Validation study for the addition of *Cicer arietinum* (Desi type) as a species to which the conductivity test for seed vigour can be applied to support Rules proposal C.15.1

Mohammad Khajeh-Hosseini[1], Carina Gallo[2], Marie-Helene Wagner[3] and Hulya Ilbi[4]

[1] Department of Crop Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. agr844@gmail.com or saleh@ferdowsi.um.ac.ir

[2] National Institute of Agricultural Research, Oliveros Experimental Station, Oliveros, Argentina

[3] Station Nationale d'Essais de Semences (SNES), GEVES, Angers, France

[4] Ege University, Faculty of Agriculture, Department of Horticulture, Bornova-Izmir, Turkey

Abstract

The conductivity test identified differences in field emergence of 11 seed lots of the Desi type of chickpea (*Cicer arietinum*). Six of these seed lots, all having a laboratory germination of more than 85%, were tested by four laboratories using the electrical conductivity test, as described in the ISTA Rules (ISTA 2015). All laboratories consistently identified the same significant differences in the seed lot conductivity and the results were repeatable within laboratories and reproducible between laboratories. This provides evidence in support of the addition of *Cicer arietinum* (Desi type), to the ISTA Rules as a species for which the conductivity test can be applied.

Introduction

The conductivity test is validated in the ISTA Rules as a test that can be applied to species of *Pisum sativum*, *Phaseolus vulgaris*, *Glycine max, Cicer arietinum* (Kabuli type) and *Raphanus sativus* (ISTA, 2018). This test is based on the leakage of solutes that occurs from all seeds that are soaked in water. These solutes include sugars, amino acids and most importantly for the test, electrolytes. Thus the incidence of leakage can be detected by measurement of the electrical conductivity (EC) of the seed soak-water. The test was developed following the observation of the correlation between solute leakage and field emergence in wrinkled-seeded vining peas (*Pisum sativum*). Low leakage and therefore low conductivity was associated with seeds that emerged well, that is seeds with high vigour; whereas low vigour seeds with poor emergence had high levels of leakage and conductivity (Matthews and Whitbread, 1968). The conductivity test has also been used as an indicator of field emergence in field beans (*Vicia faba*, Hegarty, 1977), *Phaseolus beans* (Powell *et al*., 1986), soybean (Oliveria *et al*., 1984; Yaklich *et al*., 1984) and long bean (*Vigna sesquipedalis*; Abdullah *et al*., 1991). Leakage has also been related to emergence in the light-coloured, larger seeded Kabuli type chickpea (*Cicer arietinum*) (Khajeh-Hosseini *et al.,* 2007; Khajeh-Hosseini and Rezazadeh, 2011) leading to the validated method and inclusion of the Kabuli type chickpea as a species to which the EC test can be applied (ISTA,2014). There is however another, distinctly different and well recognised type of chickpea, the Desi type which has smaller, coloured seeds (Smartt and Simmonds, 1995). The objective of this study was to demonstrate that the conductivity test can also predict the field emergence of the smaller-seeded coloured Desi type chickpea (*Cicer arietinum)* and that the test is both repeatable within laboratories and reproducible between laboratories.

Materials and Methods

Field emergence: Samples of eleven seed lots of the Desi type of chickpea (*Cicer arietinum*) were obtained from Plant Research Institute, Ferdowsi University of Mashhad. Nine seed lots originated from Iran, the other two lots from Ethiopia and Tanzania. All lots had standard germinations above 89%. Field emergence was carried out in a completely randomized block design with four replications of 25 seeds from each seed lot in a clay loam soil where seeds were sown by hand at a depth of 4 cm. The average air and soil temperatures at sowing depth during the experiment were 15 and 13.5^{o}C respectively. Emergence was counted daily for 35 days until no further increase was observed.

Comparative test: Six of the seed lots used for field emergence were used in the comparative test, five from Iran and one (lot C) from Ethiopia. The lots were selected from the original 11 so that they included two high, two medium and two low vigour lots. All lots had standard germinations above 89%. Coded samples of the seed lots were sent from Mashhad, Iran to the participating laboratories, namely Department of Crop Science, Ferdowsi University of Mashhad, Mashhad, Iran; Department of Horticulture, Ege, University, Izmir, Turkey; National Institute of Agricultural Research, Oliveros Experimental Station, Oliveros, Argentina; GEVES, Station Nationale d'Essais de Semences (SNES), Angers, France. Each laboratory completed the conductivity test using the same method as that described for chickpea (Kabuli type) in the ISTA Rules (ISTA, 2015) i.e. four weighed replicates of 50 seeds, each soaked in 250 ml deionised/ distilled water for 24 hours at 20^{o}C. The conductivity was measured after 24 hours on the same sample. The conductivity was expressed as µS cm^{-1} g^{-1} of seed.

The data from conductivity was analysed using (a) two-way Analysis of Variance, (b) calculation of z-scores and (c) the statistical tool developed by S. Grégoire according to ISO 5725-2 to calculate h-values and k-values. The statistical tool is available for download at the ISTA website:

http://www.seedtest.org/upload/cms/user/ISO572511.zip

Results

Establishment of a relationship between field emergence and conductivity readings

There was a significant negative correlation (r = -0.791**) between conductivity and the field emergence of the eleven seed lots (figure 1; Appendix 2); standard germination was not correlated with emergence (r = 0.326; *P* <0.328). When only the six seed lots used for the comparative test were considered there was also a significant negative correlation (r = -0.908*) between conductivity and field emergence. Again, standard germination was not correlated with emergence (r = 0.557; *P* < 0.250)

Comparative test

Box plot analysis revealed differences between the average EC readings for the six seed lots (figure 2A) with few outside values. There were small differences in the average values obtained by the four laboratories (figure 2B). No seed lot x lab interaction was exhibited in the side-by-side box plots (figure 2C).

The means for the seed lots following the conductivity test showed clear and significant differences in seed leachate conductivity and hence vigour (table 1). Overall seed lots of E and F had the highest conductivity of measurements, i.e. lowest vigour, followed by lots C and A, while lots B and D had the lowest conductivity indicating the highest vigour.

Application of the tolerances for conductivity from Chapter 15B of the ISTA Rules (ISTA, 2017) showed that, the replicate data (Appendix 1) for each lot in each laboratory were in tolerance with one another. There were small, but significant, differences in the overall means from the four laboratories (table 1). However, the lot means from individual labs were in tolerance.

The Coefficient of Variation (CV) for the comparative test was 7.3% a value comparable with that reported (5.9%) for the method validation of conductivity for *Cicer arietinum* (Kabuli type) (Khajeh-Hosseini *et al.,* 2014) although the value was higher. The CV was calculated by dividing the standard deviation by the overall mean conductivity of the four labs (table 1) multiplied by 100.

Calculation of the z-scores (table 2) revealed that all data fell within the values that are acceptable within ISTA proficiency tests i.e. +2.00 to -2.00.

Repeatability and reproducibility were analysed with the statistical tool developed by S. Grégoire, based on ISO 5725-2; this allows the calculation of h- and k-values. The h-values show the tendency for a laboratory to give over-estimations or under-estimations compared to the mean of all the results available whereas the k-values give a measure of the variability of the repeats. Higher values indicate greater under- or over-estimations (h-values) or greater variability between replicates (k-values).

No significant h-values were found (figure 3), indicating that the measurements were not over or underestimated. There were only one significant k-value, namely for lot A in lab 3 (figure 4) indicating that there was greater variability between replicates.

Repeatability and reproducibility values are affected by the examined species and the seed quality of the lots tested, with low vigour seeds often having higher values. It is therefore not possible to compare directly the data from comparative tests using different seed lots. However, the values obtained for repeatability and reproducibility (table 3) were lower than those obtained previously for *Cicer arietinum* (Kabuli type) (Khajeh-Hosseini *et al.,* 2014).

Discussion

Field emergence trials clearly established that the conductivity test identifies differences in vigour of Desi type chickpea. Differences in conductivity of six seed lots were consistently identified in each of four laboratories. The test was both repeatable within laboratories and reproducible in different laboratories. In addition, the replicates within the laboratories and the mean values obtained for each lot in different laboratories all fell within tolerance, using the tolerance tables in the ISTA Rules (ISTA, 2015). This provides evidence in support of the addition of *Cicer arietinum* (Desi type), to the ISTA Rules as a species for which the conductivity test can be applied.

The conductivity test therefore applies to both the Desi and Kabuli type chickpea which are distinct types of the cultivated *Cicer arietinum*. The two types have been produced as a result of dispersal of chickpea from its centre of origin in south-east Turkey, exposure of the crop to different ecological conditions during evolution and subsequent selection by man (Smartt and Simmonds, 1995). The white seeded Kabuli type give a higher range of conductivity values (Khajeh-Hosseini *et al.,* 2014) than the range seen here for the coloured Desi type. Similar contrasts in conductivity readings have been seen between cultivars of other grain legumes having coloured and white seed coats (Powell *et al*., 1986; Abdullah *et al*., 1991). Comparisons of the conductivity values obtained from different seed lots of chickpea are therefore recommended only within one type of chickpea and not between lots of one type with those of the other type.

Acknowledgements

We would like to thank Maliheh Akbarpour PhD student at Department of Crop Science, Faculty of Agriculture, Ferdowsi University of Mashhad for initial experiments on the seed lots, distributing seeds to the participating labs and contributing to data analysis.

References

Abdullah, W. D., Powell, A. A. and Matthews, S. 1991. Association of differences in seed vigour in long bean (*Vigna sesquipedalis* L.) with testa colour and imbibition damage. *Journal of Agricultural Science*, *Cambridge*, **116**, 259-264.

Hegarty, T. W. 1977. Seed vigour in field beans (*vicia faba* L.) and its influence on plant stand. *Journal of Agricultural Science, Cambridge*, **88**, 169-173.

ISTA 2014. ISTA Rules 2014. International Seed Testing Association, Bassersdorf, Switzerland

ISTA 2015. ISTA Rules 2015. International Seed Testing Association, Bassersdorf, Switzerland.

ISTA 2018. ISTA Rules 2018. International Seed Testing Association, Bassersdorf, Switzerland.

Khajeh-Hosseini, M., Rostami, M., Tatari, M., Asadi, G. and Abbasi-Alikamar, R. 2007. The electrical conductivity in chickpea seeds. *Proceedings of 28th International Seed Testing Association Congress.* Iguassu Falls, Brazil.

Khajeh-Hosseini, M. and Rezazadeh, M. 2011. The electrical conductivity of soak-water of chickpea seeds provides a quick test indicative of field emergence. *Seed Science and Technology*, **39,** 692-696.

Khajeh-Hosseini, M., Gallo, C. and Ilbi, H. 2014. Proposal for the addition of *Cicer arietinum* (Kabuli type) as a species to which the conductivity test for seed vigour can be applied. Method Validation reports 2014. International Seed Testing Association.

Matthews, S. and Bradnock, W. T. 1968. The detection of seed samples of wrinkled-seeded peas (*Pisum sativum* L.) of potentially low planting value. *Proceedings of the International Seed Testing Association*, **32**, 553-563.

Matthews, S. and Whitbread, R. 1968. An association between seed exudates and the incidence of pre-emergence mortality in wrinkle-seeded peas. *Plant Pathology*, **17**, 11-17.

Oliveira, M. de A., Matthews, S. and Powell, A.A. 1984. The role of split seed coats in determining seed vigour in commercial seed lots of soyabean, as measured by the electrical conductivity test. *Seed Science and Technology*, **12**, 659-668.

Powell, A. A., Oliveria, M. de A. and Matthews, S. 1986. Seed vigour in cultivars of dwarf French bean (*Phaseolus vulgaris*) in relation to the colour of the testa. *Journal of Agricultural Science, Cambridge*, **106**, 419-425.

Smartt, J. and Simmonds, N.W. (1995). *Evolution of Crop Plants*. Longman Group UK

Yaklich, R.W., Kulik, M.M. and Anderson, J.D. 1984. Evaluation of vigour tests in soybean seeds: relationship of ATP, conductivity, and radioactive tracer multiple criteria laboratory tests to field performance. *Crop Science*, **19**, 806-810.

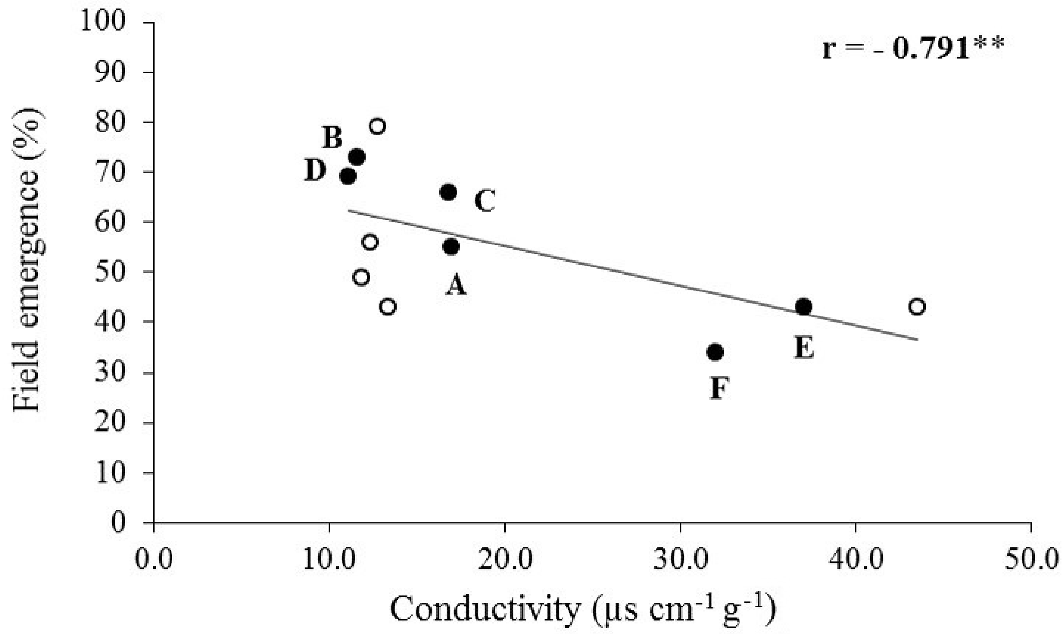

Figure 1: Relationship between conductivity and field emergence of eleven seed lots of Desi chickpea. Black circles are the seed lots selected for the comparative test.

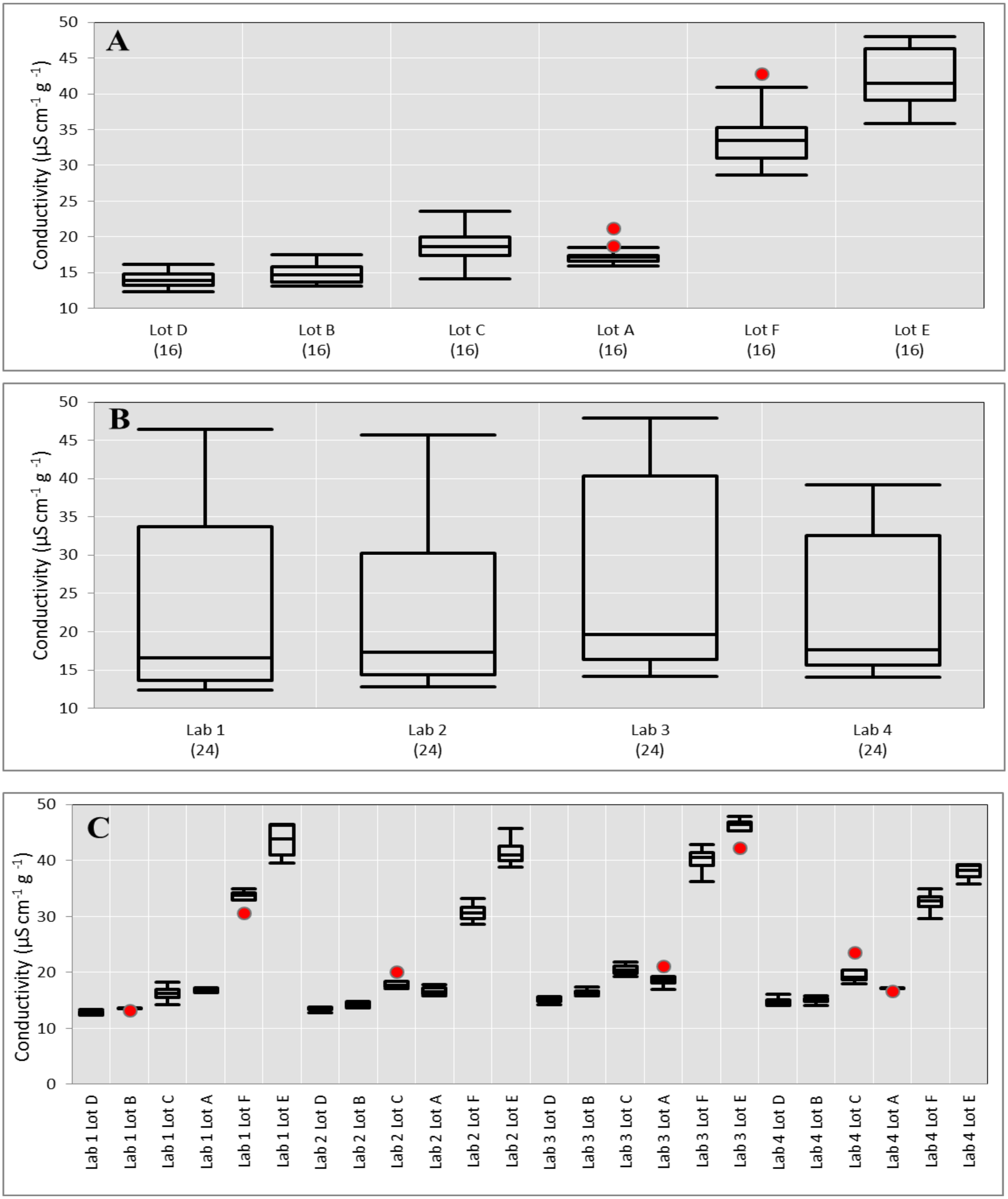

Figure 2: Box plot comparisons of the EC data from six seed lots of chickpea (*Cicer arietinum*), Desi type: (A) seed lots, (B) laboratories and (C) seed lot x laboratory

Table 1: Comparison of laboratory and seed lot means of six lots of chickpea (Desi type) tested by four laboratories using the conductivity test for 24 hours

Lab	Lot						Lab means
	A	B	C	D	E	F	
Lab 1	16.83[IK]	13.48[MN]	16.21[JL]	12.80[N]	43.40[B]	33.31[E]	22.67 [b]
Lab 2	16.70[IK]	14.21[LN]	18.15[HJ]	13.48[MN]	41.64[BC]	30.74[F]	22.49 [b]
Lab 3	18.86[GI]	16.47[JL]	20.50[G]	15.15[KM]	45.79[A]	40.03[CD]	26.13 [a]
Lab 4	17.11[IK]	15.04[KN]	20.00[GH]	14.82[KN]	37.89[D]	32.56[EF]	22.90 [b]
Lot means	17.38 [d]	14.80 [e]	18.72 [c]	14.06 [e]	42.18 [a]	34.16 [b]	

For lot and lab means, different lower case letters indicate that values are significantly different using LSD at the 5% level.
Within a row (laboratory), different upper case letters indicate that values (lots) are significantly different using LSD at the 5% level.

Table 2: Means, standard deviations (SD) and z-scores for six seed lots of chickpea (Desi type) tested by four laboratories using the conductivity test for 24 hours

Lot						
Lab	**A**	**B**	**C**	**D**	**E**	**F**
	a) means					
Lab 1	16.83	13.48	16.21	12.8	43.4	33.31
Lab 2	16.7	14.21	18.15	13.48	41.64	30.74
Lab 3	18.86	16.47	20.5	15.15	45.79	40.03
Lab 4	17.11	15.04	20	14.82	37.89	32.56
Mean	17.38	14.80	18.72	14.06	42.18	34.16
SD = S $\bar{x}$	1.0047	1.2828	1.9521	1.1090	3.3275	4.0594
b) Z-scores						
Lab 1	-0.542	-1.029	-1.283	-1.138	0.367	-0.209
Lab 2	-0.672	-0.460	-0.289	-0.525	-0.162	-0.842
Lab 3	1.478	1.302	0.914	0.981	1.085	1.446
Lab 4	-0.264	0.187	0.658	0.683	-1.289	-0.394

Table 3: Values for repeatability and reproducibility of results from the conductivity test on *Cicer arietinum* (Desi type)

Lot	Repeatability (S_r)	Reproducibility (S_R)
A	1.0124	1.3334
B	0.5786	1.3772
C	1.7208	2.4559
D	0.6627	1.2487
E	2.6864	4.0601
F	2.1987	4.4838

Table 4: Mean Conductivity and tolerance ranges (4 replicates x 50 seeds) for six lots of chickpea (*Cicer arietinum*), Desi type,

Lot		Lab 1	Lab 2	Lab 3	Lab 4
A	Maximum tolerance range	4.6	4.6	5.1	4.8
	Observed range	0.9	2.0	4.2	0.6
	Mean	16.8	16.7	18.9	17.1
B	Maximum tolerance range	3.8	4.1	4.6	4.3
	Observed range	0.4	1.0	1.7	1.7
	Mean	13.5	14.2	16.5	15.0
C	Maximum tolerance range	4.8	5.1	5.5	5.5
	Observed range	4.1	3	2.6	5.5
	Mean	16.2	18.2	20.5	20.0
D	Maximum tolerance range	3.6	3.8	4.3	4.1
	Observed range	0.9	1	1.5	2.1
	Mean	12.8	13.5	15.2	14.8
E	Maximum tolerance range	11.3	10.8	11.8	9.8
	Observed range	6.9	6.8	5.6	3.4
	Mean	43.4	41.6	45.8	37.9
F	Maximum tolerance range	8.8	8	10.5	8.5
	Observed range	4.3	4.6	6.5	5.3
	Mean	33.3	30.7	40.0	32.5

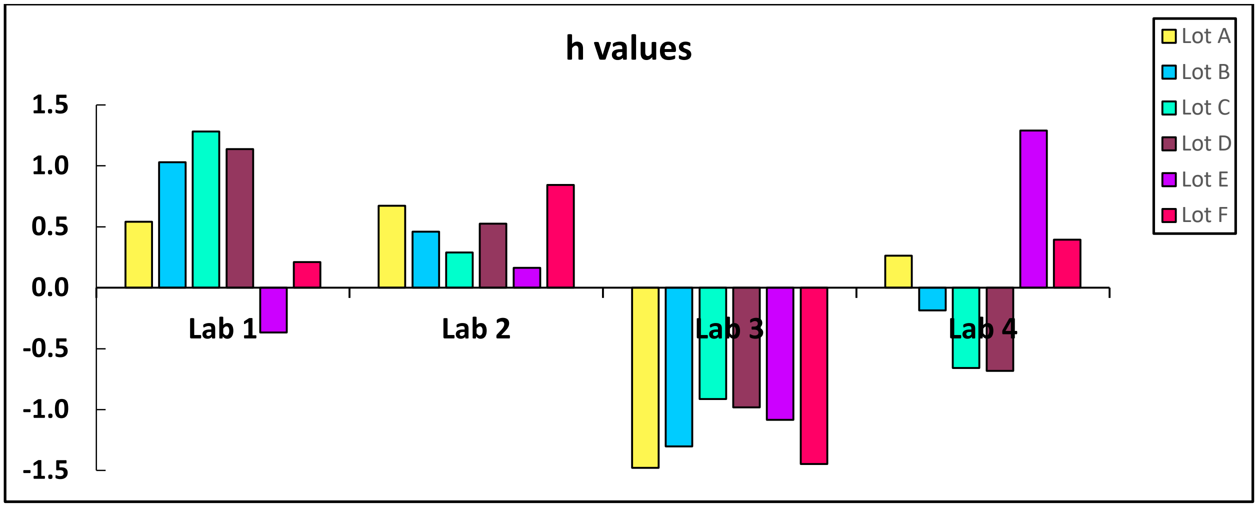

Figure 3: h-values for six seed lots of *Cicer arietinum* (Desi type) tested using the conductivity test for 24 hours in four laboratories.

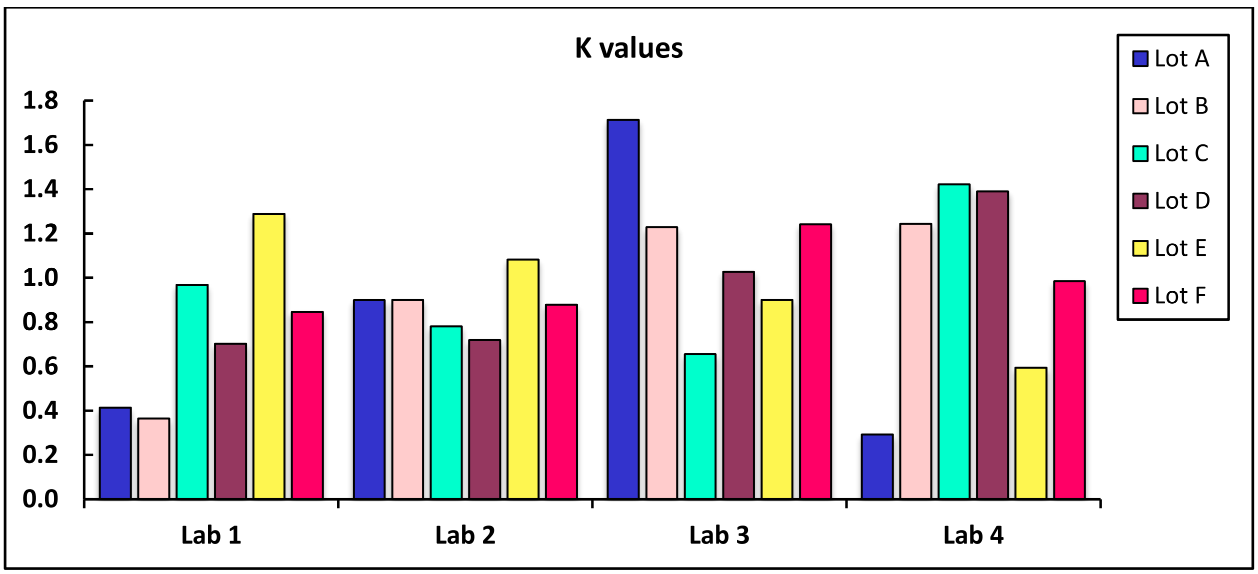

Figure 4: k-values for six seed lots of *Cicer arietinum* (Desi type) tested using the conductivity test for 24 hours in four laboratories.

Appendix 1: Data for each replicate conductivity reading for each of six lots of chickpea (*Cicer arietinum*), Desi type, taken in each of four laboratories.

Lot	Rep	Lab			
		Lab 1	Lab 2	Lab 3	Lab 4
A	1	17.19	16.10	18.57	17.27
	2	17.16	16.97	18.69	17.28
	3	16.34	15.87	21.19	16.66
	4	16.62	17.87	17.00	17.21
	Mean	16.83	16.70	18.86	17.11
	SD	0.42	0.91	1.73	0.30
B	1	13.60	13.71	17.45	14.07
	2	13.17	14.57	16.16	15.21
	3	13.56	14.75	15.80	15.05
	4	13.61	13.83	16.48	15.81
	Mean	13.48	14.21	16.47	15.04
	SD= S	0.21	0.52	0.71	0.72
C	1	16.48	17.14	20.86	18.04
	2	15.98	20.12	21.89	19.41
	3	14.17	17.54	19.29	18.98
	4	18.21	17.79	19.94	23.57
	Mean	16.21	18.15	20.50	20.00
	SD	1.67	1.34	1.13	2.45
D	1	13.30	13.78	15.70	14.76
	2	12.38	13.79	14.21	14.08
	3	13.09	13.56	15.09	14.30
	4	12.43	12.78	15.60	16.13
	Mean	12.80	13.48	15.15	14.82
	SD	0.47	0.48	0.68	0.92
E	1	41.43	40.40	46.58	35.80
	2	46.26	38.89	42.32	39.19
	3	39.52	41.60	46.31	39.09
	4	46.39	45.67	47.94	37.50
	Mean	43.40	41.64	45.79	37.89
	SD	3.46	2.91	2.42	1.60
F	1	33.91	31.15	40.95	32.49
	2	35.01	29.99	42.78	32.94
	3	30.66	28.62	36.30	34.94
	4	33.67	33.18	40.10	29.69
	Mean	33.31	30.74	40.03	32.52
	SD	1.86	1.93	2.73	2.16

Appendix 2: ANOVA table of field emergence.

Source of variation	d.f	Sum of Squares	Mean Square	F-value	P-value
Replicate	3	9.3409091	3.1136364	1.29	0.2958
Seed lot	10	476.6818182	47.6681818	19.75	<.0001
Error	30	72.4090909	2.4136364		
Total	43	558.4318182			