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Quality assessment of pea (*Pisum sativum* L.) seeds using the controlled deterioration technique

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ABSTRACT

A three-factor experiment was set at the Horticulture Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, to study the effects of the controlled deterioration (CD) on the pea seeds at the constant temperature of 35 °C. The 3 factors considered were: 3 pea seed sources (Rangpur Local/RL, Dinajpur Local/DL and Thakurgaon Local/TL); 3 ageing periods (0, 8 and 16 days); and 3 seed moisture contents (12, 16 and 20% MC). The 27 treatment combinations compared in the CRD with the 3 repetitions for the 8 arenas were: % germination, % abnormal seedlings, % dead seeds, % soil emergence and seedling evaluation test for the root and shoot lengths as well as their dry matter contents. Identical prototypes of notable (5–1% level) degradations were recorded everywhere. But the disparities were lucid under the extreme stresses. Moreover, highly noteworthy (1% level) relations were traced amid all the traits ranging from -0.9847 (soil emergence × abnormal seedling) to 0.9623 (soil emergence × normal seedling). So, the CD technique was very effectual in judging the physiological statuses of the seed sources studied. Thus, the germination test might be add-on by a vigor test, the latter of which could be assessed by quantifying the seedlings' root and shoot lengths and/or their dry matter accumulations. Moreover, in the seed quality certification, the suitable limits of vigor for the chosen traits could also be got by this technique. But the seeds of several pea varieties should be exploited to fix-up the agreeable limits of the traits. Furthermore, to save time, the ageing period could be squeezed by raising the seed MC.

Keywords: Pea; Moisture Content; Germination; Abnormal Seedlings; Dead Seeds

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1. Introduction

Legumes play vital roles in the global agriculture, chiefly by providing the greatest amount of plant proteins to humans as well as animals (mostly various pulses), vegetables (beans and peas), vegetable oils (groundnut, soybean), ornamentals (lupines, garden pea, cassia etc.), fodder (cow pea, clover etc.), timber and firewood (diverse trees), fruits (tamarind, *igna dulcis* etc.), spices (fenugreek, *Dalfiringi* etc.), medicines (medicinal plant spp.), natural dyes (indigo) and fibers (sun hemp). All the legumes fix notable quantities of atmospheric nitrogen to soils collaborating with the beneficial bacteria *Rhizobium* spp., which has unique credibility in the whole plant kingdom^[1]. Pea (*Pisum sativum* L.) is a cherished and nourishing winter vegetable as well as one of the important pulses of Bangladesh. In addition, its seeds are also utilized as snacks: fried seeds and chotpoti. Seed quality affects the performance of any crop under the field conditions in several ways^[2–4]. In ad-

dition, the initial seed quality also affects the storage life of seeds. So, an in-depth study of the seed quality is vital if crop productivity as well as the storage life of the seeds is to be enhanced or even maintained.

Seed germination, vigor and size are the 3 crucial aspects of seed quality, which influence crop yield and grade through both the indirect and the direct effects^[5]. A major component of the seed quality is the percentage of seed germination as determined by the International Seed Testing Association^[6] and performed by any seed certification program all over the world on a routine basis. The germination rate under the laboratory conditions is usually a good estimator of the field emergence^[7]. Many seeds though germinate profusely under the ideal laboratory situations fail to emerge abundantly in the crop field^[8-10]. So, the aim of the laboratory germination test is to facilitate the global trade in seeds by proving a standard measure of the field planting value under the idyllic conditions.

Differences in the field emergences of the seed lots with the similar and acceptable levels of the laboratory germination percentage had been noticed in several legumes^[3,11-13]. As such variations are observed the standard laboratory germination test fails to be the effective indicator of the field establishment. Failure of the germination percentage to relate with the field emergence leads to the very term 'Vigor' at the 1950's ISTA Congress in Washington D.C^[14]. The data evaluated by Brown and Mayer^[15] also showed that the seed germination continued at the slow rate after the fast period of the germination. Apparently, these slow germinating seeds had low vigor^[15,16]. Seed lots are considered to possess the low vigor when field emergence is low compared to other seed lots having the similar germination percentage.

Accelerated aging is a very responsive test in ranking the quality of seeds^[17-19] and the controlled deterioration test is usually illustrated by the non-linear courses of germination loss during the aging period^[20]. Several factors are liable for the vigor of

any seed. But little work has been carried out to study the effects of the ageing on the physiological statuses of pea seeds. The present study aimed to investigate some of the significant consequences of the ageing using the controlled deterioration technique on the physiological statuses of the 3 pea seed sources (Thakurgaon Local (TL), Dinajpur Local (DL) and Rangpur Local (RL)) during their germination and the subsequent establishment of the seedlings and to relate the findings with the standard germination test for seed quality certification.

2. Materials and methods

2.1 Seed characteristics

The experiment was conducted at the Horticulture Laboratory, Hajee Mohammad Danish Science and Technology University (HSTU), Dinajpur. Three different sources of pea seeds of the local varieties were obtained from the three diverse sources of the northern Bangladesh namely Rangpur, Dinajpur and Thakurgaon. They are denoted as Thakurgaon Local (TL), Dinajpur Local (DL) and Rangpur Local (RL). Cleaning was done to remove the cracked, broken and abnormal seeds, other seeds, foreign matters, etc. by hand picking. Then the pure seed fraction was transferred to three separate polyethylene bags, labeled, sealed and kept at the room temperature until exploited. At the beginning of the experimentation, the following 12 selective traits (**Table 1**) of the three seed sources were measured to have their comparative benchmark information.

2.2 Experimental design

The completely randomized design (CRD) with three replications was used. The three factors and their levels judged in the study were as seed sources (SS): TL, DL and RL; seed moisture contents (MC): 12, 16 and 20%; and ageing periods (AP): 0, 8 and 16 days. The 27 treatment amalgamations compared were as follows (**Table 2**).

Table 1. Quantitative aspects of the 3 pea seed sources used in the study

S. No.	Quantitative aspects considered	Three seed sources		
		TL	DL	RL
1	Initial moisture content (% fresh wt. basis)	12.22	13.30	14.70
2	Thousand seed weights (g)	254.66	241.86	236.55
3	Percent germination (paper towel)	91.00	91.00	89.00
4	Percent soil emergences (course sand)	80.00	79.00	79.00
5	Seedling characteristics (paper towel)			
5.a	Normal seedlings (%)	91.00	91.00	89.00
5.b	Abnormal seedlings (%)	4.00	5.00	4.00
5.c	Death seeds (%)	5.00	4.00	6.00
5.d	Root length (cm)	13.60	13.47	13.35
5.e	Shoot length (cm)	7.13	7.06	6.98
5.f	Root dry matter (g/100 seedlings)	2.62	2.57	2.52
5.g	Shoot dry matter (g/100 seedlings)	2.46	2.38	2.33

Table 2. The 27 treatment combinations compared for the study

Treatment combinations	Descriptions of the treatment groupings			Treatment combinations	Descriptions of the treatment groupings		
	SS	MC (%)	AP (days)		SS	MC (%)	AP (days)
TC ₁	RL	12	0	TC ₁₅	TL	''	16
TC ₂	''	''	8	TC ₁₆	''	20	0
TC ₃	''	''	16	TC ₁₇	''	''	8
TC ₄	''	16	0	TC ₁₈	''	''	16
TC ₅	''	''	8	TC ₁₉	DL	12	0
TC ₆	''	''	16	TC ₂₀	''	''	8
TC ₇	''	20	0	TC ₂₁	''	''	16
TC ₈	''	''	8	TC ₂₂	''	16	0
TC ₉	''	''	16	TC ₂₃	''	''	8
TC ₁₀	TL	12	0	TC ₂₄	''	''	16
TC ₁₁	''	''	8	TC ₂₅	''	20	0
TC ₁₂	''	''	16	TC ₂₆	''	''	8
TC ₁₃	''	16	0	TC ₂₇	''	''	16
TC ₁₄	''	''	8				

2.3 Initial moisture content (MC)

For both the standard germination and the soil emergence tests, randomly selected 100 seeds per replication were used. Three primary seed samples from 3 diverse places of each of the polyethylene bags were taken and the composite samples were coarsely grounded with a hand grinder (Model: G-WON). From this sample, three sub samples (forming the three replications); each consisting of 5 g were taken using an electric balance and kept in a 50 ml beaker. That was then set in the constant high temperature oven running at 130 ± 1 °C for one hour^[6]. After that, the beaker was put in a desiccator to cool down. Thirty minutes later, the sample was weighed. The % MC on the fresh weight basis of the sample was calculated out using the formula:

$$MC(\%) = \frac{\text{Loss of weight from the seed sample}}{\text{Initial weight of the seed sample}} \times 100$$

Finally, the mean values obtained from the three replicates were used as the moisture contents

for each seed source.

2.3.1 Adjustment of the seed moisture contents at the desired levels

The required number of seeds for each sub-lot was counted and then was weighed. The expected weight of the seeds at the given MC was calculated out by the formula:

$$EW = \frac{100 \times DW}{100 \times EMC}$$

Here:

EW = Expected weight of the seed at the given moisture content

DW = Dry weight of the seed sample used and

EMC = Expected moisture content to be reached at

Then the adjustment was done in two phases. Of these, the first one was applicable to all the sub-lots (sources) and the second one was used as needed.

2.3.2 Adjustment of seed moisture contents

Adequate water was taken in a desiccator until it reached the level of 4 cm below its bottom neck.

A metallic wire net was set at the neck. On the net, a piece of cotton cloth was placed. The seeds were spread onto the cloth and the lid was set properly. Finally, the desiccator was kept at the room temperature for vaporization of the water. Weighing of the seeds was done time to time to check whether the desired weight was reached by absorbing the water vapor^[6]. Under the experimental conditions, it took one to seven days to reach the desired moisture levels of the seeds. In some cases, the seeds gained slightly higher MC than expected. Hence, the seeds were re-dried using dried blue silica gel (a desiccant) following the same procedure described above.

2.4 Process of the controlled deterioration (CD)

Treatment with 100 seeds for each replication were divided into three parts by numbers (33 + 33 + 34). Each part was taken in a test tube. A small piece of tissue paper was formed in the shape of a ball and wrapped around with a small piece of polyethylene sheet. Then the mass was put in the mouth of the test tube and pressed sufficiently to make the tube airtight. Such three test tubes were marked with a glass marker as the one replication for each treatment. The test tubes were placed in the beaker (1,000 ml) and sufficient water was added to cover the seed-height. The beaker was set in an electric oven. The temperature of the oven was adjusted to $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, which was run for the 3 required incubation periods (0, 8 and 16 days). During the deterioration, a small quantity of water was added now and then to the beaker to keep the water level high enough to cover the seed-height. So, the whole thing was set to act as a hot water bath.

2.5 Seed quality test

2.5.1 Standard germination test

The rolled paper towel method (4 ply) was used for it. In each towel 25 seeds were set centrally with 2 cm apart from one another and then rolled as needed. The towels were then tied loosely with a piece of thread. Those were set on a plastic tray in the upright manner. The tray was kept on the table at the room temperature. Light watering was done

as needed with a plastic jet bottle. After 7 days, the seedlings were evaluated according to ISTA^[6]. Data were collected for normal seedlings, abnormal seedlings and dead seeds. The results were then expressed as the percentage and the transformed values were exploited for the statistical analyses.

2.6 Soil emergence test

Sufficient coarse sand was sieved to discard all the organic substances and soil particles > 0.8 and < 0.05 mm in diameter. The sieved sand was sterilized in an electric oven running at $100\text{ }^{\circ}\text{C}$ for 24 hours. Later on, the sand was cooled down. A rectangular wooden seed flat ($1.0\text{ m} \times 0.5\text{ m} \times 0.15\text{ m}$) was filled in with the sterilized sand. Then the seeds were inserted in the sand $5\text{ cm} \times 5\text{ cm}$ apart in all the directions and also at the depth of 5 cm. In this way 100 seeds were placed in the seed flat for each replication. Light watering was done as needed with a plastic jet bottle. After 14 days of setting the emergence was recorded. The result was expressed as the percentage and the transformed values were used for the statistical analyses.

2.7 Seedling (7 days old normal seedlings) evaluation test

The normal seedlings were selected. The seed residues were separated from the seedlings with a sharp knife. Roots and shoots were also separated, spread on a table and allowed to wither for two hours to facilitate the measurement of the roots and shoots with a 30 cm plastic scale. The roots and the shoots were then taken separately in 50 ml beakers and lastly dried in an electric oven at the constant temperature of $85\text{ }^{\circ}\text{C}$ for 48 hours^[6]. Finally, those were weighed and expressed on the 100-seedling basis for the statistical analyses.

2.8 Statistical analyses

The results were evaluated using the analysis of variance (ANOVA) while the paired means was compared by the *t*-test. In addition, the simple correlation was studied. The MSTAT-C package was exploited for this purpose.

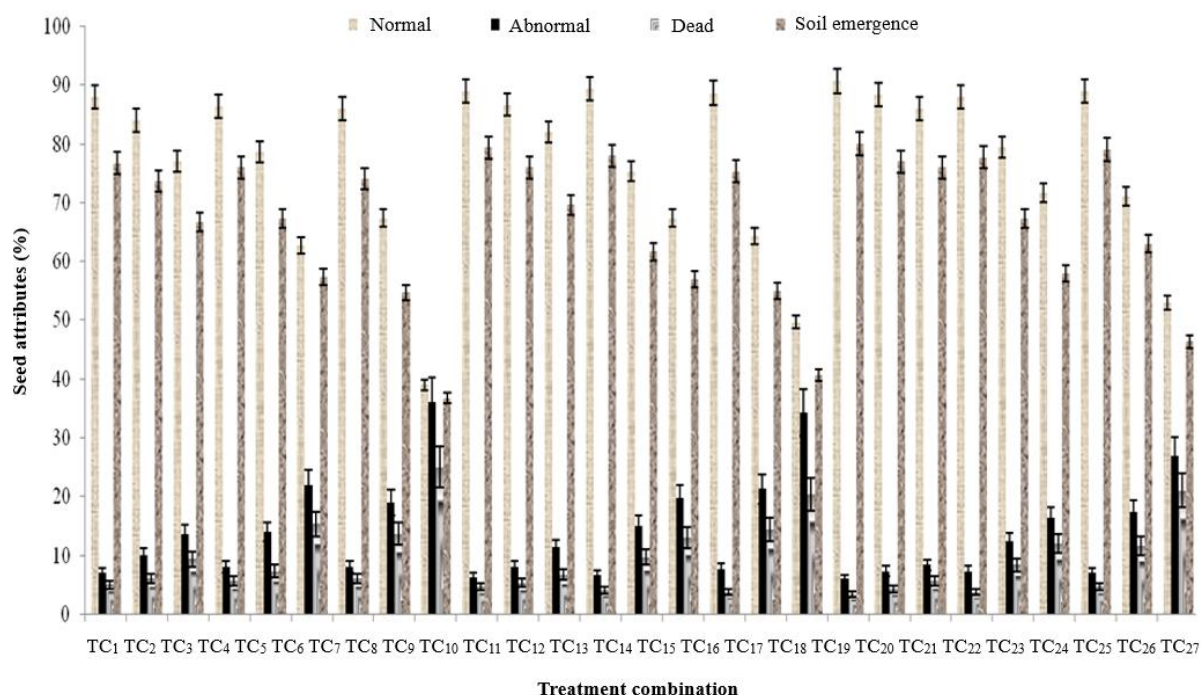


Figure 1. Various traits obtained from the standard germination and the soil emergence tests of the 3 pea seed sources as influenced by the controlled deterioration technique.

TC₁ = RL M₁₂ A₀; TC₂ = RL M₁₂ A₈; TC₃ = RL M₁₂ A₁₆; TC₄ = RL M₁₆ A₀; TC₅ = RL M₁₆ A₈; TC₆ = RL M₁₆ A₁₆; TC₇ = RL M₂₀ A₀; TC₈ = RL M₂₀ A₈; TC₉ = RL M₂₀ A₁₆; TC₁₀ = DL M₁₂ A₀; TC₁₁ = DL M₁₂ A₈; TC₁₂ = DL M₁₂ A₁₆; TC₁₃ = DL M₁₆ A₀; TC₁₄ = DL M₁₆ A₈; TC₁₅ = DL M₁₆ A₁₆; TC₁₆ = DL M₂₀ A₀; TC₁₇ = DL M₂₀ A₈; TC₁₈ = DL M₂₀ A₁₆; TC₁₉ = TL M₁₂ A₀; TC₂₀ = TL M₁₂ A₈; TC₂₁ = TL M₁₂ A₁₆; TC₂₂ = TL M₁₆ A₀; TC₂₃ = TL M₁₆ A₈; TC₂₄ = TL M₁₆ A₁₆; TC₂₅ = TL M₂₀ A₀; TC₂₆ = TL M₂₀ A₈; TC₂₇ = TL M₂₀ A₁₆; DL = Dinajpur Local; TL = Thakurgaon Local; RL = Rangpur Local; M = Moisture; and A = Ageing.

3. Results and discussions

3.1 Normal seedlings

Due to the controlled deterioration, the percentage of normal seedlings vis-à-vis the percentage of germination decreased notably at the 5% level from 90.67 (TC₁₉) to 39.00% in (TC₉) (**Figure 1**). As the conditions of the ageing, i.e., seed MC from 12–20% and the ageing periods as of 0–16 days were gradually intensified, the fall in the percentage of normal seedlings was also in the same trend. The integrity of the cellular membranes, enzymatic roles, metabolic functions and also translocation activities were severely hampered due to the degradation. So the seeds lost their credibility to produce adequate number of normal seedlings as found in the germination test^[21,22]. The reduction of germination in pea seeds following the controlled declining was noted by Iqbal and Smith^[23]. Similar findings was reported by Bahadur, Kabir, Iqbal, *et al.*^[24] in groundnut seeds weakened through the controlled degradation^[25,26] recorded the similar happenings too with such an ageing study of chick pea seeds.

The decline was also prominent in the seed sources though their magnitudes were overall less in TL (TC₁₉–TC₂₇) gone after by DL (TC₁₀–TC₁₈) while it was the least in RL (TC₁–TC₉). These results showed that TL was the best seed among the sources explored. That might be due to the fact that TL had the heaviest thousand seeds, i.e., 254.66 while those were 241.86 g and 236.55 g in DL and RL, respectively (**Table 1**). The results are also in full agreement with Patricia, Gerardo, Miller, *et al.*^[27], who showed that the large seeded varieties had higher percentage of germination. Note that the bold seeds in any species are always considered as the high vigorous ones by global seed technologists due to the more food reserve. The differences in producing % normal seedlings might also be linked with the heredity of the seed sources. While the working with the ageing of five chick pea varieties^[26] found that the percentage of germination declined notably having differential responses amid varieties.

Low seed moisture content is another vital issue for the seed quality. TL had the least initial MC

(12.22) than the rest 2 sources, i.e., 13.30% and 14.70% for DL and RL, respectively (**Table 1**). So it might play another imperative role in varying the percentage of normal seedlings among the 3 sources. It was notable that, the utmost seed quality may occur at the physiological maturity stage, i.e., when the seed attains the maximum dry weight^[28]. But the seeds start to deteriorate naturally as soon as those have reached the peak maturity on the mother plants at the rate depending basically on the seed MC and the prevailing temperature^[2].

The treatment combinations TC₁, TC₄ and TC₇ were statistically parallel among themselves. Similarly, the treatments TC₁₀, TC₁₃ and TC₁₆ were statistically alike with one another. In addition, the amalgamations TC₁₉, TC₂₂ and TC₂₅ were also similar to with each other. So it was proved that just rise in the seed MC at the zero incubation had no outcome on this trait.

Finally, all the three seed sources were quite alike for the percentage of normal seedlings under the least stress conditions (12% MC and 0 day of incubation in TC₁, TC₁₀ and TC₁₉). But as the adverse situations was most severe (20% MC and 16 days ageing in TC₉, TC₁₈ and TC₂₇), their physiological divergences were clear from the germination percentages (**Figure 1**). So, the results paved the way to opine further that to watch such disparities in the seed sources of very close physiological statuses, the ageing should be done under the extreme conditions^[23–25] also had similar findings regarding this phenomenon.

3.2 Abnormal seedlings

The percentage of abnormal seedlings ranged outstandingly at the 1% level from 36.00% in TC₉ to 6.00% in TC₁₉, i.e., up to 30.00% on account of the treatment amalgamations (**Figure 1**). As the conditions of the deterioration, *viz.* the seed MC from 12–20% and the ageing periods as of 0–16 days were strengthened, the rise in abnormalities in the 3 sources were also in the same trail. Nonetheless, the magnitude was less in TL, i.e., TC₁₉–TC₂₇ (6.00%–27.00%) gone after by DL i.e., TC₁₀–TC₁₈ (6.33%–34.33%) while in RL, i.e., TC₁–TC₉ it was at the top (7.00%–36.00%). The results again re-

vealed that TL was the best one among the 3 seed sources and might happen once more due to the bold seeds and the low initial seed MC as noted before^[3] that the effects of low vigor would likely be a great problem in those species where the manifestation of deteriorative changes occurred in the seeds well in advance of the death, e.g., in soybean. Because its seeds became defective about 1.7% normal deviates before the death^[29]. Other legumes also showed relatively high risk of having abnormalities and thereby amplified the oddities for the same trait, e.g., in cowpeas and chickpeas; seeds became defective about 0.9 normal deviates before the death^[30]. Contrarily, in the cereals, the seeds became faulty and produced abnormalities only a relatively short time before the death: 0.3 normal deviates both in wheat and maize^[31]. So the abnormalities in the legumes are more common than that in the cereals and other seeds^[24] and had similar results with the ageing of the groundnut seeds. Moreover, it is widely accepted that the loss of germination is almost the last stage of the ageing: the final catastrophe i.e., the death as proceed by the more subtle stages^[32]. Therefore, the 3 seed sources had more percentage of abnormalities than that of dead seeds. The differences in producing the abnormal seedlings might also be related to the genotypes^[26] dealing with the ageing of 5 chick pea varieties found that the deformities in the varieties were decreased notably having varied responses^[25] also had such genetic disparities with the controlled weakening of 2 chick pea varieties.

The treatments TC₁, TC₄ and TC₇ were statistically identical among themselves. Again, the combinations TC₁₀, TC₁₃ and TC₁₆ were alike with one another. Even, the combinations TC₁₉, TC₂₂ and TC₂₅ were statistically similar to each other. All these 9 treatment combinations had 12–20% seed MC but no pessimistic effects. So it again proved that just increases the seed MC at the zero incubation periods had no effect on this trait. Finally, all the 3 seed sources were alike for the percentage of abnormal seedlings under the least stress (12% MC and 0 day in TC₁, TC₁₀ and TC₁₉). But as the stress conditions were extreme (20% MC and 16 days for TC₉, TC₁₈ and TC₂₇), their physiological divergenc-

es became clear from the rise in the percentage of abnormalities (**Figure 1**). So, to study such disparities in the seed sources with very close physiological statuses, the ageing must be done under the extreme settings^[23–25] had also similar findings about these consequences.

3.2 Dead seeds

Due to the CD, the percentage of dead seeds rose up notably from 3.33% (TC₁₉) to 25.00% (TC₉) i.e., up to 21.67% in consequence of the treatments (**Figure 1**). Nonetheless, TC₉ (25.00%) was followed by TC₂₇ (21.00%) and TC₁₈ (20.33%) and the latter 2 were statistically similar with one another. The amplification in the percentage of dead seeds was also significantly countable in the 3 seed sources although the extents were less in TL i.e., TC₁₉–TC₂₇ (3.33%–21.00%) gone after by DL i.e., TC₁₀–TC₁₈ (4.67%–20.33%) while it was at the top in the entire sub-groups of RL i.e., TC₁–TC₉ (5.00%–25.00%). These results revealed that in this trait TL stood second while DL was first and RL was third among the sources explored. But in terms of all other traits studied, TL was in the first position. Again, TC₂₇ and TC₁₈ were statistically same in the group. The reliability of the cellular membranes, enzymatic roles, synthesis activities and also translocation processes were increasingly impaired due to the ageing^[21,22]. Thus, the seeds lost their reliability to produce any seedlings leading to the rise in the dead toll. Again Roberts^[3] mentioned that the effects of the low vigor would likely be a great problem in the species where the manifestation of the deteriorative changes occurred in the seeds well in advance of the death, e.g., soybean. Finally, the differences in the percentage of dead seeds amid the 3 sources might also be linked with the genotypes used as stated earlier for germination.

3.3 Percentage of soil emergence

The percentage of emergence vis-à-vis field establishment was declined significantly from 80.00% (TC₁₉) to 36.67% (TC₉) i.e., up to 43.33% as a result of the treatments (**Figure 1**). Moreover, TC₉ was statistically at par with TC₁₈ (40.67%) and TC₂₇ (46.33%). As the weakening conditions of the seeds in terms of the seed MC and the ageing days were

exaggerated, the drop in the soil emergence was also in the same track. The decline was also remarkable in the 3 sources. But the highest enormity in deprivation was less in TL i.e., 46.33% in TC₂₇ followed by DL i.e., 40.67% in TC₁₈ while in RL it was the least i.e., 36.67% in TC₉. These results revealed that TL was statistically superior to the rest 2 sources exploited. That might also happen due to the bold seeds in TL over DL and RL as well (**Table 1**) as bold seeds are always treated as the high vigorous ones by the global seed scientists as pointed before. The veracity of the cellular membranes, enzymatic activities, synthesis processes and also translocation roles were increasingly disrupted due to the degradation^[21,22]. Hence, the ageing also reduced the shoot length (**Table 3**). Moreover, the weak seedlings might not have sufficient strength to come out of the sand from the 5 cm depth giving the poor percentage of soil emergence^[23–26], and the working with the ageing of different legume seeds also had identical observations. Furthermore, there could be differential responses amid genotypes. Low seed MC is another factor for quality seeds. And TL had also the least initial seed MC than the rest 2 sources. That issue might also have a vital role for disparities in the % soil emergence capacities as cited previously for the 4 traits.

3.4 Root length

The root was stunted markedly from 12.98 cm (100.00%) in TC₂₂ to 9.14cm (70.41%) in TC₉ i.e., up to 29.58 % on account of the treatments (**Table 3**). Nonetheless, TC₂₂ was in the same statistical group of TC₁₉ (12.97 cm), TC₂₅ (12.97 cm), TC₁₀ (12.91 cm) as well as TC₁₃ (12.90 cm). As the weakening conditions of the seeds, i.e., the seed MC and the ageing days were embellished, the depletion in the root length also happened in the same manner.

The differences in the root length might also be linked with the heritable natures of the seed sources^[24–26] and the working with the ageing of different legume seeds also found notable decrease in the root length in different varieties. The declining trend was also remarkable in the 3 sources. But it was the least in TL i.e., TC₁₉–TC₂₇ from 12.97

cm–9.51 cm. The results further established that TL was statistically superior to the rest 2 sources. Finally, all the seed sources were fairly similar for their root lengths under the least stress conditions (12% MC and 0 day of incubation in TC₁, TC₁₀ and

TC₁₉). But as the adverse conditions were most severe (20% MC and 16 days ageing for TC₉, TC₁₈ and TC₂₇), their physiological divergences were clear from their root lengths (**Table 3**).

Table 3. Evaluation of the seedling parameters of the 3 pea seed sources as influenced by the controlled deterioration technique

Treatment combinations	Combinations level	Root length (cm)		Shoot length (cm)		Root dry matter (mg)		Shoot dry matter (mg)	
TC ₁	RL M ₁₂ A ₀	12.82	b-d	6.37	b	2.40	c	2.19	c
TC ₂	RL M ₁₂ A ₈	12.20	g	6.30	b-d	2.24	f	2.06	f
TC ₃	RL M ₁₂ A ₁₆	11.12	n	6.04	hi	2.05	h	1.88	i
TC ₄	RL M ₁₆ A ₀	12.80	d	6.34	bc	2.39	cd	2.18	cd
TC ₅	RL M ₁₆ A ₈	11.36	kl	6.11	fg	2.16	g	1.88	i
TC ₆	RL M ₁₆ A ₁₆	10.22	r	5.54	m	1.92	j	1.77	k
TC ₇	RL M ₂₀ A ₀	12.81	cd	6.31	b-d	2.39	c-e	2.17	cd
TC ₈	RL M ₂₀ A ₈	11.24	m	5.70	l	2.09	h	1.79	jk
TC ₉	RL M ₂₀ A ₁₆	9.41	r	4.68	p	1.80	k	1.54	m
TC ₁₀	DL M ₁₂ A ₀	12.91	ab	6.36	b	2.52	b	2.27	b
TC ₁₁	DL M ₁₂ A ₈	12.40	f	6.22	e	2.34	de	2.11	ef
TC ₁₂	DL M ₁₂ A ₁₆	11.31	lm	5.79	jk	2.09	h	1.90	i
TC ₁₃	DL M ₁₆ A ₀	12.90	a-c	6.36	b	2.55	b	2.28	b
TC ₁₄	DL M ₁₆ A ₈	11.29	i	6.12	f	2.26	f	2.07	f
TC ₁₅	DL M ₁₆ A ₁₆	9.55	p	5.73	kl	1.98	i	1.82	j
TC ₁₆	DL M ₂₀ A ₀	12.84	b-d	6.28	c-e	2.52	b	1.28	b
TC ₁₇	DL M ₂₀ A ₈	11.45	jk	5.98	i	2.17	g	1.98	g
TC ₁₈	DL M ₂₀ A ₁₆	9.37	q	4.88	o	1.93	ij	1.55	m
TC ₁₉	TL M ₁₂ A ₀	12.97	a	6.47	a	2.63	a	2.33	a
TC ₂₀	TL M ₁₂ A ₈	12.56	e	6.32	bc	2.40	c	2.13	de
TC ₂₁	TL M ₁₂ A ₁₆	11.38	j-l	6.05	g-i	2.17	g	1.96	gh
TC ₂₂	TL M ₁₆ A ₀	12.98	a	6.47	a	2.62	a	2.32	ab
TC ₂₃	TL M ₁₆ A ₈	11.70	h	6.25	de	2.34	e	2.10	ef
TC ₂₄	TL M ₁₆ A ₁₆	10.67	o	5.86	j	2.15	g	1.92	hi
TC ₂₅	TL M ₂₀ A ₀	12.97	a	6.47	a	2.63	a	2.31	ab
TC ₂₆	TL M ₂₀ A ₈	11.46	j	6.08	f-h	2.43	c	2.00	ab
TC ₂₇	TL M ₂₀ A ₁₆	9.51	p	5.11	n	2.14	g	1.59	l
CV (%)		0.66		0.66		0.39		0.39	

DL = Dinajpur Local; TL= Thakurgaon Local; RL= Rangpur Local; M= Moisture; and A= Ageing. In a column means bearing the same letter (s) do not differ significantly as per DMRT.

But the roots became stunted notably even at 8 days of ageing irrespective of the MC. So, the results set the base to opine further that to watch such disparities in the roots of the seed sources of very alike physiological habits, the ageing should be done under the medium to the extreme situations^[23–25] which also had similar arguments about this phenomenon.

3.5 Shoot length

The shoot became dwarf distinctly at the 5% level from 6.47 cm in TC₂₅ (and TC₂₂) to 4.68 cm in TC₉ i.e., up to 27.66% because of the treatments (**Table 3**). As the weakening conditions of the seeds, i.e., the seed MC and the ageing days were inflated, the depletion in the shoot length also occurred in the same manner. Again, the reliability of the cellu-

lar membranes, enzymatic activities, metabolic paths and also translocation routes were ever more damaged due to the worsening^[21,22]. So, the seedlings from such weak seeds could lose their competence to have the long shoots^[23–26] and the working with the ageing of different legume seeds also found notable decreases in the shoot lengths. Again, the differences might also be associated with the seed sizes and the inherent qualities of the seed sources utilized as narrated previously in connection with the other parameters.

The declining trend was also remarkable in the 3 pea seed sources. But the reduction was least in TL i.e., TC₁₉–TC₂₇ from 6.47–5.11 compared to those of DL i.e., TC₁₀–TC₁₈ from 6.36–4.88 and RL i.e., TC₁–TC₉ from 6.37 cm–4.68 cm; the results further confirmed that the seed source TL was quite

superior to the rest 2 supplies. Finally, all the 3 seed sources were rather identical for the shoot length under the least stress conditions (12% MC and 0 day of incubation in TC₁, TC₁₀ and TC₁₉). But physiological divergences were clear from their shoot lengths at most severe stress (20% MC and 16 days ageing for TC₉, TC₁₈ and TC₂₇) (**Table 3**). So, the results founded the pedestal to conclude again that to study such disparities in the seed sources of very alike physiological natures, the ageing should be done under the severe stresses^[23–25] which also had similar arguments about this occurrence.

3.6 Root dry matter

The root dry matter also went down distinctly at the 1% level from 2.63 mg in TC₂₅ to 1.80 mg in TC₉ i.e., up to 31.56% owing to the treatment amalgamations (**Table 3**). As the weakening conditions of the seeds, i.e., the seed MC and the ageing days were exaggerated, the depletion in the root dry matter synthesis also occurred in the same style. Again, the divergences in the root dry matter might also be deeply associated with the genotypes of the 3 seed sources explored^[24–26,33], and the working with the ageing of various legume seeds also found notable decrease in it. The declining trend was also remarkable in the 3 sources. But the fall was least in TL i.e., TC₁₉–TC₂₇ from 2.63 mg–2.14 mg compared to others. These results notably confirmed that TL was statistically finer than the rest 2 cases. Finally, all the 3 seed sources were somewhat akin for the root dry matters under the least stresses (12% MC and 0 day of ageing in TC₁, TC₁₀ and TC₁₉) but were diverse at severe stresses (20% MC and 16 days of ageing for TC₉, TC₁₈ and TC₂₇) (**Table 3**). So, the results gave the foot to opine additionally that to judge such disparities in the seed sources of very close physiological natures, the degradation should be done under the severe settings^[23–25] which also had similar attitudes regarding this happening.

3.7 Shoot dry matter

The shoot dry matter became markedly less at the 5% level from 2.33 mg in TC₁₉ (100%) to 1.54 mg (66.09%) in TC₉ i.e., up to 33.90% due to the treatments studied (**Table 3**). As the ageing conditions of the seeds, i.e., the seed MC and the incuba-

tion days were inflated, the lessening in the gathering of shoot dry matter also occurred in the same line. As the integrity of the cellular membranes, enzymatic reactions, metabolic functions and also translocation activities were gradually slowed down owing to the deprivation^[21,22]. The seeds could lose their competence to have long shoots and consequently bound to produce less amount of dry matter in it^[23–26] and the working with the ageing of diverse legume seeds also found notable decrease in the shoot dry matters. Again, the differences in its dry matter might also be governed by the genotypes of the seed sources explored. The declining trend was also amazing in the 3 sources. But the rates were the slightest in TL i.e., TC₁₉–TC₂₇ from 2.33 mg –1.59 mg compared to the others. The results established that the seed source TL was clearly superior among the 3 seed sources exploited in the present experimentation. Again, all the 3 seed sources were quite parallel for the shoot dry matter accumulations under the least adverse situations (12% MC and 0 day of ageing in TC₁, TC₁₀ and TC₁₉) but were varied at intensified condition (20% MC and 16 days ageing for TC₉, TC₁₈ and TC₂₇) (**Table 3**). So, the results opened the avenue to remark again that to assess such disparities in the seed sources of very identical physiological ranks, the weakening should be done under the extreme settings. Similar experiences pertaining to this phenomenon had also been focused by working with the controlled weakening of diverse legume seeds^[23–25].

3.8 Correlation studies

Highly significant ($P > 0.001$) associations were found between all the parameters compared and the figures ranged, overall, from -0.9847 to 0.7060 (**Table 4**). Of those relationships, 12 were negative while 16 were positive. Out of those, the topmost positive value was 0.9828 amid the dead seeds × abnormal seedlings while the least positive figure was 0.7060 for the shoot dry matter × normal seedlings. On the other hand, the utmost negative value was -0.9847 for the soil emergence × abnormal seedlings but the poorest negative integer was -0.7908 in case of the shoot dry matter × dead seeds. Another vital finding was that among all the values

pertaining to the normal seedlings (i.e., germination percentage), its affiliation with the soil emergence was at the climax, i.e., 0.9623. So, it vividly pinpointed that the standard germination test is really an effective indication of the field emergence. While dealing with the CD of pea seeds, Iqbal and Smith^[23] also found such positive and negative affinities among the different studied parameters. And the common findings for Iqbal^[33] and the present

study of Bahadur *et al.*^[24] were of similar natures dealing with the ageing of groundnut seeds had also found identical associations among the common traits. Roberts and Osei-Bonsu^[2] argued that when care was taken to assess the liaison in various biologically meaningful terms, it was clear that most of the vital attributes of seed vigor were closely associated to one another.

Table 4. Correlation matrix for the 8 quantitative aspects of the 3 pea seed sources studied following the controlled deterioration skill

	Normal seedling	Abnormal seedling	Dead seed	Soil emergence	Shoot length	Root length	Shoot dry matter
Abnormal seedling	-0.9575						
Dead seed	-0.9632	0.9828					
Soil emergence	0.9623	-0.9847	-0.9821				
Shoot length	0.9033	-0.9488	-0.9444	0.9329			
Root length	0.7745	-0.8599	-0.8629	0.8821	0.8619		
Shoot dry matter	0.7060	-0.7954	-0.7908	0.8135	0.8321	0.9212	
Root dry matter	0.8330	-0.9061	-0.9057	0.9108	0.9373	0.9418	0.9302

Note: all the values are highly significant ($P < 0.001$)

4. Conclusions

From the results it was fairly clear that the seed source TL was the most vigorous one. On the contrary, the source DL occupied the second place while the source RL was the last one in the queue in terms of the quality. Such significant positions could probably be due to disparities in the thousand seed-weights: 254.66 g, 241.86 g and 236.55 g in those sources, respectively. The results further pointed out that all the tests performed during the course of the study gave comparable as well as consistent consequences. As such, the uses of all other tests might be limited to specific situations where those either substitute the standard germination test, or complement it. For example, in the developing countries where the labor is cheap as well as plentiful but funding, equipment and technical hands are limited, the same seeds could be tested using the standard germination test and the normal seedlings obtained from that test could then be evaluated for their root and shoot characteristics. So, the controlled deterioration technique is a unique skill to study the physiological statuses of the seeds even having initially very close natures. Nonetheless, further research works should be done for the consistency of the acceptable limits of each of the parameter for the seeds of all the cultivated varieties of pea as those traits might differ among themselves.

Finally, to save time, the ageing period could also be squeezed by increasing the seed moisture contents.

Conflict of interest

The authors declare that they have no conflict of interest.

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