}	22.8	22.4	21.0	20.2	17.8	14.4	T_1	22.8	12.9	10.9	10.7	9.8	6.2
Root length (cm)	T_{19}	5.5	5.1	5.0	4.8	4.6	4.4	T_1	5.5	4.0	3.5	3.3	3.1	3.0
Seedling dry weight (mg)	T_{19}	210.0	199.7	197.5	194.2	185.7	185.6	T_1	210.0	188.9	175.3	150.3	136.0	116.3
Seed vigour index-II	T_{19}	18129.3	17147.7	16626	16130	15264.5	15148.2	T_1	18129.3	14202.2	11917.6	8816.9	7524.8	6084.4
Germination index	T_{19}	36.2	35.82	35.6	35.41	35.18	35.11	T_1	36.2	24.27	24.08	18.2	16	12.9
Chlorophyll content	T_{19}	18.18	18.14	18.08	18	17.88	17.76	T_1	18.18	7.12	5.66	5.03	4.67	4.08
α-amylase	T_{19}	0.62	0.6	0.56	0.52	0.5	0.47	T_1	0.62	0.4	0.2	0.14	0.1	0.08
Dehydrogenase activity	T_{19}	0.682	0.676	0.664	0.658	0.640	0.636	T_1	0.682	0.610	0.442	0.368	0.332	0.312
Peroxidase enzyme activity	T_1	0.1	0.34	0.39	0.58	0.64	0.68	T_{19}	0.1	0.13	0.15	0.3	0.32	0.36
Electrical conductivity	T_1	0.203	0.268	0.408	0.61	0.648	0.682	T_{19}	0.203	0.207	0.212	0.234	0.286	0.306
Germination index	T_{19}	36.2	35.82	35.6	35.41	35.18	35.11	T_1	36.2	24.27	24.08	18.2	16	12.9

Treatment details are given under Materials and Methods. DAS, Days after sowing.

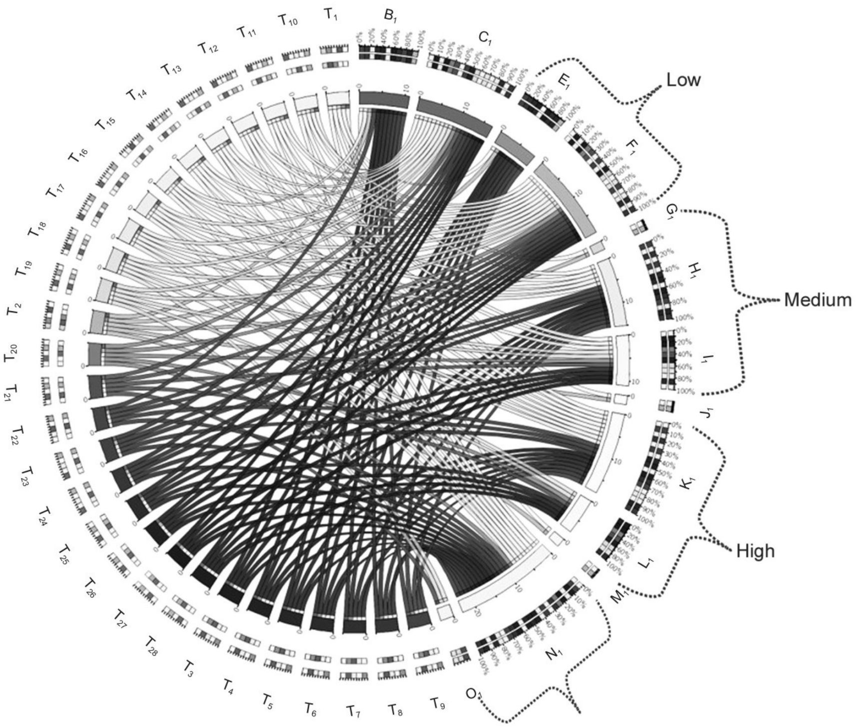

Fig. 2b Circos plot representation on effect of greengram seeds on germination percentage at 60, 120, 180, 240 and 270 DAS (days after storage).
Treatment details are given under Materials and Methods. E_1, F_1, G_1, H_1, I_1, J_1, K_1, L_1, M_1, N_1 and O_1 signifies the germination percentage obtained individually from the treatments T_1–T_{28} at different storage period (60, 120, 180, 240 and 270 DAS); E_1–F_1 indicates low germination percentage obtained among the treatments (T_1–T_{28}); G_1-I_1 signifies average germination percentage; and K_1–O_1 indicated higher germination percentage obtained among the treatments (T_1–T_{28}).

treatment effect, seeds treated with Emamectin benzoate (40 mg/kg and 3 gm/kg silica) stored in HDPE interwoven bags had the maximum chlorophyll content which corroborate with the results of Marichamy *et al.* (2020) who recorded the highest total chlorophyll content in amaranth seeds after 4 days of storage packed in 200-gauze polyethylene bag. The treatment T_{19} (HDPE interwoven bags in combination with 40 mg/kg of Emamectin benzoate and silica pouch) had the highest field emergence (77.88%) whereas T_1 (jute bag) had the lowest value (52.0%). The findings of our studies corroborate with those from the results obtained by Monira *et al.* (2012).

Estimation of Biochemical indicators

Lipid peroxidation value (µg/g): According to the observations made, the T_1 (jute bag without treatments) sample had the highest MDA content (0.68 g/gm) (Table 1). However, T_{19} (HDPE interwoven bags with Emamectin benzoate (40 mg/kg along with 3 gm/kg of silica gel) had the lowest MDA concentration (00.32 g/gm). The findings of Xing *et al.* (2023) confirmed that an increase in lipid peroxidation value is associated with decreased vigour and germination that was supported by our results. Similar findings showed that increasing accumulation of total peroxide and malon-di-aldehyde content was positively connected with lower germinability, as reported by Onder *et al.* (2022).

Alpha-amylase enzyme activity (maltose released/min in mg): Interestingly, the T_{19} had shown better performance in terms of α-amylase enzyme activity with a value of 0.6 mg maltose released/min; whereas, the minimum value was 0.08 mg (T_1), respectively (Table 1). Our results are supported by the findings of Nie *et al.* (2022) in rice, who clearly indicated that α-amylase is the most important enzyme and its activity is highly associated with germination percentage and seedling growth parameters in rice. While using Kittock and Law's (1968) method, initial OD value was recorded to be 0.682 and the

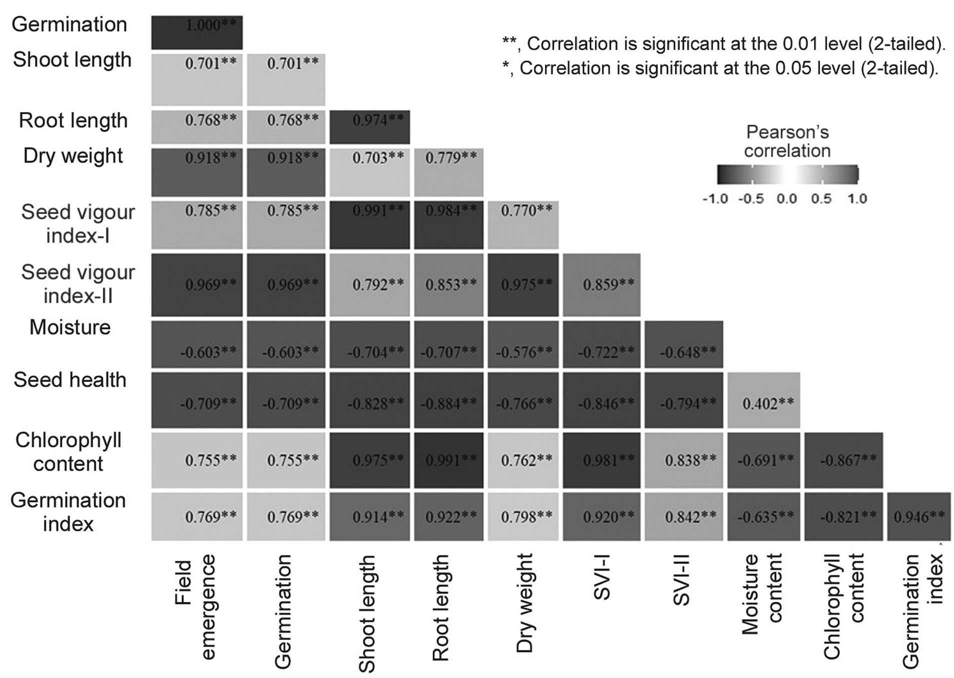

Fig. 3 Correlation studies of physiological seed quality characters.

highest value (0.636) was obtained from T_{19}. Meanwhile, minimum OD value (0.312) was observed from the seeds treated with jute bags (T_1) (Table 1). Seed lot treated with 40 mg/kg of Emamectin benzoate and 3 g/kg of silica gel packed in HDPE interwoven bags (T_{19}) had the lowest value of electrical conductivity (0.306 S/cm/g). However, the greatest value (0.682 S/cm/g) was obtained from the seed lot treated with a jute bag without treatments (T_1). The electrolyte leakage rates in the seeds aged 8 and 18 days were much higher than those in the other seeds; this finding is attributed to changes in the membrane's structure and lipid composition during seed preservation (Lin *et al.* 2022). Strong positive correlations between field emergences and germination, shoot length, root length, seedling length, seedling dry weight, SVI-I and SVI-II, chlorophyll content, and germination index were observed; however, negative correlations between field emergence% and moisture content were found (Fig. 3). These results are in agreement with Zhang *et al.* (2022).

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