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Quality assessment of bush bean (*Phaseolus vulgaris L.*) seeds using the controlled deterioration technique

Mohammad R. Reza*, **T.M.T. Iqbal, Souvic Sarker**

Horticulture Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

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Abstract

A three-factor experiment was set at the Horticulture Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur during April-May 2014 to study the effects of the controlled deterioration (CD) on the bush bean seeds at the constant temperature of 35° C. The 3 factors considered were: two bush bean varieties; four ageing periods (0, 4, 8, and 16 days); and three seed moisture contents (12, 16 and 20% moisture content). The twenty four treatment combinations compared in the Complete Randomized Design (CRD) with the three replications for the eight parameters 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 clear under the extreme stresses. Moreover, highly noteworthy (1% level) relations were traced amid all the traits ranging from -0.466 (seed dry matter x abnormal seedling) to 0.983 (normal seedling x root length). So, the controlled deterioration technique was very effectual in judging the physiological statuses of the varieties studied. Thus, the germination test might be add-on by a vigour 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 vigour for the chosen traits could also be got by this technique. But the seeds of several bush bean varieties should be exploited to fix-up the agreeable limits of the traits. In addition to that, the variety BB1 could also be used in hybridization program as a parent to have better seeds of inherent quality. Finally, to shorten time, the ageing epoch could also be condensed by elevating the seed moisture contents to a certain stage.

* **Corresponding Author:** Mohammad R. Reza

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 from various pulses), vegetables (beans and peas), vegetable oils (groundnut, soybean), ornamentals (lupines, garden pea, cassia, etc.), fodder (cow pea, clover, berseem, etc.), timber and firewood (diverse trees), fruits (tamarind, *Igna dulkis,* etc.), spices (fenugreek, *dalfiringi*, etc.), medicines (several spp.), natural dyes (indigo, etc.) and fibres (sun hemp). All the legumes fix notable quantities of atmospheric nitrogen to soils utilizing the bacteria *Rhizobium* spp. - a unique credibility in the whole plant kingdom to improve soil fertility (Purseglove, 1977). And numerous leguminous species have major roles in the national economy of Bangladesh. Of those, bush bean (*Phaseolus vulgaris L.*) is a significant one. It is originated in the Central America (Smart, 1976). It is an introduced vegetable in Bangladesh for which the soil and climatic conditions from November - March is favorable for the production of its high quality tender beans of the international standard.

Its about 15cm long green tender pods are consumed by tossing in salads, stir-fried, included in soups and stews, or served as a side dish. Every 100g of its edible part contains 26 K cal, 1.7g protein, 4.5g carbohydrate, 0.1g fat, 50 mg calcium, 28 mg phosphorus, 12 mg potassium, 221 IU Vitamin-A and 11 mg vitamin C(Hortex Foundation, 2002). Actually this vegetable is grown for its immature pods. Today, many varieties of bush bean are grown throughout the world.

Seed quality affects the performance of any crop under the field conditions in several ways (Roberts and Osei-Bonsu, 1988; Roberts, 1986; Keefe and Draper, 1986). In addition, 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 any seed is to be enhanced or even maintained.

A single definition of the seed quality fit for all crops under varied agro-ecological conditions is neither possible nor usable. So, the elements of the seed quality should be taken into the account are: (1) Percent seeds that germinate and, therefore, initial seedling density. (2) Germination rates and growth of seedlings, which would determine the sizes of plants being produced. (3) Health conditions of seedlings. (4) Uniformity of the seedlings and finally (5) Ultimate yield and grade of the produce.

Seed germination, vigour and size are the three vital aspects of seed quality, which influence any crop yield and grade through both the indirect and the direct effects (Ellis, 2004). A major component of the seed quality is the % germination determined as per the International Seed Testing Association (ISTA, 1999) and performed by each and every seed certification program all over the world on the routine basis. The germination rate under the laboratory conditions is usually a good estimator of the field emergence (Pourhadian and Khajehpour, 2010). Many seeds though germinate profusely under the ideal laboratory situations fail to emerge abundantly in the crop field (Wang *et al.*, 2004; Kolasinska *et al.*, 2000; Kulik and Yaklich, 1982). 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 are noticed in several legumes (Borba, 1987; Roberts, 1986; Perry, 1982; Hampton and Scott, 1982). As such variations are usually 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 **'Vigour'** at the 1950's ISTA Congress in Washington D. C. (Perry, 1978). The data evaluated by Brown and Mayer (1986) also showed that the seed germination continued at the slow rate after the fast period of the germination. Apparently, those slow germinating seeds had the low vigour (Ranal and Santana, 2006; Brown and Mayer, 1986). Seed lots are considered to possess the low vigour 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 (Khan *et al.*, 2007; Hill *et al.*, 2007 and Kibinza *et al.*, 2006). And the controlled deterioration test is usually illustrated by the nonlinear courses of germination loss during the aging period (Kruse, 1999). Several factors are liable for the vigour of any seed. But little work is carried out to study the effects of the ageing on the physiological statuses of bush bean seeds.

Keeping that view in mind, the study was set with these three objectives **1.**To investigate some of the significant effects of ageing using the controlled deterioration technique on the physiological status of the two cvs. of bush bean (Bari Bush Bean 1 and Bari Bush Bean 2) seeds during their germination and subsequent establishment of the seedlings **2.**To relate the findings with the standard germination test; at present that is considered as the legal basis for the judgment of every seed quality certification program all over the globe and **3.**To assess the inherent seed quality of both the two cvs. for breeding purposes for the development of high quality bush bean varieties.

Materials and methods

The materials utilized and the methods exploited in doing this work are presented here under various heads including their descriptions. The experiment was set-up at the Laboratory, Department of Horticulture, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The experimentation was carried out from April - May, 2014.

The varieties

Two varieties of bush bean seeds were procured from the Regional Agricultural Research Institute, Bangladesh Agricultural Research Institute (BARI), Thakurgaon. The varieties are BARI Bush Bean 1 (BB1) and BARI Bush Bean 2 (BB2).

At the beginning of the experimentation, the following twelve selective traits of the two bush bean varieties were measured to have their comparative worktable mark information. Initial moisture content (% fresh wt. basis) 10 and 10; Thousands seed weights(g) 238.43 and 205.56; Percent germination(paper towel)- Normal seedlings (%) 90 and 89, Abnormal seedlings (%) 5 and 6, Dead Seeds(%) 5 and 5 ,Shoot length (cm) 26.13 and 22.52, Root length(cm) 10.1 and 9.17, Shoot dry matter (g/100 seedlings) 6.74 and 6.47, Root dry matter (g/100 seedlings) 1.89 and 1.54 respectively for BB1 and BB2.

Experimental design

The Completely Randomized Design (CRD) with three replications was used. The three factors and their levels judged in the study were as varieties: BB1 and BB2; Seed moisture contents (MC):12, 16 and 20%; and ageing periods (AP): 0, 4, 8 and 16 days.

Factors exploited

The three factors and their levels to be judged in the study were as Varieties (2) BB1 and BB2, Seed moisture contents (%) 12, 16 and 20; Ageing periods (days) 0, 4,8 and 16.

Treatment combinations

There were 24 treatment combinations. BB1 had 12 treatment combinations i.e. TC_1 to TC_{12} and BB2 were 12 i.e. TC_{13} to TC_{24}. TC_1 had one moisture content i.e. 12% and four ageing period i.e. 0, 4, 8 and 16 similar to TC_2 as well as TC_{24}.

Number of seeds used per replication

For both the standard germination and the soil emergence tests, randomly selected 100 seeds per replication were used.

Tests utilized to evaluate the seed quality following the deterioration, parameters noted and the methods of data collection

The following tests were exploited for that purpose A) Standard germination (4-ply rolled paper towel method) for-Normal seedlings (percent germination) and Abnormal seedlings (percent) and Dead seeds (percent decayed) B) Soil emergence (in seed-flat containing sterilized coarse sand) and C) Seedling evaluation test (with 7 days old normal seedlings taking from the standard germination test accompanied) for: Length of the root (per seedling), Length of the shoot (per seedling), Dry matter of the root (per 100 seedlings basis) and Dry matter of the shoot (per 100 seedlings basis) and D) All the above eight parameters were also correlated with one another.

Standard germination test

The rolled paper towel method was used for it. In each towel 25 seeds were set centrally lengthwise 2cm apart from one another and then rolled loosely as required. The towels were then also tied loosely with a piece of thread. Those were set on a plastic tray in the upright direction. The tray was kept on the table at the room temperature. Light watering was done as required with a plastic jet bottle. After 7days, the seedlings were evaluated according to ISTA (1999). For that, data were collected for normal seedlings, abnormal seedlings and dead seeds. The results were then converted to the percentage and the converted values were exploited for the statistical analyses.

Soil emergence test

The soil emergence test was conducted using the heat sterilized coarse sand as the substratum. The particulars of that were as follows:

Sufficient coarse sand was sieved to avoid all the organic substances, and soil particles > 0.8 and < 0.05mm in diameter. The sieved sand was sterilized in the electric oven running at 100°C for 24hours. Later on, the sand was cooled down. A rectangular wooden seed flat (0.5 × 0.5 × 0.15M) was filled in with the sterilized sand. Then the seeds were inserted in the sand 5 × 5cm apart in all the directions and also at the depth of 5cm. In that way 100 seeds were placed in each seed flat for each replication. Light watering was done as needed with a plastic jet bottle (250ml). After 7 days of setting, the emergence was recorded. The result was expressed as the percentage and the transformed values were used for the

statistical analyses.

Seedling (7days old normal seedlings) evaluation test

For that, the normal seedlings obtained from the standard germination test were used. Then the root and shoot lengths, and their dry matters were measured.

Firstly, 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 waited to wither for two hours to facilitate the measurement of the roots and shoots with a 30cm plastic scale. The roots and the shoots were then taken separately in 50ml beakers and lastly dried in the electric oven at the constant temperature of 85°C for 48 hours (ISTA, 1999). Finally, those were weighed and expressed on the 100-seedling basis for the statistical analyses.

Statistical analyses

The results were calculated using the analysis of variance (ANOVA) while the paired means were compared using the 'T' test. In addition, the simple correlation was studied. The MSTAT-C package was exploited for that.

Results and discussion

The findings of the present study being reported are available in this chapter under the nine different headings and in the 2 tables. Moreover, the results are also discussed and supported by the experiences of a range of scientists studied the quality of different seeds all over the globe, specially the legumes using the controlled deterioration technique.

Normal seedlings

Due to the controlled deterioration, the % normal seedlings vis-à-vis the % germination decreased remarkably at the 5% level of probability (Appendix 1) from 90.67 (TC_1) to 5.33% in (TC_{24}), i.e., up to 85.34% due to the treatment combinations (Table 1). As the conditions of the ageing, i.e. seed moisture contents from 12 - 20% and the ageing periods as of 0 - 16days were gradually intensified, the fall in the % normal seedlings was also in the same track.

The integrity of the cellular membranes, enzymatic roles, metabolic functions and also translocation activities were hampered increasingly due to the degradation. So, the seeds lost their credibility to produce adequate number of normal seedlings as found in the germination test (Roberts, 1972; Khan *et al.*, 2004). Iqbal and Smith (1996) noted such reduced germination in pea seeds following the controlled declining. Bahadur *et al.* (2005) obtained parallel types of results in groundnut seeds weakened through the controlled degradation. Kabir *et al.* (2005) and Kapoor *et al.* (2010) recorded the similar kind of happenings too with such an ageing study of chick pea seeds.

Table 1. Various traits attained from the standard germination and the soil emergence tests of the seeds of two bush bean varieties as influenced by the control deterioration technique.

Treatment combinations	Nature of the combinations	Normal seedlings (%)		Abnormal seedlings (%)		Dead seeds (%)		Soil emergence(%)	
TC_1	$BB1M_{12}A_0$	90.67	a *	5.33	h	4.00	j	23.33	a
TC_2	$BB1M_{12}A_4$	86.67	ab	9.33	gh	4.00	j	22.33	a
TC_3	$BB1M_{12}A_8$	86.67	ab	8.00	h	5.33	j	22.00	ab
TC_4	$BB1M_{12}A_{16}$	81.33	a-c	9.33	gh	9.33	ij	18.33	c
TC_5	$BB1M_{16}A_0$	84.00	ab	9.33	gh	9.33	ij	21.33	ab
TC_6	$BB1M_{16}A_4$	77.33	b-d	9.33	gh	13.33	hi	19.00	c
TC_7	$BB1M_{16}A_8$	70.67	d	12.00	e-h	17.33	h	15.67	d
TC_8	$BB1M_{16}A_{16}$	54.67	ef	18.67	b-e	26.67	g	12.33	ef
TC_9	$BB1M_{20}A_0$	57.33	e	18.67	b-e	24.00	g	11.67	fg
TC_{10}	$BB1M_{20}A_4$	42.67	gh	21.33	a-d	36.00	f	10.00	gh
TC_{11}	$BB1M_{20}A_8$	33.33	h	27.33	a	42.67	e	6.33	i
TC_{12}	$BB1M_{20}A_{16}$	13.33	i-k	10.67	f-h	76.00	b	2.00	k
TC_{13}	$BB2M_{12}A_0$	89.33	a	6.67	h	4.00	j	22.67	a
TC_{14}	$BB2M_{12}A_4$	85.33	ab	9.33	gh	5.33	j	20.00	bc
TC_{15}	$BB2M_{12}A_8$	68.00	d	16.00	d-g	16.00	h	14.33	de
TC_{16}	$BB2M_{12}A_{16}$	48.00	e-g	24.00	a-c	28.00	g	10.00	gh
TC_{17}	$BB2M_{16}A_0$	85.33	ab	8.00	h	6.67	j	18.33	c
TC_{18}	$BB2M_{16}A_4$	73.33	cd	10.67	f-h	16.00	h	13.00	ef
TC_{19}	$BB2M_{16}A_8$	40.00	gh	24.00	a-c	36.00	f	9.33	h
TC_{20}	$BB2M_{16}A_{16}$	21.33	i	25.33	ab	53.33	d	4.66	ij
TC_{21}	$BB2M_{20}A_0$	46.67	fg	18.67	b-e	34.67	f	8.66	h
TC_{22}	$BB2M_{20}A_4$	18.67	ij	17.33	c-f	64.00	c	5.00	i
TC_{23}	$BB2M_{20}A_8$	10.67	jk	12.00	e-h	77.33	b	2.66	jk
TC_{24}	$BB2M_{20}A_{16}$	5.33	k	8.00	h	86.67	a	1.66	k
LSD		9.203		6.612		6.027		2.01	
CV (%)			9.80		8.45		8.65		9.36

BB1= BARI Bush Bean 1, BB2= BARI Bush Bean 2, M = Moisture, and A = Ageing.

*In a column means bearing the same letter(s) do not differ significantly as per LSD at the 5% of probability.

The decline was also prominent in the varieties though their magnitudes were overall less in BB1 (TC_1 - TC_{12}) while it was least in BB2 (TC_{13} - TC_{24}). These results showed that BB1 had the best seeds of the two varieties investigated. That might be due to the fact that BB1 had the heaviest thousand seed weight, i.e. 238.43 while BB2 had 205.56 g . The results are also in the same track with that of Patricia *et al.* (2005) who showed that the large seeded varieties had higher percentage of germination. It is noted that the bold seeds in any species are always chosen as the high vigourous ones by global seed technologists due to the reserve of more food materials. The differences in producing % normal seedlings might also be linked with the heredity of the varieties. While working with the ageing of five chick pea varieties, Kapoor *et al.* (2010) found that the % germination declined notably having differential responses among varieties. Low seed moisture content is an important factor for the seed quality. BB1 and BB2 had the same moisture content i.e. 10.00 % . It is a basic thing that the utmost seed quality may occur at the physiological maturity stage, i.e. when the seed achieves the maximum dry weight (Siddique *et al.*, 1987). 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 moisture content and the prevailing temperature (Roberts and Osei-Bonsu, 1988).

Table 2. Evaluation of the seedling parameters of the seeds of the two varieties of bush bean as influenced by the controlled deterioration technique.

Treatment combinations	Nature of the combinations	Root length (cm)		Shoot length (cm)		Root dry matter (g)*		Shoot dry matter (g)*	
TC_1	BB1$M_{12}A_0$	9.80	a *	25.93	a	1.73	a	6.71	a
TC_2	BB1$M_{12}A_4$	9.65	ab	25.35	ab	1.53	ab	6.69	a
TC_3	BB1$M_{12}A_8$	9.60	ab	24.47	bc	1.44	b	6.42	a-c
TC_4	BB1$M_{12}A_{16}$	8.63	d	23.38	cd	1.09	c	6.10	bc
TC_5	BB1$M_{16}A_0$	9.47	a-c	25.15	ab	1.44	b	6.57	ab
TC_6	BB1$M_{16}A_4$	8.85	cd	23.06	de	1.04	c	5.94	c
TC_7	BB1$M_{16}A_8$	7.99	e	21.70	e	0.91	cd	5.22	d
TC_8	BB1$M_{16}A_{16}$	5.57	g	15.19	g	0.74	de	3.77	e
TC_9	BB1$M_{20}A_0$	7.11	f	18.70	f	0.99	c	5.10	d
TC_{10}	BB1$M_{20}A_4$	4.74	h	13.80	gh	0.61	e	4.79	d
TC_{11}	BB1$M_{20}A_8$	3.88	i	11.69	i	0.58	e	4.16	e
TC_{12}	BB1$M_{20}A_{16}$	2.54	jk	5.94	k	0.22	f	2.92	f
TC_{13}	BB2$M_{12}A_0$	9.09	b-d	21.77	e	1.52	ab	6.44	a-c
TC_{14}	BB2$M_{12}A_4$	8.94	cd	21.76	e	1.43	b	6.32	a-c
TC_{15}	BB2$M_{12}A_8$	7.09	f	19.21	f	1.11	c	5.20	d
TC_{16}	BB2$M_{12}A_{16}$	5.05	gh	15.20	g	1.13	c	4.19	e
TC_{17}	BB2$M_{16}A_0$	8.87	cd	21.66	e	1.39	b	6.06	bc
TC_{18}	BB2$M_{16}A_4$	6.81	f	18.13	f	1.03	c	4.77	d
TC_{19}	BB2$M_{16}A_8$	4.74	h	14.81	g	0.61	e	3.99	e
TC_{20}	BB2$M_{16}A_{16}$	2.99	j	6.25	k	0.31	f	3.03	f
TC_{21}	BB2$M_{20}A_0$	4.68	h	13.38	h	0.71	de	3.88	e
TC_{22}	BB2$M_{20}A_4$	2.73	jk	9.07	j	0.26	f	2.35	g
TC_{23}	BB2$M_{20}A_8$	2.41	jk	4.95	k	0.29	f	1.99	g
TC_{24}	BB2$M_{20}A_{16}$	2.29	k	3.63	l	0.12	f	1.92	g
LSD		0.5811		1.308		0.2205		0.4590	
CV (%)		5.52		4.72		4.63		5.83	

*100 shoot/roots basis

BB1= BARI Bush Bean 1, BARI Bush Bean 2, M = Moisture, and A = Ageing

*In a column means bearing the same letter(s) do not differ significantly as per DMRT at the 5% level of probability.

The treatment combinations TC_1, TC_5 and TC_9 were statistically parallel among themselves. Similarly, the treatments TC_{13}, TC_{17} and TC_{21} were statistically alike with one another. So, it proved that just rise in the seed moisture content at the zero incubation had no result on that trait.

Table 3. Correlation matrix for the eight quantitative aspects of the two bush bean varieties studied following the controlled deterioration technique.

	SL	RDM	SDM	NS	ABS	DS	SE
RL*	0.981**	0.948**	0.971**	0.983**	-0.581**	-0.942**	0.982**
SL		0.931**	0.966**	0.975**	-0.467**	-0.961**	0.952**
RDM			0.942**	0.952**	-0.500**	-0.927**	0.951**
SDM				0.965**	-0.466**	-0.949**	0.957**
NS					-0.521**	-0.977**	0.969**
ABS						0.328**	-0.594**
DS							-0.922**

** Significant at 1% level of probability

*SL= Shoot length, SDM= Shoot dry matter, NS= Normal seedling

DS= Dead seed, RL= Root length, RDM= Root dry matter

ABS= Abnormal seedling, SE= Soil emergence.

Finally, the two varieties were quite alike for the % normal seedlings under the least stress conditions (12% moisture content and 0 day incubation in TC_1, and TC_{13}). But as the adverse situations were most severe (20% moisture content and 16 days ageing in TC_{12}, and TC_{24}), their physiological divergences were clear from the germination percentages (Table 1). So, the results pinpointed the way to opine further that to watch such disparities in the seeds of very close physiological statuses, the ageing should be done under the extreme conditions. Iqbal and Smith (1996), Bahadur *et al.* (2005) and Kabir *et al.* (2005) also had similar findings regarding that phenomenon.

Abnormal seedlings

The percent abnormal seedlings ranged notably at the 1% level of probability from 27.33in TC_{11} to 5.33 in TC_1, i.e. up to 22.00% on account of the treatment combinations tested (Table 1). As the conditions of the deterioration, viz. the seed moisture contents from 12-20% and the ageing periods as of 0-16days were strengthened, the rise in abnormalities in the seeds of the two varieties were also in the same stream. Nonetheless, the magnitude was less in BB1 i.e. TC_1 - TC_{12} (5.33 - 27.33) while it was at the top in BB2 i.e. TC_{13} - TC_{24} (6.67 - 25.33%). The results again showed that BB1 was the best one between the two varieties and might happen once more due to the bold seeds.

Again, Roberts (1986) mentioned that the effects of low vigour 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 (Ellis and Roberts, 1981). 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 (Osei-Bonsu, 1981). 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 (Ellis and Roberts, 1980). So, the abnormalities in the legumes are quite common than the cereals and other seeds. Bahadur *et al.* (2005) 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 (Heydecker, 1972). So, the two varieties had the % more abnormalities than the % dead seeds. The differences in producing the abnormal seedlings might also be related to the genotypes. Kapoor *et al.* (2010) dealing with the ageing of 5 chick pea varieties found that the deformities in the varieties decreased notably having varied responses. Kabir *et al.* (2005) also had such genetic disparities with the controlled weakening of 2 chick pea varieties.

Lastly, the treatments TC_1, TC_5 and TC_9 were statistically identical among themselves. Again, the combinations TC_{13}, TC_{17} and TC_{21} were alike with one another. All these six treatment combinations had 12-20% seed moisture contents but no negative effects. So, it again proved that just increase in the seed moisture content at the zero incubation period had no effect on that trait. Finally, the seeds of the two varieties were alike for the % abnormal seedlings under the least stress (12% moisture content and 0 day in TC_1, and TC_{13}). But as the stress conditions were extreme (20% moisture content and 16days for TC_{12} and TC_{24}), their physiological deviations became clear from the rise in the % abnormalities (Table 3). So, to study such differences in the seeds of the varietiess with very close physiological statuses, the ageing must be done under the extreme settings. Iqbal and Smith (1996), Bahadur *et al.* (2005) and Kabir *et al.* (2005) had also similar types of experiences about these consequences.

Due to the controlled deterioration, the % dead seeds rose up notably from 4.00 (TC_1) to 76.00 (TC_{12}) i.e. up to 72.00% in consequence of the treatments judged (Table 1). Nonetheless, TC_{12} (76.00) and TC_{24} (86.67 %) were statistically comparable with one another. The magnification in the % dead seeds was also significantly countable in the seeds of the two varieties although the extents were less in BB1 i.e. TC_1 -TC_{12} (4.00 - 76.00) while it was at the top in the entire sub groups of BB2 i.e. TC_{13} -TC_{24} (4.00 - 86.67%). These results revealed that in that trait BB1 was supreme between the two varieties investigated.

The ability of the cellular membranes, enzymatic roles, synthesis activities and also translocation processes impaired increasingly due to the ageing (Roberts, 1972; Khan *et al.*, 2004). Thus, the seeds lost their credibility to produce any seedlings leading to the rise in the death toll. Again, Roberts (1986) mentioned that the effects of the low vigour 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. Because its seed became defective about 1.7 normal deviates before its death (Ellis and Roberts, 1981). Other legumes also showed relatively high risk of having abnormalities and thereby amplified oddities for the same traits, e.g. in cowpeas and chick peas, the seeds became defective about 0.9 normal deviates before their death (Osei-Bonsu, 1981). Contrarily, in cereals, the seeds became faulty and produce abnormalities only a relatively short time before the death: 0.3 normal deviates both in wheat and maize (Ellis and Roberts, 1980). Moreover, it is widely accepted that the loss of germination is all most the last stage of the deterioration: the final catastrophe i.e. the death as proceed by the more subtle stages (Heydecker, 1972). So, the abnormalities in the legumes are quite common and it might be the main cause of the less % of dead seeds than the % abnormal seedlings in all the cases of the two varieties. Finally, the differences in the % dead seeds between the two varieties might also be associated with the genotypes used as stated earlier for germination.

Percent soil emergence

The percent emergence vis-à-vis field establishment dropped markedly at the 1% level of possibility (Appendix I) from as high as 23.33 (TC_1) and 22.33 (TC_2) to 1.66 (TC_{24}) i.e. up to 21.67% as a result of the treatments compared (Table 1). Moreover, TC_{12} (2.00%) was statistically at per with TC_{24} (1.66%).

As the weakening environments of the seeds in terms of the seed moisture ccontents and the ageing days were inflated, the drop in the soil emergence was also in the same manner. The decline was also remarkable in the two varieties too. But the highest enormity in deprivation was less in BB1 i.e. 2.00 in TC_{12} while in

BB2 it was the least i.e. 1.66% in TC_{24}. These results revealed that BB1 was statistically supreme than the rest variety exploited. That might also happen due to the bold seeds in BB1 over BB2 as well as bold seeds are always treated as the high vigourous ones by the worldwide seed scientists as urged before.

The veracity of the cellular membranes, enzymatic activities, synthesis processes and also translocation roles were increasingly disrupted due to the degradation (Roberts, 1972; Khan *et al.*, 2004). Hence, the ageing also reduced the shoot length (Table 2). Moreover, the weak seedlings might not have sufficient strength to come out of the sand from the 5cm depth giving the poor % soil emergence. Iqbal and Smith (1996), Bahadur *et al.* (2005), Kabir *et al.* (2005) and Kapoor *et al.* (2010) working with the ageing of different legume seeds had also identical type of observations. Furthermore, there could be differential responses between the genotypes utilized. Low seed moisture content is another factor for quality seeds.

Root length

The roots became stunted distinctly from 9.80 (100.00) in TC_1 to 2.29cm (23.36%) in TC_{24} i.e. up to 76.64% on account of the treatments compared (Table 1). Nonetheless, TC_2 (9.65) was in the same statistical group of TC_3 (9.60), as well as TC_5 (9.47cm). As the weakening conditions of the seeds, i.e. the seed moisture contents and the ageing days were exaggerated, the depletion in the root length also happened in the same way.

Moreover, the reliability of the cellular membranes, metabolic processes, enzymatic roles and the translocation actions were even more damaged due to the weakening process (Roberts, 1972; khan *et al.*, 2004). So, the seedlings from such seeds could lose their capability to produce long roots. Iqbal and Smith (1996) working with the ageing of different legume seeds also found notable decrease in the root length.

Furthermore, the differences in the root length might also be linked with the heritable nature of the varieties. Bahadur *et al.* (2005), Kabir *et al.* (2005) and Kapoor *et al.* (2010) working with the ageing of different legume seeds also found notable decrease in the root length in different varieties. The declining trend was also outstanding in the two varieties. But it was least in BB1 i.e. TC_1 - TC_{12} from 9.80 - 2.54cm than the others. The results further proved that BB1 was statistically superior to the rest one.

Finally, the varieties were fairly similar for their root lengths under the least stress conditions (12% moisture content and 0 day incubation in TC_1 and TC_{13}). But as the imposed adverse conditions were most severe (20% moisture content and 16 days ageing for TC_{12} and TC_{24}), their physiological divergences were clear from their root lengths (Table 4). But the roots became stunted from four days and particularly even at eight days of ageing irrespective of the moisture contents. So, the results set the base to opine further that to watch such differences in the roots of the varieties of very alike physiological habits, the ageing should be done under the medium to the extreme situations. Iqbal and Smith (1996), Bahadur *et al.* (2005) and Kabir *et al.* (2005) also had similar arguments about that phenomenon.

Shoot length

The shoot became dwarf markedly at the 5% level of possibility (Appendix II) from as high as up to 25.93 in TC_1 to as low as 3.63cm in TC_{24} i.e. up to 86.00% because of the treatments judged (Table 2). As the weakening environments of the seeds, i.e. the seed moisture contents and the ageing days were magnified, the depletion in the shoot length also occurred in the same manner. Again, the reliability of the cellular membranes, enzymatic activities, metabolic paths and also translocation routes were ever more damaged due to the worsening (Roberts, 1972; Khan *et al.*, 2004). So, the seedlings from such weak seeds could lose their competence to have the long shoots. Iqbal and Smith (1996), Bahadur *et al.* (2005), Kabir *et al.* (2005) and Kapoor *et al.* (2010) 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 varieties utilized as described previous in connection with the other parameters.

The declining trend was also remarkable in the seeds of the varieties of two bush bean. But the reduction was least in BB1 i.e. TC_1 - TC_{12} from 25.93 - 5.94 compared to BB2 i.e. TC_{13} - TC_{24} from 21.77-3.63cm; the results further confirmed that the variety BB1 was quite superior to the rest one.

Finally, the seeds of the two varieties were rather identical for the shoot length under the least stress conditions (12% moisture content and 0 day incubation in TC_1 and TC_{13}). But physiological deviations were clear from their shoot lengths (Table 1) at most severe stress condition (20% moisture content and 16 days ageing for TC_{12} and TC_{24}). So, the outcomes founded the pedestal to conclude again that to study such differences in the seed lots of very similar physiological natures, the ageing should be done under the severe stress conditions. Iqbal and Smith (1996), Bahadur *et al.* (2005) and Kabir *et al.* (2005) also had similar arguments about that occurrence.

Root dry matter

The root dry matter also went down markedly at the 5% level of possibility (Appendix II) from as high as 1.73 in TC_1 to as downhill as 0.12 mg in TC_{24} i.e. up to 93.06% due to the treatment combinations (Table 2). As the weakening situations of the seeds, i.e. the seed moisture content and the ageing days were exaggerated, the depletion in the root dry matter synthesis also occurred in the same way. Again, the divergences in the root dry matter might also be extremely associated with the genotypes of the two varieties explored. Iqbal (1989), Bahadur *et al.* (2005), Kabir *et al.* (2005) and Kapoor *et al.* (2010) working with the ageing of various legume seeds also found notable decrease in it. The declining trend was also notable in the seeds of the two varieties. But the fall was least in BB1 i.e. TC_1 - TC_{12} from 1.73-0.22mg compared to the next varieties. These results notably established that BB1 was statistically best than the rest one.

Finally, the two varieties were fairly similar for the root dry matters (Table 1) under the least stress conditions (12% moisture content and 0 day ageing in TC_1 and TC_{12}) but were diverse at severe stress conditions (20% moisture content and 16 days ageing for TC_{12} and TC_{24}). So, the results presented the foot to opine additionally that to evaluate such inequalities in the varieties of very close physiological nature, the degradation should be done under the severe settings. Iqbal and Smith (1996), Bahadur *et al.* (2005) and Kabir *et al.* (2005) also had similar attitudes regarding this happening.

Shoot dry matter

The shoot dry matter became distinctly less at the 5% level of possibility (Appendix II) from as much as 6.71 in TC_1 (100) to as poor as 1.92 g (71.38) in TC_{24} i.e. up to 28.62% due to the treatments studied (Table 2).

As the ageing conditions of the seeds, i.e. the seed moisture content and the incubation days were embellished, 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 gradually slowed down owing to the deprivation (Roberts, 1972; Khan *et al.*, 2004), the seeds could lose their competence to have long shoots and consequently bound to produce less amount of dry matter in it. Iqbal and Smith (1996), Bahadur *et al.* (2005), Kabir *et al.* (2005) and Kapoor *et al.* (2010) 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 directed by the genotypes of the varieties explored. The declining trend was also amazing in the two varieties. But the rates were least in BB1 i.e. TC_1 - TC_{12} from 6.71-1.92mg compared to another one. The results proved that the variety BB1 was clearly superior among the two varieties exploited in the present experimentation.

Again, the two varieties were quite similar for the shoot dry matter gatherings under the least adverse conditions (12% moisture content and 0 day ageing in TC_1 and TC_{13}) but varied at intensified conditions (20% moisture content and 16 days ageing for TC_{12} and TC_{24}) (Table 2). So, the results highlighted the possibility to mention again that to evaluate such differences in the varieties of very identical physiological ranks, the weakening should be done under the extreme settings. Similar experiences pertaining to that phenomenon had also been focused by Iqbal and Smith (1996), Bahadur *et al.* (2005) and Kabir *et al.* (2005) working with the controlled weakening of diverse legume seeds.

Correlation studies

Highly significant (at 1% level) associations were found between all the parameters compared and the figures ranged from -0.466 to 0.983 (Table 3). Among the relationships, 12 were negative but 16 were positive. Out of those, the topmost positive value was 0.983 between the normal seedlings × root lengths while the least positive figure was 0.328 for the shoot dead seeds × abnormal seedlings. On the other hand, the utmost negative value was -0.977 for the normal seedlings × dead seeds but the poorest negative integer was -0.466 in case of the abnormal seedlings x seed dry matters. 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. 0.983. So, it vividly pinpointed that the standard germination test is really an effective indication of the field emergence. While dealing with the Controlled deterioration of bush bean seeds, Iqbal and Smith (1996) also found such positive and negative affinities among the different studied parameters. And the common findings for Iqbal (1989) and the present study were of similar natures. Bahadur *et al.* (2005) dealing with the ageing of groundnut seeds had also found identical associations among the common traits. Roberts and Osei-Bonsu (1988) 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 vigour were closely associated to one another.

Summary and conclusions

From the results it is fairly clear that the varietyBB1 was the most vigorous one while BB2 was the leastone in the queue in terms of the quality. Such significant positions could perhaps be due to differences in the thousand seed-weights: 238.43 and 205.56g in the varieties BB1 and BB2, respectively. Note that the bold seeds in any crop variety are always accepted as the high vigourous ones by the seed technologist all over the world and so, sieving of seeds with screens of slots of variable dimensions is done during their processing to have only those ones.

The outcomes further revealed that all the tests performed during the course of the experiment expressed 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 like Bangladesh where the labour is cheap as well as plentiful but finance, equipment and skilled hands are inadequate, 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.

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