Relationship between variation in quality of individual seeds and bulk seed quality in common bean (*Phaseolus vulgaris* L.) seed lots

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Abstract

The variation in individual seed electrical conductivity (EC) (μ S cm^{-I} g^{-I}) of 24 seed lots of two common bean cultivars produced at two locations was quantified using the parameters mean – median, standard deviation (SD), and the range o–75%. Also coefficient of variation (CV) was tested, which was regarded not to be a good indicator of this type of variation. Bulk seed lot quality of this material with a very high germination percentage was determined using EC and percentage viable seeds. At physiological maturity (PM), a low variation in individual seed EC as quantified by mean – median, SD and the range o–75% was associated with good quality as measured by a low bulk EC and a high percentage of viable seeds. At harvest maturity, associations were less clear than at PM, partly because individual seed variation was smaller and also because bulk EC values differed only slightly among most seed lots. The relationships between individual seed variation and bulk quality were different for the two sites, as shown by a statistically significant improvement of the adjusted R² of the regression when site was included in the regression model, but the relationships were not affected by cultivar. No relationship was found between CV for individual seed EC and bulk quality.

Additional keywords: coefficient of variation, electrical conductivity, median, range, standard deviation, tetrazolium, variability

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Introduction

In field crops, the quality of a seed lot is the resultant of a combination of quality characteristics of individual seeds within that lot. We shall use the term bulk quality to refer to the quality of a seed lot so as to distinguish it from the quality of the individual seeds within that lot. Large differences in quality among individual seeds can be accompanied by a low bulk quality. This is the case, for instance, when due to ageing the quality of a stored seed lot decreases and the differences in time of germination among individual seeds within the lot increase (e.g. Siddique & Goodwin, 1983; Hosnedl & Horakova, 1998). It is not clear whether this association between a higher seed-to-seed variation and a poorer bulk quality also exists at the time of harvesting over a large number of seed lots produced under different growing conditions, or whether it is mainly the quality level of the seeds *per se* that is governing bulk quality. Individual seed differences in quality among seed lots are well known for various crops after harvesting (e.g. Levengood *et al.*, 1975; Steere *et al.*, 1981; Siddique & Goodwin, 1983; Moore III *et al.*, 1988). Such differences must result from differences in crop production methods and growing conditions.

The uniformity of seed development within the crop is a major factor through which crop production practices and growing conditions will affect seed-to-seed variation. During the growth of field crops, maximum seed quality is generally regarded to be attained at physiological maturity (PM), i.e., at the end of seed filling (e.g. Egli, 1998). The crop is harvested at harvest maturity (HM), when seeds have dried to a moisture content that allows harvesting without considerable damage. By that time, seed quality may already have deteriorated. Because seed development within a crop is not uniform, there are differences in the moment individual seeds reach PM. In common bean (Phaseolus vulgaris L.), seeds from earlier pods reach PM earlier, i.e., in less days after sowing than seeds from later pods, whereas the seeds from earlier pods have more time between PM and harvesting, and decline more slowly in moisture content (Muasya et al., 2002a). Seeds from earlier pods also tend to attain maximum seed quality sooner after sowing than those from later pods (Muasya et al., 2002b) and are thus exposed longer to the prevailing weather conditions between PM and HM. In soya bean, longer exposure of early pods to deteriorating conditions was thought to explain the lower viability at harvest of seeds from earlier compared with later pods (Illipronti Jr et al., 2000). Consequently, differences in development of seeds within a crop could lead to a seed lot in which individual seeds differ in age, moisture content and quality, as they differ in time to their maximum attainable quality level. Longer periods of flowering or seed formation may increase the variation in age among seeds within the crop (Gavras, 1989; Padrit et al., 1996) and consequently the variation in quality. Growing conditions will affect the length of these periods. We therefore assume that also at the time of harvesting a relationship may exist between the magnitude of the variation in individual seed performance and the final bulk seed quality of different seed lots. Muasya et al. (2006) recently identified easy parameters that properly quantified different types of variation in individual seed quality, thus enabling further studies in this field.

This research aims at investigating whether a higher variation in seed quality between individual seeds at physiological and at harvest maturity that results from different production conditions is associated with a poorer bulk quality. If these associations are relevant, production methods could be developed that reduce the variation among seeds.

Materials and methods

Experimental site and set-up

Twenty-four seed lots from two common bean cultivars were produced using the same cultivation practices. The crops were sown on three dates in each of two seasons at two locations in Kenya: Kitui and Eldoret. Kitui is situated in a semi-arid lowland area, Eldoret in a highland area. Rainfall during the growing periods was 117–845 mm at Kitui and 287–546 mm at Eldoret. Average daily temperatures were 21.4–26.1 °C at Kitui and 13.0–14.2 °C at Eldoret. At each site and in each season the experiment was laid out as a split plot with four blocks. The two cultivars, Rosecoco and Mwezi Moja, were assigned to the main plots, the sowing dates to the subplots. Both cultivars are determinate but Rosecoco shows prolonged flowering whereas the flowering period of Mwezi Moja is short.

At planting, each gross plot of 16 m² was fertilized with calcium ammonium nitrate, triple super phosphate and muriate of potash at rates of 80 kg N, 100 kg P and 20 kg K per hectare, respectively. Two seeds per hill were planted at a hill spacing of 0.5 x 0.1 m. At full emergence the seedlings were thinned, leaving one per hill. Within each plot, two areas of 40 plants were harvested at physiological maturity (i.e., when pods had changed colour from green to green yellow and seeds had their final red purple colour) and harvest maturity (i.e., when pods had changed colour from green yellow to straw yellow).

Pods were picked and shelled by hand. Seeds with abnormal development and size were discarded and only normal looking seeds were selected and dried in a continuous-flow drier at 30 °C until 14% moisture content. They were then stored at 2 °C and 75% relative humidity for on average three months until further analysis.

Electrical conductivity tests

Electrical conductivity (EC) testing was carried out because it is one of the most reproducible vigour test methods and can be applied to both bulk seed samples and indvidual seeds. EC was determined after equilibrating the seeds for 3 days at room temperature (19–25 °C). Their moisture content was then constant at 12%, as determined by a moisture meter (Unitron®, Scandinavia A/S). To measure bulk EC, four replicate samples (one from each block) of 50 seeds were weighed and left to soak in 250 ml of distilled water at 20 °C for 24 hours. Electrical conductivity (μ S cm⁻¹) was measured using a Fieldlab-LF conductivity meter and an LF 513T electrode dip-type cell (Schott Gerate Glass Company, Mainz, Germany). The EC per gram of seed weight (μ S cm⁻¹

g^{-I}) at 12% moisture content in 250 ml of water was then calculated (Hampton & TeKrony, 1995). To measure individual seed EC, four samples of 20 seeds each were taken and combined into one sample of 80 seeds. Each seed was weighed individually and left to soak in 50 ml of distilled water at 20 °C for 24 hours, using the same method as for measuring bulk EC. The electrical conductivity per gram of seed weight (μ S cm^{-I} g^{-I}) at 12% moisture content in 50 ml of water was then calculated (Hampton & TeKrony, 1995).

Tetrazolium tests

Seed viability was assessed by the tetrazolium test because it is a rapid, easy and highly reproducible test that can be carried out with minimal equipment, whereas its results correlate well with germination tests (e.g. Dahiya *et al.*, 1997). Four replicate samples (one from each block) of 20 seeds each were equilibrated at room temperature (19–25 °C) for one day before being left to soak in water at room temperature for 24 hours. The seeds were then cut longitudinally through the middle of the embryonic axis and left to soak in a 0.5% tetrazolium (2,3,5-triphenyltetrazolium chloride) solution at 30 °C for three hours, briefly washed in distilled water and examined under hand lens magnification (Hampton & TeKrony, 1995). The fractions seeds evaluated as 'sound' or 'weak viable' were combined to calculate viability, i.e., the percentage of viable seeds.

Calculations and statistical analysis

The following parameters of the frequency distributions of EC (μ S cm⁻¹ g⁻¹) of individual seeds were calculated: mean – median, population standard deviation (SD), coefficient of variation (CV, percentage), and the range 0–75%, i.e., the difference between the minimum and the upper quartile, which excludes the 25% highest values.

Simple and multiple linear regression analyses were carried out using Genstat 5 (Release 4.1). Variation parameters were allocated as explanatory variates (x) and bulk EC and percentage viable seeds as the response variates (y). Cultivars and sites were stepwise added as factors to the regression model. Percentage variance (adjusted R²) over all seed lots and adjusted R² after adding cultivar, site, or site and cultivar as factors to the regression model were calculated. If adding a factor to the model significantly increased adjusted R², linear regression was carried out for each level of the factor and the statistical significance of the regression coefficient was determined. Seed lot values that were out of range were inspected, but kept in the models.

Results

Quality of the seed lots in terms of percentage seed germination was very high. In such a situation germination tests will not reveal differences in quality. Precise and statistically reliable information on germination percentages, however, is not available due to malfunctioning of the equipment involved.

The linear regressions of bulk seed quality (bulk EC and percentage viable seeds) on individual seed variation in EC (quantified by mean – median, SD and the range o–75%) found at physiological maturity (PM) were statistically significant (Table 1; Figure 1). A large variation in individual seed EC was associated with a low quality, i.e., a high EC and a low viability (Figure 1). Adding site as a factor to the model significantly improved the proportion of variance accounted for by the regression (Table 1), showing that the relationship differed between the two sites. There was no statistically significant increase in R² when cultivar was added as a factor to the regression model (Table 1), showing that the relationship was similar for the two cultivars. Bulk quality did not significantly increase or decrease with increasing variation in individual EC when this was measured as CV (Table 1).

The parameters quantifying variation among individual seeds were lower at harvest maturity (HM) than at PM for most seed lots and the variation in bulk EC over seed lots was less than at PM (Figure 2). Nevertheless, over all seed lots a higher variation as measured by mean – median, SD, and the range o–75% was associated with a lower bulk EC (Table 2; Figure 2). When site was added as a factor to the models, the R² of the regression increased, except for o–75% (Table 2). Regression analysis for the individual sites showed that a positive association between bulk EC and variation measured as mean – median was statistically significant for Kitui only whereas the positive association between bulk EC and SD was only significant for cv. Mwezi Moja in Kitui (Table 2; Figure 2). Over all seed lots, no statistically significant associations were found at HM between variation and percentage viable seeds (Table 2), but adding site to the regression model significantly increased R² (Table 2; Figure 2). Analysis per site showed that for both sites a higher variation as quantified by mean – median or the range o–75% was associated with a lower percentage viable seeds (Figure 2).

Discussion

Associations between individual seed quality variation and quality of the seed lot

All statistically significant associations found between variation in individual seed EC and bulk quality as indicated by bulk EC and percentage of viable seeds showed that a higher individual seed variation was associated with a poorer bulk quality (Figures 1 and 2). In other words, our results show that lack of uniformity among seeds is usually associated with a poorer seed lot quality over a large number of seed lots grown under different conditions. This is consistent with the decrease in, for instance, germination uniformity found in seed lots that deteriorate in quality because of ageing (Hosnedl & Horakova, 1998). Statistically significant associations, however, were only found when variation was quantified by mean – median, SD or the range o–75% at PM (Figure 1). These associations were weaker at HM (Table 2; Figure 2). Statistically significant associations were not found when variation between individual seeds was measured as CV (Figures 1 and 2). This is consistent with an earlier finding that CV was not regarded to be a good parameter for quantifying individual seed variation in

Table 1. Coefficients of determination (adjusted R²) of linear curves fitting bulk electrical conductivity (µS cm⁻¹ g⁻¹) and percentages viable seeds at physiological maturity to different parameters describing individual seed variation before and after adding cultivar (cv) and site as factors to the regression model (n = 24). The most suitable models are underlined.

Variation	R ²				Statistical signifi	Statistical significance $^{\rm I}$ of net change after adding:	ge after adding:	
patameter Over all seed lot Bulk electrical conductivity	Over all seed lots	After adding cv. as a factor to the model	After adding site as a factor to the model	After adding cv. and site as factors to the model	Cv. as a factor	Site as a factor	Cv. as a factor Site as factor after site after cv.	Site as factor after cv.
Mean – median 0.441*** I	I ***IV**	0.408**	0.691***	0.640***	SZ	*	SZ	-k
SD^{2}	0.416***	0.380**	0.658***	0.638***	NS	* *	NS	*
CV 3	- 4	1	0.005 NS	o.069 NS	NS	NS	NS	NS
Range 0–75% 0.537***	0.537***	0.514***	0.656***	0.648***	NS	*	NS	NS
Percentage viable seeds	seeds							
Mean – median 0.312**	0.312**	0.255**	0.624***	0.682***	NS	***	NS	* *
SD	0.278**	o.209 NS	0.579***	0.666**	NS	**	NS	**
CV	ı	ı	o.io6 NS	0.303 NS	NS	NS	NS	*
Range 0-75%	0.426***	0.401**	0.628***	0.694***	NS	**	NS	* *

I NS = not statistically significant $(P \ge 0.05)$; * = statistically significant at $0.01 \le P < 0.05$; ** = statistically significant at $0.001 \le P < 0.01$; *** statistically significant at P < 0.001.

SD = population standard deviation.

³ CV = coefficient of variation.

^{4 - =} residual variance exceeded variance of response variate.

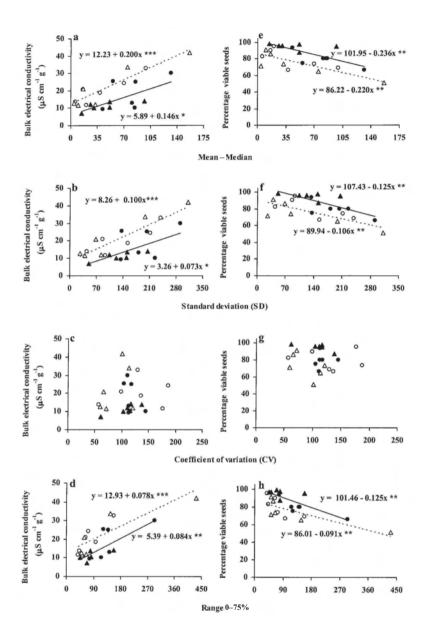


Figure I. Relationship between parameters describing individual seed quality variation at physiological maturity and bulk quality as measured by electrical conductivity and percentage viable seeds for cv. Rosecoco in Eldoret (a), cv. Mwezi Moja in Eldoret (b), cv. Rosecoco in Kitui (c) and cv. Mwezi Moja in Kitui (d). The curves represent the most suitable models as shown by the R² values in Table I. NS = not statistically significant ($P \ge 0.05$); *= statistically significant at 0.01 $\le P < 0.05$; ** = statistically significant at 0.001 $\le P \le 0.01$; and *** = statistically significant at P < 0.001.

Table 2. Coefficients of determination (adjusted R²) of linear curves fitting bulk electrical conductivity (µS cm⁻¹ g⁻¹) and percentages viable seeds at physioogical maturity to different parameters describing individual seed variation before and after adding cultivar (cv.) and site as factors to the regression model (n = 24). The most suitable models are underlined.

Variation	\mathbb{R}^2				Statistical signifi	Statistical significance $^{\rm I}$ of net change after adding:	ge after adding:	
	Over all seed lots	After adding cv. as a factor to the model	After adding site as a factor to the model	After adding cv. and site as factors to the model	Cv. as a factor	Site as a factor	Cv. as a factor after site	Site as factor after cv.
Bulk electrical conductivity	nductivity							
– mediar		0.286*	0.426**	0.491**	NS	-}¢	SN	-k
SD 2	o.179*	0.314*	0.406**	0.631***	NS	*	*	*
CV 3	4	I	I	o.233NS	NS	NS	NS	*
Range 0–75%	0.277*	0.325*	0.407**	o.294 NS	NS	NS	NS	NS
Percentage viable seeds	seeds :							
Mean – median	1	ı	0.801***	0.875***	NS	***	*	***
SD	ı	ı	0.761***	0.862***	NS	***	-;×	* * *
CV	1	ı	0.690***	0.839***	NS	***	* *	***
Range 0-75%	0.130 NS	o.090 NS	0.829***	0.869***	NS	***	NS	***

I NS = not statistically significant $(P \ge 0.05)$; * = statistically significant at $0.01 \le P < 0.05$; ** = statistically significant at $0.001 \le P < 0.01$; *** statistically significant at P < 0.001.

SD = population standard deviation.

³ CV = coefficient of variation.

^{4 -=} residual variance exceeded variance of response variate.

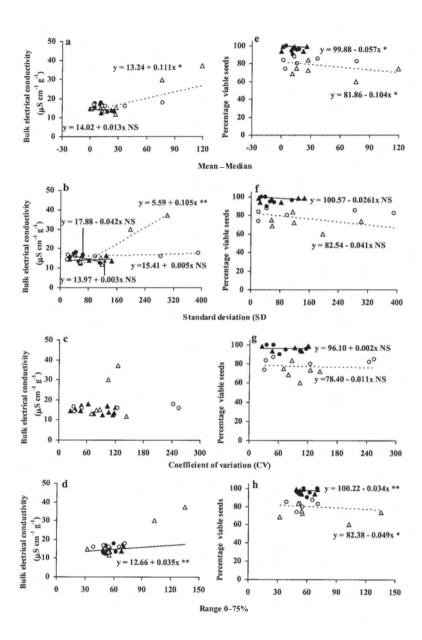


Figure 2. Relationship between parameters describing individual seed quality variation at harvest maturity and bulk quality as measured by electrical conductivity and percentage viable seeds for cv. Rosecoco in Eldoret (\bullet), cv. Mwezi Moja in Eldoret (Δ), cv. Rosecoco in Kitui (\circ) and cv. Mwezi Moja in Kitui (Δ). The curves represent the most suitable models as shown by the R² values in Table 2. NS = not statistically significant ($P \ge 0.05$); *= statistically significant at 0.01 $\le P < 0.05$; ** = statistically significant at 0.001 $\le P < 0.05$; and *** = statistically significant at P < 0.001.

seed lots from different origins (Muasya *et al.*, 2006). Earlier, variation quantified by mean – median or SD was found to be higher when there were seeds within a population showing extremely high values deviating from the bulk of the population, whereas the range 0–75% was not sensitive to a few outliers and measured variation in bulk of the seed population adequately (Muasya *et al.*, 2006).

Effects of the production site

Adding site to the regression model usually significantly increased the percentage variance accounted for at both PM and HM (Tables I and 2), which shows that the associations between bulk quality and variation were different for the two sites (Figures I and 2). At a comparable level of variation, bulk quality was better in Eldoret than in Kitui, and consequently also the quality level of individual seeds *per se*. This is probably partly related to higher average daily temperatures and unreliable rainfall in Kitui. Drought and high-temperature stress during seed filling reduce germination and vigour of soya bean seeds (Dornbos Jr & Mullen, 1991). However, when associations were statistically significant, quality at both sites was better when variation was lower.

Seed quality differences between physiological maturity and harvest maturity

Associations between bulk seed quality and individual seed variation were generally clearer at PM than at HM. This is partly explained by the fact that most of the seed lots were within a narrower range of bulk quality and also showed a smaller variation between individual seeds and a narrower range of variation at HM than at PM (Figures 1 and 2). The reasons for this range in bulk and individual seed quality being narrower at HM than at PM are probably related to the phenomenon that between PM and HM the quality of several seed lots did not decrease as was expected (cf. Egli, 1988), but was still increasing. When reaching HM more seeds will have attained their maximum quality.

Reducing seed-to-seed variation

In this series of experiments, also within a site the differences in seed-to-seed variation have resulted from differences in weather conditions during production, because cultivation practices had been kept the same for all seed lots. General strategies for reducing the seed-to-seed variation within a production site and growing season could concentrate on reducing differences between and within plants. The first could be achieved through using uniform high quality planting material and proper seedbed preparation, thus ensuring uniform emergence and plant establishment. Thinning, as was applied in our experiments, could further reduce differences among plants. Within-plant differences could be reduced by methods aiming at synchronizing the development and maturation of seeds. Among these could be methods to reduce the length of the flowering period and/or the number of orders of inflorescences, e.g. by non-excessive nitrogen fertilization, or using a not too wide spacing. Application of desiccants can accelerate natural drying (e.g. Kelly & George, 1998) and desiccants

are applied mainly to improve yield, facilitate harvesting or advance harvesting when conditions during the maturation drying period are not reliable. Desiccants thus decrease the length of the maturation period and improve the uniformity of seed lots (e.g. Marchiori Jr *et al.*, 2002), especially in indeterminate crops, but this better uniformity does not necessarily lead to improved seed quality. However, desiccants are reported not to reduce seed quality if applied properly (e.g. Marchiori Jr *et al.*, 2002; Greven *et al.*, 2004), but the possibility of reducing seed quality remains (Kelly & George, 1998; Greven *et al.*, 2004).

Alternatively, selective harvesting of seeds at the optimum harvest time is a possibility to reduce variability in large-seeded crop species even further. This, however, will be restricted to regions and crops in which manual labour is current.

Conclusions

- I. At physiological maturity, a lower bulk quality as measured by bulk EC or percentage viable seeds was found to be linearly related to a higher variation in individual seed EC (μ S cm $^{-1}$ g $^{-1}$) when the latter was quantified by the parameters mean median, SD or o–75%. There was no linear relation between bulk quality and variation in individual seed EC as measured by CV over seed lots.
- 2. Associations between quality and individual seed variation were also found at harvest maturity, but they generally were less clear than at physiological maturity, because at harvest maturity individual seed variation was lower and seed lots varied less in bulk quality.
- 3. The associations between individual seed variation and bulk quality were different for seed lots produced at different sites, indicating that not just the degree of variation, but also the level of individual seed quality determines bulk quality.
- 4. Generally, the associations between bulk quality and individual seed variation were not different for the two cultivars tested.

References

- Dahiya, O.S., R.P.S. Tomer & Ashwani-Kumar, 1997. Evaluation of viability and vigour parameters with respect to field emergence in chickpea (*Cicer arietinum L.*). Seed Research 25: 19-24.
- Dornbos Jr, D.L. & R.E. Mullen, 1991. Influence of stress during soybean seed fill on seed weight, germination, and seedling growth rate. *Canadian Journal of Plant Sciences* 71: 373–383.
- Egli, D.B., 1998. Seed biology and the yield of grain crops. CAB International, Wallingford, 178 pp. Gavras, M.F., 1989. The influence of mineral nutrition, stage of harvest and flower position on seed yield and quality of *Phaseolus vulgaris* L. *Dissertation Abstracts International. Biological Sciences and Engineering* 50: 1687B–1698B.
- Greven, M.M., B.A. McKenzie, J.G. Hampton, M.J. Hill, J.R. Sedcole & G.D. Hill, 2004. Factors affecting seed quality in dwarf bean (*Phaseolus vulgaris* L.) before harvest maturity. Seed Science and Technology 32: 797–811.
- Hampton, J.G. & D.M. TeKrony, 1995. Handbook of Vigour Test Methods (3rd edition). International

- Seed Testing Association, Zurich, 117 pp.
- Hosnedl, V. & D. Horakova, 1998. Vigour and germination of deteriorated pea seeds. In: Opportunities for High Quality, Healthy and Added-value Crops to meet European Demands. Proceedings 3rd European Conference on Grain Legumes, 14–19 November 1998, Valladolid. European Association for Grain Legume Research, Paris, p. 294.
- Illipronti Jr, R.A., W.J.M. Lommen, C.J. Langerak & P.C. Struik, 2000. Time of pod set and seed position on the plant contribute to variation in quality of seeds within soybean seed lots. *Netherlands Journal of Agricultural Science* 48: 165–180.
- Kelly, A.F. & A.A.T. George, 1998. Encyclopaedia of Seed Production of World Crops. John Wiley & Sons, Chichester, 403 pp.
- Levengood, W.C., J. Bondie & C. Chen, 1975. Seed selection for potential viability. *Journal of Experimental Botany* 26: 911–919.
- Marchiori Jr, O., M.H. Inoue, A.L. Braccini, R.S. Oliveira Jr, M.R. Avila, M. Lawder & J. Constantin, 2002. Quality and yield of canola (*Brassica napus*) seeds after pre-harvest desiccant application. *Planta Daninha* 20: 253–261. (in Portuguese)
- Moore III, F.D., P.A. Jolliffe, P.C. Stanwood & E.E. Roos, 1988. Use of Richards function to interpret single seed conductivity data. *HortScience* 23: 396–398.
- Muasya, R.M., W.J.M. Lommen & P.C. Struik, 2002a. Differences in development of common bean (*Phaseolus vulgaris* L.) crops and pod fractions within a crop. I. Seed growth and maturity. *Field Crops Research* 75: 63–78.
- Muasya, R.M., W.J.M. Lommen & P.C. Struik, 2002b. Differences in development of common bean (*Phaseolus vulgaris* L.) crops and pod fractions within a crop. II. Seed viability and vigour. *Field Crops Research* 75: 79–89.
- Muasya, R.M., W.J.M. Lommen, E.O. Auma & P.C. Struik, 2006. Evaluation of variation in individual electrical seed conductivity in common bean (*Phaseolus vulgaris*) seed lots. *Seed Science and Technology* 34. (in press)
- Padrit, J., J.G. Hampton, M.J. Hill & B.R. Watkin, 1996. The effects of nitrogen and phosphorus supply to the mother plant on seed vigour in garden pea (*Pisum sativum L.*) cv. Pania. *Journal of Applied* Seed Production 14: 41–45.
- Siddique, M.A. & P.B. Goodwin, 1983. Conductivity measurements on single seeds to predict the germinability of French beans. *Seed Science and Technology* 13: 643–652.
- Steere, W.C., W.C. Levengood & J.M. Bondie, 1981. An electronic analyser for evaluating seed germination and vigour. *Seed Science and Technology* 9: 567–576.