



Prediction of faba bean (*Vicia faba*) seed viability through single seed conductivity test

RESHMA SHAHEEN¹, KALYANI SRINIVASAN², R C AGARWAL³, B S PHOGAT⁴, SHAHID UMAR⁵,
MANJU UPRETY⁶, R S RATHI⁷ and RAMESH KUMAR⁸

National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012

Received: 8 September 2010; Revised Accepted: 14 March 2011

ABSTRACT

Electrical conductivity of seed leachates is a well developed test to assess the quality of seeds. Membrane integrity and incidence of dead tissue are both influenced by the two major factors determining seed vigour in grain legumes mainly seed ageing and the incidence of imbibition damage. The conductivity of individual seed is not as well known as the conductivity of a seed-lot for analyzing the extent of deterioration of seed. The present investigation was carried out to examine the relationship between conductivity of individual faba bean (*Vicia faba* L) seeds of three cultivars and their germination. Seed lots of different vigour were obtained through controlled ageing for varying periods. After measuring the leachate conductivity, seeds were allowed to germinate. The conductivity of seeds which were scored as abnormal or dead was predominantly greater than those which produced normal seedlings. The usefulness of this non-destructive method for assessment and prediction of seed viability is discussed.

Key words: Conductivity, Seed leachate, *Vicia faba*

The fundamental principle of the electrical conductivity test is that deteriorated seeds lose more amino acids, electrolytes and soluble sugars due to the loss of membrane integrity. The most widely used index of membrane integrity is the rate and amount of electrolyte leakage as reflected in electrical conductivity of water in which the seeds are soaked under controlled conditions. The inverse relationship between electrical conductivity, and germinability and vigour, in the seeds of several crop species including grain legumes is well established (Srinivasan and Saxena 2001, ISTA 1995, Nautiyal 2009). Factors that influence the conductivity

reading of seed leachates include soaking temperature, soak duration, initial seed moisture content, seed size and seed weight (Loeffler *et al.* 1988; Thapliyal and Connor, 1997; Hepburn *et al.* 1984). It is, therefore, important to standardize the test procedure for each species. Mirdad (2006) found that the conductivity of soak water using a bulk of 50 seeds related well to germination. Deswal and Sheoran (1993) found the method to be very sensitive and simple to use with a large number of samples. The conductivity test was subsequently adapted to measure the leachate from single seeds (Hamman *et al.* 2001). Muasya *et al.* (2006) studied twenty four seed lots of two common bean cultivars to evaluate the distributions found in individual seed electrical conductivity to determine which parameters would best quantify the observed variation between seeds and to explore whether cultivar or production environment affected the variation. Such measurements from single seeds were used to predict seed viability and subsequent germination using automatic seed analyzers. The test provides an indication of the membrane integrity of single seeds, so that a few inferior seeds do not prejudice the overall evaluation of a bulk sample (Thornton *et al.* 1990, Tyagi 1992). According to Wang *et al.* (2004) the electrical conductivity provided a more sensitive parameter in ranking seed lot quality and showed a higher correlation with field treatment than the standard germination test in two forage legume species, viz *Vicia*

¹Research Associate (e mail: reshmaisrar@yahoo.com),
²Principal Scientist, Division of Germplasm Conservation, (e mail: kalyani@nbpgr.ernet.in);

³National Coordinator (e mail: agrawal_rakesh_chandra@yahoo.com), NAIP Project, KAB-II, New Delhi 110 012

⁴Principal Scientist (e mail: phogat@nbpgr.ernet.in), Division of Germplasm Evaluation;

⁵Reader (e mail: s_umar9@hotmail.com), Department of Botany, Faculty of Science, jamia Hamdard, New Delhi 110 062

⁶Technical Officer (e mail: dr.manjuuprety@yahoo.co.in), Division of Germplasm Conservation

⁷Senior Scientist (e mail: ranbirsinghrathi@gmail.com), NBPGR, Shillong,

⁸Senior Scientist and Incharge (mail: rk_phagna@rediffmail.com), Regional Maize Research and Seed Production Centre, Begusarai (Bihar)

benghalensis and *Medicago sativa*.

Loss of solute from seeds reflects the loss of integrity of cell membranes and/or presence of dead tissues within the seed. Membrane integrity and incidence of dead tissue are both influenced by the two major factors determining seed vigour in grain legumes mainly seed ageing and the incidence of imbibition damage (or soaking injury) and greater sensitivity of aged seeds to membrane damage.

The bulk conductivity test provides an average evaluation of conductivity of several replicates of bulk samples of seeds. Wide variation between replications could occur, however, as a result of high leakage from individual seeds that show extensive physical or physiological deterioration or damage. In this case an evaluation of leakage from individual seeds may be desirable.

Measurement of single seed conductivity has also been proposed as a means of predicting the germination of a seed sample. And not strictly accurate – it has been proposed as a useful estimate of seed vigour of certain spp, not standard germination. In this case, any seed having leachate conductivity above a so called partition value, is said to be non-germinable. However a common partition value can not be applied to all seeds within a species due to differences in leakage that arise due to genotype and seed size. In addition area of dead tissue within the seed that contributes to high leakage may not be located in area of the seed that prevents germination. Recently Makkawi *et al.* (2008), could arrive at a partition value of 180 μ S/cm in the case of lentil seeds by analyzing the conductivity of single seeds. Low quality seeds tended to have higher conductivity value as ageing increased.

This study investigated the relationship between the conductivity of individual faba bean seeds subjected to controlled ageing for various periods, and their germination after measuring conductivity.

MATERIALS AND METHODS:

Freshly harvested seeds of three cultivars of faba bean, viz Vikrant, 'PRT 7' and 'PRT 12' were used in the present study. Seeds were conditioned to fixed moisture content of 20% by adding required quantity of water to pre weighed seed in aluminium foil pouches. The pouches were sealed and stored at 4°C for 5 days in order to equilibrate moisture within and among the seeds. Seed moisture content was determined following standard protocols (ISTA 1995). After attaining 20% moisture content, seeds were divided into five sets of 50 seeds each. four sets were sealed in aluminium foil packets and subjected to artificial ageing by keeping them at a constant temperature of 45°C for different intervals, viz 48 hrs, 72 hr, 96 hr and 120 hr. One set was treated as non-aged, control. Samples after fixed period of time were withdrawn and each seed from the packet was weighed individually and kept in 5 mL double distilled water (conductivity 1–3 μ S/cm) in small glass vials and incubated

at 25°C in an environmental shaker for 16–18 hr at 120 rpm. After this the seed steep water was decanted into a clean beaker and the conductance of the same was measured in a pre-calibrated conductivity meter. The conductivity of water was subtracted from each reading and the net conductance of the leachate is expressed as μ S/cm/gm weight of the seed.

After measuring the leachate conductivity, the same seeds were subjected to germination test. Each seed was identified clearly in order, throughout the whole experiment so that its conductivity could be related with the subsequent performance in the germination test.

The germinability was determined by moist rolled paper towel test and incubated at 25°C \pm 1°C. Seedling evaluation was done on the 14th day according to International Seed Testing Association rule (ISTA 1995). Three germination categories were recognized:

- Normal seedlings: having normal root and shoot growth.
- Abnormal seedlings: having normal shoot but stunted or no root as manifested in its morphology.
- Dead seeds: seeds that failed to germinate and were rotten. Both normal and abnormal seeds were considered to be viable and dead seeds to be non-viable.

The experiment was conducted twice in 2008 and 2009. The data was analyzed using SPSS package.

RESULTS AND DISCUSSION

Since the variation in single seed weights was significant, the conductivity was expressed as μ S/cm/gm fresh weight of the seed. The results revealed that among the three cultivars, 'PRT 7' recorded maximum mean initial conductivity of 1 775.50 (unaged seeds), followed by 'Vikrant' (1 626.58), Table 1. Minimum conductivity for un-aged seeds was observed for 'Vikrant'. In PRT 12 and 'PRT 7', there was a gradual increase in the conductivity with increase in the ageing duration, while in Vikrant the increase was slower and more gradual. Table 3 indicates the distribution of non-viable seeds between various EC ranges, which have been coded for the convenience from 1 to 8. (1 indicates minimum range of 0–1 000, and 8 indicates the maximum range of > 7 000 and 2–7 with EC values between 1001 and 7 000.

In PRT 12 (Table 2), 26.67% of non-viable seeds exhibited a conductivity between 1 001–2 000 while 11.11% exhibited 2 001–3 000, when aged for 48 hr. The trend was similar at 72 hr with more of seeds shifting to 2001–3 000 range. At 96 hr of ageing however the percentage of non-viable seeds increased to 33% out of which maximum per cent (33%) exhibited 2 001–3 000 μ S/cm/gm of conductivity and 17% exhibited higher range of 3 001–4 000 μ S/cm/gm. At 120 hr all the seed were dead and the conductivity values also accordingly shifted to other higher ranges with 11.11% of seeds exhibiting 5 001–6 000 μ S/cm/gm. As regards the conductivity of viable seed lot of 'PRT 12', at 0 hr of ageing, when the viability was 100%, 73.33% exhibited conductivity

Table 1 General statistics of three cultivars at different ageing period and EC

Variety	Treat	Mean	Std. Error of Mean	Minimum	Maximum	Range	Std. deviation
PRT 12	0	900.17	29.84	614.23	1337.28	723.05	200.15
	48	1660.56	64.38	922.86	2615.10	1692.24	431.90
	72	1740.62	81.37	798.14	2987.59	2189.45	545.88
	96	2277.42	130.98	804.08	4346.52	3542.44	878.66
	120	3179.44	182.80	1436.52	5785.19	4348.68	1226.26
	Total	1951.64	70.63	614.23	5785.19	5170.96	1059.39
PRT 7	0	1157.04	32.88	642.96	1775.50	1132.54	220.55
	48	2036.73	186.14	840.72	8504.00	7663.28	1248.68
	72	2393.67	130.77	1076.49	5149.50	4073.01	877.20
	96	2843.07	178.19	1263.61	6226.00	4962.40	1195.31
	120	3068.53	177.55	522.94	5558.10	5035.15	1191.03
	Total	2299.81	81.17	522.94	8504.00	7981.06	1217.50
Vikrant	0	881.91	34.41	546.24	1626.58	1080.34	230.82
	48	2095.14	134.98	1037.93	4411.76	3373.83	905.45
	72	2097.61	114.63	1048.87	4513.73	3464.86	768.94
	96	2388.93	138.29	1233.74	4933.65	3699.90	927.65
	120	2714.53	127.48	1219.51	4652.00	3432.49	855.13
	Total	2035.62	66.23	546.24	4933.65	4387.40	993.41
Total	0	979.71	21.52	546.24	1775.50	1229.25	250.04
	48	1930.81	80.73	840.72	8504.00	7663.28	937.97
	72	2077.30	67.58	798.14	5149.50	4351.36	785.16
	96	2503.14	88.84	804.08	6226.00	5421.92	1032.24
	120	2987.50	95.81	522.94	5785.19	5262.25	1113.22
	Total	2095.69	42.44	522.94	8504.00	7981.06	1102.56

within 1 000 $\mu\text{S}/\text{cm}/\text{gm}$, while the rest exhibited between 1 001–2 000. The 48 hr aged lot where the viability had decreased to 62.2%, maximum of seeds (55.5%) exhibited conductivity in the range 1 001–2 000, while a small per cent (4.44%) showed conductivity in the range of 2 001–3 000. At 72 hr the distribution was more or less similar with slightly higher percentage of seeds exhibiting higher range of conductivity of 2001–3000 (8.89%). At 96 hr of ageing, all the viable seeds, ie 26.67% exhibited conductivity between 1 001–2 000 $\mu\text{S}/\text{cm}/\text{gm}$ fresh weight.

'PRT 7' exhibited an entirely different trend as compared to 'PRT 12' with different percentage of seeds showing different conductivity ranges after different durations of ageing. One constant feature however was that in all durations the percentage of non-viable seeds showing conductivity range of 2 001–3 000 was maximum, ie 20% at 98 hr, 33.3% at 72 hr, 40% at 96 hrs and 48.89% at 120 hr. Also the percentage of seeds showing the higher range of 3001–4000 increased from 0% at 48 hr to 8.89% at 120 hr. In the category of viable seeds in this variety, the '0' hr was characterized by 80% of seeds exhibiting conductivity values between 1 001–2 000. With increase in duration of ageing, more percentage of seeds shifted to higher conductivity ranges of 2 001–4 000.

Variety Vikrant showed a slightly different trend where the non-viable seeds at various durations exhibited conductivity values not exceeding 5 000 $\mu\text{S}/\text{cm}/\text{gm}$ while

that of viable seeds at various durations not exceeding 3 000 $\mu\text{S}/\text{cm}/\text{gm}$. The frequency distribution of soak water conductivity of the three cultivars clearly brings out the shift in the conductivity range from lower to higher as ageing advanced and more number of seeds became non-viable (Fig 1).

The conductivities above which seeds failed to germinate differed between the three faba bean cultivars, viz 'PRT 7', 'PRT 12' and 'Vikrant' used and therefore there was no common partition value which could be considered as an absolute boundary between germinable and non-germinable seeds. Genetic differences between the cultivars, and the extent of ageing undergone by each cultivar along with differences in other factors such as seed coat colour, seed size, seed weight etc. might explain the differences observed in the ranges of the conductivity readings. Although the germination of individual seeds could not be predicted, samples with poor germination in different cultivars after controlled ageing, were identified by their higher mean single seed conductivity. In spite of the differences in mean conductivity in these experiments, the range in conductivity of non-germinated seeds overlapped the range in conductivity values of the germinated seeds (Table 1, Fig 1). Many seeds that germinated had the same conductivity as seeds in all of the non-germinated categories, and hence single seed conductivity measurements did not accurately predict the quality of the seed. Measurements from entire seed samples

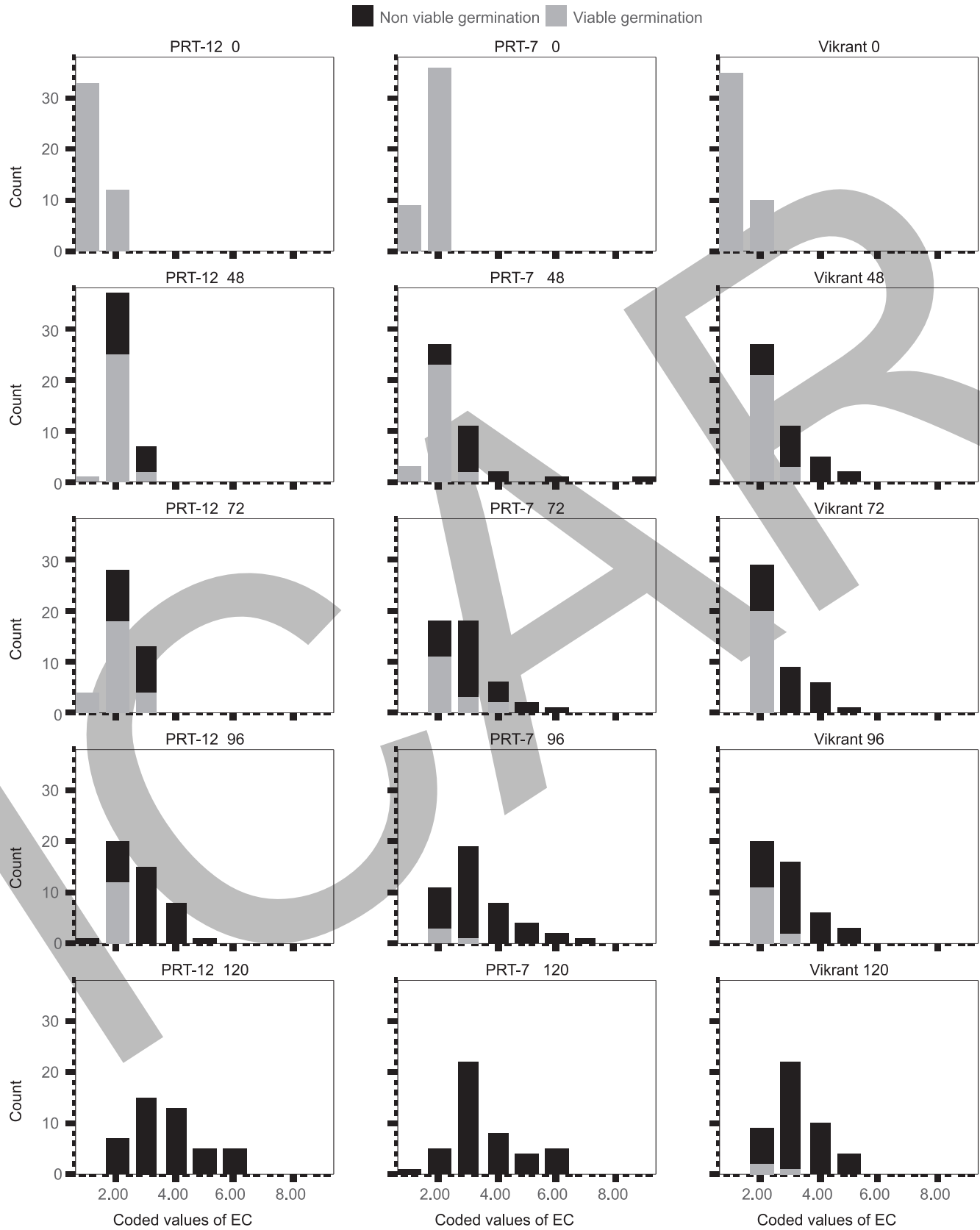


Fig 1 Effect of ageing on the Frequency of distribution of soak water conductivity of 3 cultivars

x

Table 2 Two-way distribution of seeds of three cultivars into viable/non-viable categories and coded EC values

PRT-12	0 hrs						48 hrs						72 hrs						96 hrs						120 hrs					
	Viable			Non-Viable			Viable			Non-Viable			Viable			Non-Viable			Viable			Non-Viable			Viable			Non-Viable		
	Count	%		Count	%		Count	%		Count	%		Count	%		Count	%		Count	%		Count	%		Count	%				
1*	33	73.33	-	-	-	-	2.22	4	8.89	-	-	-	1	2.22	1	2.22	-	-	-	-	-	-	-	-	-	-	-			
2	12	26.67	25	55.56	12	26.67	18	40.00	10	22.22	12	26.67	8	17.78	8	17.78	15	33.33	15	33.33	15	33.33	15	33.33	7	15.56				
3	-	-	2	4.44	5	11.11	4	8.89	9	20.00	9	20.00	8	17.78	8	17.78	8	17.78	8	17.78	8	17.78	13	28.89	13	28.89				
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Total	45	100.00	28	62.22	17	37.78	26	57.78	19	42.22	12	26.67	33	73.33	45	100	45	100	45	100	45	100	45	100	45	100				
PRT-7	9	20.00	3	6.67	4	8.89	11	24.44	7	15.56	3	6.67	8	17.78	1	2.22	1	2.22	1	2.22	1	2.22	1	2.22	1	2.22				
1	36	80.00	23	51.11	9	20.00	3	6.67	15	33.33	1	2.22	18	40.00	8	17.78	18	40.00	18	40.00	18	40.00	22	48.89	22	48.89				
2	2	4.44	2	4.44	2	4.44	2	4.44	4	8.89	2	4.44	4	8.89	2	4.44	4	8.89	2	4.44	2	4.44	4	8.89	4	8.89				
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Total	45	100.00	28	62.22	17	37.78	16	35.56	29	64.44	4	8.89	41	91.11	45	100.00	45	100.00	45	100.00	45	100.00	45	100.00	45	100.00				
Vikrant	35	77.78	21	46.67	6	13.33	20	44.44	9	20.00	11	24.44	9	20.00	2	4.44	7	15.56	2	4.44	2	4.44	7	15.56	7	15.56				
1	10	22.22	3	6.67	8	17.78	8	17.78	9	20.00	2	4.44	14	31.11	1	2.22	21	46.67	1	2.22	1	2.22	21	46.67	21	46.67				
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
Total	45	93.33	24	53.33	21	46.67	20	44.44	25	55.56	13	28.89	32	71.11	3	6.67	42	93.33	3	6.67	3	6.67	42	93.33	42	93.33				

*Coded EC values (EC values from 0 to 1000-1, 1001 to 2000 - 2, 2001 to 3000 - 3, 3001 to 4000 - 4, 4001 to 5000 - 5, 5001 to 6000 - 6, 6001 to 7000 - 7 >7000-8)

may conceal a few very poor seeds or a greater of mediocre seeds. Such differences would be revealed by the distribution of conductivity values from individual seeds. However, the present work emphasizes the need for much more biological investigation into the relationship between individual seed conductivity measurements and germination before these can be used in a routine manner by seed testing laboratories.

Membrane integrity is thought to be an important component of seed vigour, but failure of single seed conductivity to reliably predict performance in the germination test (Hepburn *et al.* 1984) or during emergence from the soil suggests that other seed characteristics may also be associated with performance failure. Some seeds in an experiment with apparently intact membranes (low conductivity) could not perform, while conversely, some seeds with deteriorated membranes (high conductivity) performed very well. The ability of a planted seed to germinate and produce a seedling capable of emerging from the soil is a multi-faceted process, so perhaps it is not surprising that the success or failure of a single seed cannot be assigned to a single mechanism or predicted by a single technique. The strongest relationship between seed leachate conductivity and vigor to be found in the literature is in pea seed (Bustamante *et al.* 1984).

In general, studies of seed leachate conductivity and performance compare the average conductivity of a particular seed lot with the performance of that population as a whole. Even those studies in which the conductivity of individuals within that population are measured using a single seed conductivity analyzer, seedlings and their conductivity values are combined as percentages within a specified group e.g., conductivity frequency histograms are compared to seedling-type (emerged non-emerged etc.) (Hepburn *et al.* 1984, Thornton *et al.* 1990).

In the earlier studies Thornton *et al.* (1990) and Wang *et al.* (1994) have reported that seed lots with higher numbers of seeds exhibiting low electrical conductivity values were readily germinable and higher values coincided with the percentage of dead seeds. Hepburn *et al.* 1984 on the other hand found little association between individual seed conductivity and its germination ability. Several workers have reported the high negative correlation between standard germination and bulk conductivity of seed soaked water (Loeffler *et al.* 1988). Several others have documented the relationship between high electrical conductivity of seeds steeped water and field emergence (Viera *et al.* 1999).

A perfect partition value could not be arrived at in the present study, above and below which the germinable and non-germinable seeds could be asserted respectively. Nevertheless a highly significant regression value of $r = 0.65$ (significant at 1% (2-tailed)) was obtained for the negative correlation between germinability and EC value of the seeds. There is a significant difference between the ageing groups,

if the data of all cultivars is aggregated, which indicates significant EC values at different level of ageing.

REFERENCES

- Bustamante L, Seddon R D and Rennie W J. 1984. Pea seed quality and seedling emergence in the field. *Seed Science and Technology* **12**: 551–8.
- Deswal D P and Sheoran I S. 1993. A simple method for seed leakage measurement: Applicable to single seeds of any size. *Seed Science and Technology* **21**: 179–85.
- Hamman R, Halmajan H and Egli D B. 2001. Single seed conductivity and seedling emergence in soybean. *Seed Science and Technology* **29**: 575–86.
- Hepburn H A, Powell A A and Mathews S. 1984. Problems associated with routine application of electrical conductivity measurements of individual seeds in the germination testing of peas and soybeans. *Seed Science and Technology* **12**: 403–13.
- International Seed Testing Association. 1995. (*in*) *Handbook of Vigour Test Methods*, 3rd edn. Hampton J G and Tekrony D M (Eds), ISTA, Zurich, Switzerland.
- Loeffler T M, Tekrony D M and Egli D B. 1988. The bulk conductivity test as an indicator of soybean seed quality. *Journal of Seed Technology* **12**: 37–55.
- Makkawi M, Balla M El, Bishaw Z and Van Gastel J G. 2008. Electrical conductivity in lentil seed leachates using a single-seed analyzer. *Journal of New Seeds* **9**: 267–83.
- Mirdad Z, Powell A A and Mathews S. 2006. Prediction of germination in artificially aged seeds of *Brassica spp* using the bulk conductivity test. *Seed Science and Technology* **34**: 273–86.
- Muasya R M, Lommen W J M, Auma E O and Struik P C. 2006. Evaluation of variation in individual seed electrical conductivity in common bean (*Phaseolus vulgaris*) seed lots. *Seed Science and Technology* **34**: 621–32.
- Nautiyal P C. 2009. Seed and seedling vigour traits in groundnut (*Arachis hypogaea* L.). *Seed Science and Technology* **37**: 721–35.
- Srinivasan K and Saxena S. 2001. Priming seeds for improved viability and storability in *Raphanus sativus* cv. chinese pink. *Indian Journal of Plant physiology* **6**: (3)271–74.
- Thapliyal R C and Connor K E. 1997. Effects of accelerated aging on viability, leachate exudation and fatty acid content of *Dalbergia sissoo* (Roxb.) *Seed Science and Technology* **25**: 311–19.
- Thornton J M, Powell A A and Matthews S. 1990. Investigation of the relationship between seed leachate conductivity and the germination of Brassica seed. *Annals of Applied biology* **117**: 129–35.
- Tyagi C S. 1992. Evaluating viability and vigour tests in soybean seed with an automatic seed analyzer. *Seed Science and Technology* **20**: 687–94.
- Vieira R D, Piazza J A and Percent D. 1999. Electrical conductivity and field performance of soybean seeds. *Seed Science and Technology* **21**: 15–24.
- Wang Y R, Hampton J G, and Hill M J. 1994. Red clover vigour testing-Effects of three test variables. *Seed Science and Technology* **22**: 99–105.
- Wang Y R, Yu L, Nan Z B and Liu Y L. 2004. Vigor test used to rank seed lot quality and predict field emergence in four forage species. *Crop Science* **44**: 535–41.