Crop physiological analysis of seed quality variation in common bean (*Phaseolus vulgaris* L.)

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Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. ir. L. Speelman,
in het openbaar te verdedigen
op woensdag 12 september 2001
des namiddags te vier uur in de Aula.

Reuben M. Muasya (2001)

Crop physiological analysis of seed quality variation in common bean (*Phaseolus vulgaris* L.)
Muasya, R.M. – [S.L.: s.n.]. Ill.
PhD Thesis Wageningen University. – With ref. –
With summaries in English and Dutch
ISBN: 90–5808–469–8
Subject headings: *Phaseolus vulgaris* L., physiological maturity, harvest maturity, seed quality, viability, vigour, variation

Propositions

1. Seeds from early pods of common bean crops achieve higher vigour during development than seeds from late pods.
   This thesis

2. In common bean crops, variation in seed moisture content between individual seeds changes drastically after physiological maturity and reaches a peak before harvest maturity.
   This thesis

3. The degree of variation in individual seed quality can determine the quality of a seed lot.

4. In common bean, statistics can lie about physiology.

5. Love is corrective.

6. The development of a PhD thesis follows a pattern similar to that of the development of viability in common bean.

Propositions belonging to the PhD thesis of Reuben M. Muasya
Crop physiological analysis of seed quality variation in common bean (*Phaseolus vulgaris* L.)

Wageningen, 12 September 2001.

Abstract

Reuben M. Muasya, 2001. Crop physiological analysis of seed quality variation in common bean (*Phaseolus vulgaris* L.). PhD thesis, Wageningen University, The Netherlands, 169 pp., English and Dutch summaries.

Common bean yield is low in Kenya and use of poor quality seed by small-scale farmers has been identified as a major yield constraint. This research project aimed at increasing insight into development of common bean seed quality and its variation during crop production and into how conditions during production affect these.

In experiments involving bean cultivars Rosecoco and Mwezi Moja, physiological maturity (PM), i.e. the moment of maximum seed dry weight, was achieved at 58% seed moisture content. Harvest maturity (HM) was defined to occur at 20% moisture content. At PM, the percentage viable seeds as measured by tetrazolium test was still increasing. It became maximum closer to HM implying that seed development does not stop at PM. Seed vigour as measured by electrical conductivity (EC) was maximum at PM and remained constant until HM.

Seeds in pods of different earliness and seeds of the whole crop all achieved maximum viability at the same moment beyond PM. The maximum viability achieved also was the same in all seed classes. Maximum seed vigour was achieved at PM in individual seed classes and was achieved earlier in seeds from earlier pods than from later pods. The vigour of seeds from the individual earliness classes at their optimum moment of harvesting was higher than the vigour of seeds from all pods combined. Individual seed variation in dry weight, moisture content and EC over time was lower in seeds from earlier pods than from all pods combined.

Seed lots produced under different weather conditions and at two sites differed in quality within and between seed lots. Within seed lots, variation in individual seed quality as quantified by mean - median, range 0 - 100%, variance and standard deviation (SD) in individual seed EC was high when there were seeds with extremely high values deviating from the bulk of the seeds. Seed lots with deviating values did not necessarily have large variation in the bulk of the seeds, as quantified by the ranges 0 - 75% and 25 - 75%. Low variation in individual seed EC as quantified by mean - median, SD and the range 0 - 75%, was associated with good quality as measured by low bulk EC and/or high percentage viable seeds. Associations were clearer at PM than at HM. Relationships between individual seed variation and bulk quality were different at the two sites implying both the degree of variation and the level of individual seed quality can determine bulk quality. No relationship was found between CV% in individual seed EC and bulk quality.

High temperature and less rainfall both could reduce seed quality. Over the ranges studied, high temperature was more detrimental than limited rainfall. Weather conditions seemed to reduce seed quality mainly through reducing the maximum quality attainable during crop development. Quality deterioration "in planta" was less important. Variations in weather conditions during production did not lead to lower quality seed lots through increasing variation within the crop, as measured by duration of flowering or pod set or plant-to-plant variation in number of flowers. Production conditions conducive to low seed yield or low individual seed weights were also conducive to low percentage of viable seeds. However, high seed yield does not guarantee high percentage viable seeds.

Although the moment all seeds within a crop or crop fraction achieve the final red purple colour pattern was identified as a good indicator of PM, practically the use of pod and seed colour changes was an unreliable method for determining when to harvest. Results imply that processes determining the changes in colour and those determining changes in seed moisture content are differentially affected by external conditions. Based on the results of this research, picking pods from individual pod classes based on 20% seed moisture content could improve the uniformity within the harvested seeds and subsequently the final quality of the seeds harvested. This was shown for seeds from early pods.

Keywords: Physiological maturity, harvest maturity, earliness, common bean, *Phaseolus vulgaris* L., morphological markers, variation, moisture content, dry weight, viability, vigour, electrical conductivity, tetrazolium, seed lot, seed filling, maturation drying, temperature, rainfall.

Preface

This thesis is a result of 4 years 'Sandwich' PhD research work on common bean seed quality and variation conducted at both Moi University, Kenya, and Wageningen University, The Netherlands, under the funding of an MHO/NUFFIC linkage programme.

First I thank God for all He has done in my life and especially for giving me good health, strength and determination during the 4 years of this project. To Him I return all the glory and honour for this work.

It is my great pleasure to express my heartfelt thanks to the following individuals and institutions that directly or indirectly contributed to the success of my work.

Special acknowledgement are due to Moi University, for granting me this study opportunity and to Wageningen University for granting me the admission and facilitating my programme both in Kenya and in The Netherlands. Special acknowledgements also go to Kenya Forestry Research Institute (KEFRI), Kitui Centre, and Kitui Farmers Training Centre (KFTC) for allowing me to carry out experiments in their laboratories and experimental sites, respectively.

My greatest respect and appreciation go to my promotor Prof. dr Paul C. Struik, advisor of my PhD programme for his special interest, professional guidance, unreserved support and encouragement. I honestly lack words good enough to describe all my appreciation to Paul for his guidance and support throughout this thesis work.

I am deeply grateful to my first co-promotor Dr Willemien J. M. Lommen for her keen interest in the progress of my work. Willemien was deeply involved in my work at every stage. She was always available for consultation during fieldwork and during the preparation of the manuscript. Her valuable ideas and devotion to my work always gave a new dimension of looking at the data. Willemien, thank you for your patience even during the 16-hour journey from Eldoret to Kitui during the fieldwork. You were very tired and could hardly speak but you made it. My acknowledgements also go to my second co-promotor Prof. Elmada O. Auma for his constant visits to my experimental plots and directions during my fieldwork. His assistance with administration and financial matters in the initial stages of this project was very valuable to the success of the project. Prof. Auma's critical comments were always useful.

I greatly appreciate the vital role played by Dr Henk Hilhorst, the Wageningen University co-ordinator of the MHO Seed Technology project in ensuring equipment and research funds were available during my fieldwork. Henk gave this project a special attention to ensure its success. I am also grateful to the Moi University co-ordinator of the project and head of the Department of Crop Production and Seed

Technology Dr Patrick Mathenge for ensuring funds were always available for research expenses. I also extend my thanks to all members of the Department of Crop Production and Seed Technology for always being helpful and supportive during my fieldwork.

My heartfelt gratitude goes to Ms Gon Van Laar for taking her precious summer time to design a layout of my thesis and for her editorial suggestions. To all the staff of Crop and Weed Ecology and Plant Production Systems, your friendly gestures and readiness to help whenever I approached you were always inspirational to me. My thanks also go to Drs Gerrit Gort and Drs Albert Otten of Mathematics Department, Wageningen University, for their assistance with statistical analysis and curve fitting.

Very special thanks go to Catherine Muui, Scholastica Mbatha, Lilian Ochola, Martin Odari, David Ndunda, Teresia Ndendwa, Bridie Ndunda and Jackson Teti, my research assistants both in Eldoret and Kitui experimental sites for their dedication to data collection and laboratory analysis. I would also like to thank all the staff of KEFRI and KFTC Kitui for their support during my fieldwork. Thanks are due to Philip Ngotho for allowing me to use his house as a store during my experiments in Kitui.

I am grateful to all my PhD colleagues in the Crop and Weed Ecology Group and especially my officemates René Akanvou, Mahmoud Otroshy, Roberto La Rovere, Woldeamlak Araia and Admasu Tsegaye for their support and constant consolation when the going got tough. Special thanks go to Linnet Gohole, my colleague from the Moi University, Kenya, for her constant encouragement during my fieldwork and the write up of this thesis.

The physical and spiritual encouragement and support from all the End Time Message believers in Eldoret, Kenya, and in Rotterdam always gave me strength to go on with my work. Special thanks to Marinus Benschop, Teunis Eilander, Dinah ter Velde and the families of Nelson Kemboi, Harry van der Stel, Nuri Hagenouw, Jan Bikker, Frank Gorre, Roy Nabi, George Cruden, Albert de Kogel and Tom Kandagor for their support and encouragement. Special appreciation to Sandy Gorre for helping with data input at a time when the work was overwhelming.

I wish to extend my appreciation to my parents for their encouragement and understanding especially when we lost a relative while I was away preparing this thesis.

Finally, I am greatly indebted to my wife Jane and the children Joseph, Emmanuel and Faith for their encouragement, patience and understanding during my constant absence from them for the whole period of this study. With honour I dedicate to them this thesis.

Reuben M. Muasya

Wageningen, 12 September 2001

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Chapter 1

General introduction

1. General introduction

Common bean as a food crop

Common bean (*Phaseolus vulgaris* L.) is the principal food legume of more than 500 million people in Latin America and Africa, and for more than 100 million of these people it is the leading source of dietary protein (FAO, 1984; Krista et al., 1991). While the demand for common bean has been growing, the world production of beans has been declining (FAO, 1998). The world total production of common bean is estimated to be 19.0 million metric tonnes per year of which 27% comes from Latin America and 10% from Africa (FAO, 2000).

In Kenya, common bean is the most important pulse and second to maize as food crop (GOK, 1998). The national annual demand for common bean has been estimated at 500,000 metric tonnes, but the actual annual production is only about 125,000 metric tonnes (Ndiritu, 1990; GOK, 1998). The total area under bean cultivation in Kenya is estimated to be 500,000 ha (GOK, 1998) leading to actual bean yields of 250 kg ha^{-1}, partly under mixed cropping. In pure stands, yields of 700 kg ha^{-1} have been reported (Songa et al., 1995). This yield is low compared to a potential yield of up to 5000 kg ha^{-1}. Such high yields have already been achieved in other countries, e.g. Mexico under field conditions (Rodriquez and McDonald Jr, 1989).

Use of poor quality seed, low soil fertility, adverse weather conditions and incidence of pests and diseases have been identified as some of the major constraints to bean production in the developing tropics (Wortmann and Allen, 1994; Gridley and Danial, 1995). While substantial research work has been done on breeding for improved cultivars, response to soil fertility, and pest and disease control (e.g. Maiuki, 1988; Makini and Danial, 1995; Tyagi et al., 1996), production of good quality seeds has not been focused on.

Since most farmers use farm-saved seed increased insight into development of common bean seed quality during crop production and into how conditions during production affect the seed quality could ultimately lead to production of better quality seed, and consequently towards increased common bean yields in Kenya.

Reasons for poor seed quality in Kenya

Seed planted

It is estimated that 30% of poor quality of the seed produced in Kenya is already due to the use of poor quality seed for planting, assuming all other factors are favourable

Table 1. Estimated sources (in percentages) of the seeds planted by common bean farmers in Kenya.

Seed source	Percentage
Authentic seed merchants	5.0
Farm saved seed	82.0
Borrowed from neighbours	4.2
Grain from the market	8.6
Other sources	0.2

Source: Muhammed et al., 1985; Songa et al., 1995.

(Combes, 1983). Table 1 shows the sources of seeds that farmers use for planting. The common bean seed is produced by both formal and informal seed sectors. Only 5% of the seed comes from authentic sources (Table 1). Commercial seed companies produce certified bean seeds and fix a price for it to meet the cost of multiplication and distribution. However, most of the small-scale bean farmers cannot afford the prices, and consequently opt to alternative seed sources. Due to the limited demand, companies also multiply less stocks of certified seed. This creates scarcity of the seed even to farmers who can afford it (Cromwell et al., 1992). Therefore farmers opt to use farm-saved seed (Combes, 1983) or buy market bean grains and use them as seed. The bean seed the small-scale farmers use is therefore produced and stored as a grain crop without adhering to standard seed quality regulations e.g. isolation and roguing of offtype plants and without focusing on high viability or seed vigour (as defined in Table 2), weeds, pests and diseases.

The use of this type of seed can lead to production of a seed of reduced vigour because both poor genetic make-up and seed borne diseases in the seed sown can be transmitted to the seed produced (van Rheenen et al., 1981). Seeds of reduced vigour also express a more variable emergence with some seedlings emerging later than others (e.g. TeKrony and Egli, 1991) and could lead to a more variable crop producing a mixture of immature to over-mature seeds. This may result in high percentage of abnormal seedlings and reduced vigour (Mariga and Copeland, 1989).

Biotic stresses

Because of the high cost of chemicals and labour, weed, pest and disease control by the small-scale farmers in Kenya depend on the financial ability of the farmer. Weeding is usually done manually. In a growing season, a crop may be weeded repeatedly. Pests and diseases are mainly controlled by chemical application during critical stages of crop growth. Usually most of the farmers attempt to control pests and

Table 2. Definitions of terms used in this thesis.

Term	**Definition**
Viability	The capability of the seed to germinate and produce a normal seedling under optimum conditions (Dornbos Jr, 1995).
Vigour	The sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence (ISTA, 1995). Vigour is positively related to the ability of a seed population to establish an optimum plant stand, in both optimum and suboptimum soil environments (Dornbos Jr, 1995).
Tetrazolium test	Test for evaluating both viability and vigour. In this test triphenyl tetrazolium chloride is reduced by the terminal oxidase systems in living plant tissue from a colourless solution to a red, water-insoluble formazan compound which is precipitated within live cells while in dead cells no reaction takes place and they remain colourless (ISTA, 1995). In this research this test was only used for establishing viability.
Electrical conductivity test	Test for evaluating seed vigour based on the association between the amount of electrolyte leakage from the seed or seeds and vigour. The test involves steeping of seeds in a certain quantity of deionized water for a standard period of time. The seed(s) release(s) electrolytes into the water. By applying an electromotive potential across electrodes the electric current passing through the solution can be measured (Pandey, 1992; ISTA, 1995). High values indicate low vigour.
Seed filling	The stage of seed development during which seeds accumulate dry weight (Egli, 1998). This stage starts at pod set and terminates when the seed achieves physiological maturity.
Physiological maturity (PM)	The stage of seed development of a seed or seed crop beyond which there is no further increase in seed dry weight (Crookston and Hill, 1978; Egli, 1998).
Harvest maturity (HM)	The stage of seed development when the seed has dried to a moisture level at which the seed can be harvested without a high risk of mechanical damage (TeKrony et al., 1980b; Egli, 1998).
Maturation drying	The growth phase after PM during which the seed undergoes moisture loss (TeKrony and Egli, 1997).

diseases after observing symptoms of attack but at this stage the damage on the crop has already taken place.

Inadequate control of the biotic stresses usually leads to seeds contaminated by weeds, pests and diseases. Controlling weeds, pests and diseases during crop and seed development ensures they are not carried over to the seed and subsequently to the next crop. Insect populations, e.g. of bean fly, build-up during crop growth, and if not controlled, may extend to storage (Beebe and Corrales, 1991) leading to reduced seed quality during storage. Control of fungal infection in the field by systemic fungicides sprays and other cultural practices e.g. removing diseased plants, leads to seeds of improved seed germination and field emergence (Ellis et al., 1976). Based on these reports it is evident that failure to control these biotic stresses may lead to production of seeds poor in quality or which may develop poor quality while in storage.

In Kenya substantial research work has been done (e.g. Tyagi et al., 1996; Otsyula et al., 1998; Desaeger and Rao, 2000; Koech and Whitebread, 2000) with the aim of producing pest and disease resistant varieties of common bean as well as identifying the most suitable control measures. Nevertheless, control of weeds, pests and diseases in common bean crops in Kenya still depends on the financial ability of the farmer because of the high cost of chemicals and labour.

Fertilization

Most of the small-scale common bean farmers in Kenya produce their crop without any fertilizer or use amounts lower than the recommended rates (Table 3). In Kenya, bean-growing areas have been reported to lack N and P (Keya, 1975; Ssali and Keya, 1980, 1983; Chui, 1988, 1989; Wortmann and Allen, 1994).

The common practice by farmers in Kenya will lead to poor seed quality because nutrient stress during mother plant development and at seed fill are generally associated with reduced seed quality of the progeny (Egli et al., 1987; Dornbos Jr, 1995). On the other hand, other reports suggest that fertilization may reduce seed quality, e.g. Heenan and Campbell (1980). It is estimated that 80% of the farmers plant seeds which have been produced without adequate fertilization and may express poor germination and vigour (Songa et al., 1995; personal observation).

Higher fertility level may not only improve seed quality but also will increase yield and therefore provide the farmers with means to procure more inputs. Whereas an improved nutrient supply may increase the maximum quality of individual seeds, it could also prolong the flowering and maturation periods (Gavras, 1989; Padrit et al., 1996). This could create large differences in viability and vigour between early and late produced seeds when all pods are harvested simultaneously and could, therefore, reduce the uniformity of the seed lot produced.

Table 3. Environmental conditions and recommended fertilizer rates during bean production in the lowland and highland agro-ecological zones of Kenya.

	Highland agro-ecological zones, e.g. Eldoret	Lowland agro-ecological zones, e.g. Kitui
Altitude (m asl)	2000 - 2200	900 - 1000
Annual rainfall (mm)	900 - 1100	500 - 750
Long rains duration	March - June	March - June
Long rains reliability (% of years with rains)	70%	30%
Short rains duration	July - September	October - December
Short rains reliability (% of years with rains)	90%	50%
Average temperature (°C)		
Daily minimum	10 - 18	18 - 20
Daily maximum	20 - 25	25 - 35
Daily average	15 - 22	22 - 28
Bean crop duration range (d)	90 - 120	90 - 120
Soil pH	<5.0	5.0 - 5.5
Soil fertility	low	medium to low
Recommended fertilizer rates		
N (kg N ha^{-1})	80	80
P (kg P_2O_5 ha^{-1})	100	100
K (kg K_2O ha^{-1})	20	20

Source: Jaetzold and Schmidt (1982) and personal observation.

Weather conditions during crop growth and maturation

The typical weather conditions of the main bean producing areas for both lowland and highland areas of Kenya are shown in Table 3. The bean growing seasons vary depending on the agro-ecological zone. Beans are grown in both long and short rainfall seasons. A bean crop is planted at the onset of rains or shortly thereafter depending on how prepared the farmer was before the rains start. Sowing may also be delayed for a week or two depending on when the farmer wants to harvest his crop. In the lowland agro-ecological zones, the traditional sowing time during the long rains is March with the crop maturing in June. During the short rains the crop is sown in October and matures in January. In the highlands the crop is planted in the long rains in March and maturation takes place in June when the seasonal rains are still going on. The short rain crop is planted in July/August and matures in October at lower rainfall than in the first season.

In some years, inadequate rainfall compounded by variability in amount and distribution may cause the crop to undergo water stress either during vegetative, flowering, podding or seed filling stages. Water stress during mother plant development and at seed filling has been associated with reduced seed quality of the progeny (Egli et al., 1987; Dornbos Jr, 1995). Suboptimal weather conditions during seed growth and development could accelerate physiological deterioration of the seed leading to poor germination and seedling vigour (Miles, 1985; Spears et al., 1997). Delayed harvesting at high temperatures and rainfall led to accelerated aging in legumes (Powell et al., 1984).

Adverse weather conditions, e.g. water stress during crop growth, could also lead to variations between plants in the timing of flowering, podding, seed filling and eventually result in lack of uniformity between seeds within seed lots at harvest. On the other hand ideal weather conditions during the vegetative and flowering stages could lead to long flowering and podding periods and subsequently to large differences between early and late seeds within the crop. This could lead to a high individual seed variation when all seeds are harvested at one moment.

Knowledge on how weather conditions in Kenya affect common bean seed production will be useful in manipulating the sowing and harvesting dates to coincide with ideal weather conditions for a seed crop or adopt different methods which could reduce variation within a seed lots.

Time of harvest

In Kenya, time of harvesting is predicted by the change in colour of the pods from green to yellow and finally to straw yellow. Bean harvesting starts when pods have changed colour to straw yellow and may proceed until the pods are completely dry to the extent of shattering.

The late harvest could lead to exposing the seed to deleterious conditions and enhance deterioration. According to TeKrony et al. (1980a, b) and Lassim and Chin (1987), legume seed achieves maximum dry seed weight, germinability and vigour at physiological maturity (PM) (Fig. 1 and Table 2). After PM the period of maturation drying (see Table 2) commences. Seed quality is lost gradually after PM as the natural seed deterioration progresses (Dornbos Jr, 1995). A seed crop is normally harvested at harvest maturity (HM) (see Table 2). HM was defined for soybean as the first time the seed dries to a moisture content of 15 - 20% (TeKrony et al., 1980b; Copeland and McDonald, 1995). In mungbean, HM was reported at 19% (Dharmalingam and Basu, 1990) and in dry bean, reports vary between 17% (van de Venter et al., 1996) and 20 - 25% (Kelly, 1988). Especially in unfavourable weather conditions (e.g. high rainfall),

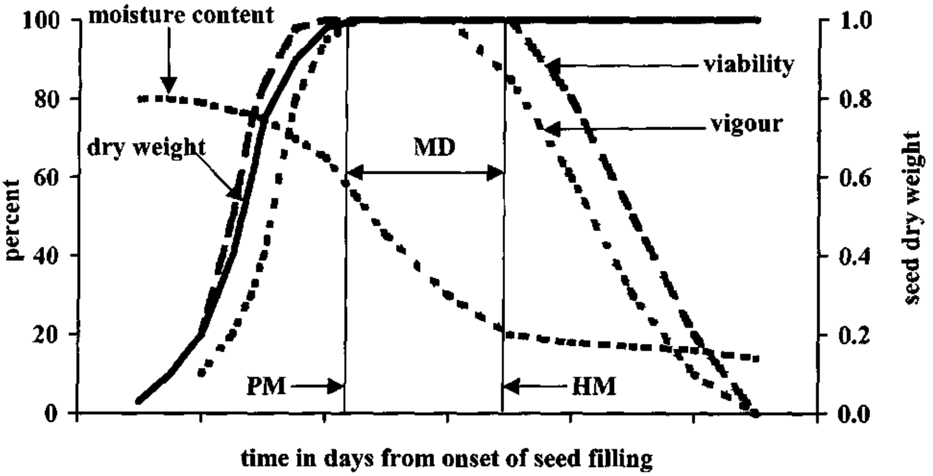

Fig. 1. Development of seed dry weight, viability, vigour (relative values), and moisture content (%) in time from seed filling to storage and also indicating the period of maturation drying (MD) between the moment of physiological maturity (PM) and harvest maturity (HM) as reported by Miles (1985), Dornbos Jr (1995), TeKrony and Egli (1997) and Egli (1998).

the earlier the crop is harvested after PM the better the seed quality (Dornbos Jr, 1995).

Understanding how seed weight, moisture content, pod and seed colour, vigour and viability develop within a crop and how this development is related to the moment during development when seed quality is maximum was thought to be important for deciding when to harvest a high quality common bean seed crop.

Method of harvesting and handling

At harvest, whole plants are uprooted then further dried in the sun. Thereafter they are threshed by beating them with sticks to remove the seeds (grains) from the pods.

This practice may lead to poor seed quality for several reasons. Simultaneous harvesting of seeds, as it is practiced by farmers in Kenya, may lead to harvesting of early and late seeds together resulting in seeds varying in age at harvest because within a crop, the timing of development of individual seeds differs. Some seeds may reach PM while seed filling in others is still going on (Marcos-Filho, 1980; Chamma et al., 1990). This may lead to a seed lot consisting of seeds differing in age and may show high individual seed variation and subsequently poor bulk quality. Exposing the seeds in the hot sun during drying will accelerate quality deterioration of the seed (Powell et al., 1984; Dornbos Jr, 1995). The practice of threshing dried beans by pounding them usually leads to many broken seeds and seeds whose coat has been ruptured. In other countries this practice has lead to 20% quality losses (Powell et al., 1984) but with improved modern methods of shelling or hand shelling pounding could be avoided.

Knowledge on the appropriate method of harvesting may minimize common bean seed quality losses due to handling damage in Kenya.

Conditions after harvest

Common beans in Kenya are again dried in the sun after threshing which exposes the seeds to high temperature. Then they are stored at room temperature in bags.

The high temperature may again accelerate seed quality deterioration in the same way as before threshing, whereas the normal storage conditions will enhance the ageing of the seed. Poor storage conditions have been reported to cause up to 10% loss in seed quality in the tropics (Genchev, 1997). The most important factors slowing down the rate of ageing (and therefore the retention of seed viability) after harvest are low seed moisture content and low temperature (e.g. Coolbear, 1995). Improvements to seed quality retention during storage could also be achieved by ensuring a better initial quality seed at the start of storage (Dornbos Jr, 1995). This calls for insight in how to obtain maximum quality at harvesting moment before storage.

Possible ways of improving the seed planted

Several factors have been identified that are likely to contribute to the poor quality of common bean in Kenya. These could be outlined as the low quality of seed planted, high incidences of weeds, pests and diseases, low fertilization rate, prevailing weather conditions during crop and seed development and maturation, late time of harvesting, poor method of harvesting and handling, and drying and storage conditions after harvest. Several of these could be improved by known methods, e.g. weeds, pest and disease control, method of harvesting and conditions after harvesting.

The physiological quality of the seed planted, however, could also be improved, e.g. by optimizing the timing and method of harvest. A better understanding of processes and mechanisms underlying seed quality formation in a crop is necessary before suggestions for improvement can be given. Understanding how seed weight, moisture content, pod and seed colour, vigour and viability develop within a crop and how this development is related to the moment during development when seed quality is maximum was thought to be important for deciding when to harvest a high quality common bean seed crop. In order to optimize physiological seed quality under diverse weather conditions, understanding is needed of the processes through which weather conditions could affect seed quality. For this, better insight is needed into growth and development of common bean seed and seed quality, within-crop variation and into how weather conditions in Kenya affect within crop variation, and within- and between seed lots variation.

Research problem

Knowledge on the time course of seed and seed quality development of common bean is essential in estimating the appropriate date when seeds could be harvested with minimal loss in seed viability and vigour.

At the moment of maximum seed dry weight (physiological maturity (PM)) the ovule vascular system connecting the plant and seed is disconnected. As shown in Fig. 1, maximum seed viability and vigour coincide at PM (Knittle and Burris, 1976; TeKrony et al., 1979; Dornbos Jr, 1995; TeKrony and Egli, 1997). Viability is quickly gained during seed development and is maintained for some time after the maximum is achieved but vigour increases gradually, and later than viability as seed filling progresses and decreases earlier than viability (Miles, 1985) (Fig. 1). After PM the period of maturation drying commences. Seed quality is lost gradually after PM as the natural seed deterioration progresses (Dornbos Jr, 1995).

At PM, the moisture content in the seed is still high and therefore harvesting is not practical without damaging the seed. For example in soybeans, TeKrony et al. (1980b) observed PM when the seed moisture content was as high as 54 - 62%. In common bean van de Venter et al. (1996) reported mass maturity when seed moisture was 52%. Because of high moisture contents, the seed crop has to undergo maturation drying before harvesting is practical. A seed crop is normally harvested at harvest maturity (HM). HM was defined for soybean as the first time the seed dries to a moisture content of 15 - 20% (TeKrony et al., 1980b; Copeland and McDonald, 1995), in mungbean it is 19% (Dharmalingam and Basu, 1990) and in dry bean reports vary between 17% (van de Venter et al., 1996) and 20 - 25% (Kelly, 1988).

Within-crop differences may arise in timing and duration of the period of seed filling. For instance seeds from pods differing in age may complete seed filling at different moments leading to differences in the moment seeds achieve their maximum quality. Differences in timing of development of seeds within a crop also imply that some seeds may have started undergoing deterioration by the time other seeds reach PM.

It is unknown if seed in pods differing in earliness differ in maximum attainable viability and vigour at their moment of maximum quality. The quality of seed from a uniform selection of pods of similar age might be higher than from all pods combined for two different reasons (a) seeds from certain selection of pods are all closer to the moment at which quality is maximum and (b) maximum quality achieved for seeds from pods from a certain earliness could differ. The difference between the maximum quality of individual pods and the average seed quality of the crop will be higher if a crop is less uniform. The difference in maximum quality achieved in seeds from pods differing in earliness is not known. It is also not known how large the difference is in quality between seeds from the whole crop and from pods differing in earliness in common bean.

Understanding the relationship between the development of seed dry weight, moisture percentage and seed viability and vigour for seeds from whole crop and from pods of different earliness is important in determining the optimum time and method of harvesting, e.g. simultaneous or sequential harvesting.

Weather conditions are likely to affect seed quality in different ways. Weather might affect maximum seed quality obtainable at PM, deterioration of the seed between PM and HM and/or it may change the uniformity of the crop. Differences within and between crops in uniformity due to differences in duration of flowering, podding, seed filling and maturation drying may lead to larger or smaller variation in quality between individual seeds and consequently to lower or higher maximum quality achieved at a certain developmental moment. Although no direct relationship has been reported between moisture stress during vegetative stage and seed quality, Meckel et al. (1984) reported that moisture stress during vegetative stage caused alterations in plant size and yield and could lead to variations between plants. This variation could result in lack of uniformity between plants leading to seeds varying more in age at harvest and consequently reducing the maximum quality of the seed lots produced. High temperatures and rainfall during maturation drying after PM could lower the maximum seed quality achieved through accelerated ageing (Miles, 1985). Weather conditions in Kenya are likely to affect seed quality through differences in rainfall and temperature between and within sites.

Identifying the moment of PM and HM while the crop is in the field is important in deciding when to harvest a seed crop. Morphological markers of PM have been identified, e.g. yellow or black pod colour in soybean (Crookston and Hill, 1978; Egli, 1998) and brown seed colour in common bean (Chamma et al., 1990). In soybean 95% brown pod colour has been used to assess HM (Fehr et al., 1971; Gbikpi, 1981). Morphological markers for HM though important to the farmers for assessing when to harvest common bean in Kenya have not been properly identified and reported.

Research objectives

The research objectives were to increase insight into:

- The differences within a common bean crop in development of physical and seed quality attributes over time in seeds from the whole crop and in pods differing in earliness.
- How high is the variation between seeds and how it develops in common bean crops and in pods differing in earliness with time.
- Variation within common bean seed lots produced under different conditions and whether this variation is related to the final bulk seed quality at physiological maturity and harvest maturity.
- Effects of weather conditions on timing and dynamics of crop stages, plant-to-plant variation and final bulk quality at physiological maturity and harvest maturity, and the difference in bulk quality at PM and HM.

Thesis structure

The general introduction (Chapter 1) outlines the common bean as a crop and the reasons why yields are low in Kenya. Poor seed quality is identified as a major limitation to common bean production in Kenya and the factors contributing to poor seed quality are outlined. Descriptions of the scientific problem and research objectives are presented.

Development with time of common bean seed characteristics and quality in pods of different earliness and in the whole crop are reported in Chapters 2 and 3. In Chapter 2, the development of common bean seed, the moments when the seed achieves physiological maturity and harvest maturity and the identification of these moments using moisture content and morphological colour markers for seeds from whole crop and from pods differing in earliness are reported. Chapter 3 outlines the development of seed viability and vigour over time, shows when quality is maximum and relates quality to seed moisture content in seeds from the whole crop and in seeds from pods differing in earliness.

How large the variation is among seeds from pods differing in earliness and from whole crop, how this variation develops during crop growth, and the relationship between variation in seed characteristics and the developmental moments is described in Chapter 4.

A description of the variation in individual seed quality of seeds in seed lots produced under different set of conditions and the relationship between individual seed variation in quality and bulk quality is reported in Chapters 5 and 6. Chapter 5 describes the distribution patterns of individual seed quality and shows how different variation parameters measure individual seed variation in quality within seed lots and Chapter 6 describes the association between the individual seed variation in quality and bulk seed quality at PM and HM.

How weather conditions affect duration of crop stages, plant to plant variation, individual seed variation and final bulk quality at PM and HM is reported in Chapter 7.

The general discussion treats the highlights of common bean seed development with time and within-crop differences, variation in quality within and between seed lots and how weather conditions affect this variation, identification of when to harvest a higher quality crop and the implications of the research results for improving common bean seed quality in Kenya (Chapter 8).

Chapter 2

Seed development in common bean (*Phaseolus vulgaris* L.) crops and in different pod fractions within a crop[*]

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[*] Muasya, R.M., W.J.M. Lommen & P.C. Struik, 2001. Seed development in common bean (*Phaseolus vulgaris* L.) crops and in different pod fractions within a crop. Submitted to Field Crops Research.

2. Seed development in common bean (*Phaseolus vulgaris* L.) crops and in different pod fractions within a crop

Abstract

Two experiments with common bean cultivars Rosecoco and Mwezi Moja aimed at increasing insight into differences in development with time of seeds from pods of different earliness within a crop. The pods of different earliness were made up of, early, medium, and late pods as compared to the whole crop. In all pod levels, cultivars and seasons, seeds achieved physiological maturity (PM, the moment of maximum dry weight) in the time period when fresh weight was maximum. PM was achieved at 58% seed moisture content in seeds from all pod classes and cultivars. The moment of PM tended to be earlier in seeds from earlier pods. The moment all seeds within a crop or pod fraction achieved their final red purple colour pattern indicated PM well, though not completely accurate. Harvest maturity (HM 20% seed moisture content) was earlier in earlier pods in cv. Rosecoco but not different in pods from different earliness in cv. Mwezi Moja. The period of seed drying from 58 - 20% moisture content was longer in seeds from earlier pods in cv. Mwezi Moja, but not in cv. Rosecoco. The course of decline in moisture content between 58 - 20% differed over pod classes, but was not systematically affected by pod earliness. The timing in seed development in the whole crop could be explained by the timing in seed development in pods of different earliness. Relationships among seed moisture content and seed or pod colour in whole crops differed from those in fractions of pods differing in earliness.

Keywords: Colour, dry weight, harvest maturity, moisture content, physiological maturity, pod earliness

Introduction

Seeds attain maximum dry weight at the moment of physiological maturity (PM) (Shaw and Loomis, 1950; TeKrony and Egli, 1997). At PM the vascular system connecting the plant and seed is disrupted, marking the end of the seed filling period of the plant (TeKrony and Egli, 1997). Seeds of most crops that are harvested as dry seeds attain maximum viability and vigour around PM (TeKrony et al., 1980b; Lassim and Chin, 1987; TeKrony and Egli 1997). At PM seed moisture content is still too high for mechanical harvesting and threshing, for instance 54 - 62% in soybean (TeKrony et al., 1980a), 52% in dry bean cv. Teebus (van de Venter et al., 1996) and 54% in faba beans (Pokojska and Grzelak, 1996). The seed has to undergo a period of maturation drying before harvesting and threshing are possible without causing physical damage to the seed. Seed viability and vigour are lost gradually after PM as natural seed deterioration progresses (Dornbos Jr, 1995). Harvest maturity (HM) is the earliest moment the seed moisture has declined to a level that makes the crop harvestable for its dry seeds (TeKrony and Egli, 1997). For soybean this moment is when moisture content is 15 - 20% (TeKrony et al., 1980b; Copeland and McDonald, 1995), in mungbean it is 19% (Dharmalingam and Basu, 1990) and in dry beans reports vary between 17% (van de Venter et al., 1996) and 20 - 25% (Kelly, 1988).

Within a crop, the timing of development of individual seeds differs. Some seeds may have reached PM before seed filling in all seeds is completed (Marcos-Filho, 1980; Chamma et al., 1990). This variation within a crop complicates the assessment of an exact date of PM for the whole crop (TeKrony and Egli, 1997). Differences in timing of development of seeds within a crop also imply that some seeds may already have undergone degeneration when other seeds reach PM. A different timing of development leads to a different time period between seed PM and harvest and thus to a shorter or longer exposure to adverse internal or external conditions during maturation drying. Higher viability has been reported for soybean seeds from later than from earlier pods which was attributed to shorter exposure of late pods to deteriorating conditions (Illipronti Jr et al., 2000b). Differences possibly could also occur within a crop in the course of development and maturation drying. These for instance may also lead to differences in seed weight (Nanda et al., 1996) and result from differences in seed filling rate and duration. Differences within a crop in the course of maturation drying after PM lead to different internal conditions of seeds between PM and HM. At the relatively high moisture content after PM and before HM metabolic activity e.g. respiration will not stop immediately (Egli, 1998) and may even reduce dry weight (Ashley and Counce, 1993). However, in-plant storage at high moisture content may also lead to faster ageing than in storage. The internal conditions of seeds between PM and HM may also be influenced by the prevailing weather conditions during that time e.g. rain may increase seed moisture content during maturation drying. Differences in development of seeds within a crop therefore imply that the viability and vigour of seeds from selected pods might be higher at their optimum harvest moment than when all pods are harvested combined at the optimum moment. Thus far, there is no clear insight in the development and maturation drying of common bean seeds within a crop.

An important question in seed crops is how to predict the moments of PM and HM based on visual changes within the crop or individual pod. Morphological markers based on colour may provide an easy criterion. Morphological markers of PM have been identified, e.g. kernel black layer in maize (Daynard and Duncan, 1969), yellow or black pod colour in soybeans (Crookston and Hill, 1978; Egli, 1998) and brown seed colour in common beans (Chamma et al., 1990). Morphological markers for HM have also been identified e.g. 95% brown pod colour in soybean (Fehr et al., 1971; Gbikpi, 1981). Morphological markers for HM in common beans have not been properly identified and reported.

This research aims at increasing insight into the differences in development with time of common bean seeds from pods of different earliness within a crop, and into differences in the relationships among developmental characteristics (fresh and dry weight, moisture content, seed length, and pod and seed colour) for seeds within a whole crop and in pods of different earliness.

Materials and methods

Experimental set-up

Two experiments with common bean seeds were planted at the Chepkoilel campus farm of Moi University in Eldoret, Kenya in two growing seasons in 1998. The Season 1 crop was planted on 24 April and harvested from 29 June to 13 August, the Season 2 crop was planted on 17 August and harvested between 5 November and 21 December.

The site was located 2180 m asl and had a cambisol (Ferralitic) soil, well drained, non-humic and shallow, with low nutrient availability and moisture storage (Anonymous, 1987) and pH 4.6 (Muasya, 1996). Season 1 received a total of 554 mm of rainfall and 1471 °Cd temperature sum while Season 2 received 317 mm of rainfall and 1769 °Cd temperature sum. At planting, each plot of 16 m^2 was fertilized with 0.49 kg calcium ammonium nitrate (26% N), 0.76 kg triple super phosphate (48% P_2O_5) and 0.13 kg muriate of potash (60% K_2O), achieving 80 kg N, 100 kg P and 20 kg K per hectare. The experimental design was a split plot with four blocks. Two cultivars, Rosecoco and Mwezi Moja, were assigned randomly to main plots. Both are determinate cultivars, but Rosecoco shows prolonged flowering while the flowering period of Mwezi Moja is short. Both cultivars have white seeds with a red purple colour pattern. Harvesting dates (1 - 14) were assigned to subplots. The first harvesting dates were 67 and 76 days after sowing (DAS) in the first and second season, respectively. Subsequent harvests took place twice a week until 111 and 121 DAS of the first and second season, respectively. The gross plot size was 4×4 m while the net experimental area was 3.40×2.50 m and included 170 plants spaced at 0.5×0.1 m. Within the net experimental area, an area of 2 m^2 was marked for observations of the whole crop (crop level) while the remaining area of 6.5 m^2 was used for observations on three classes of pods (pod level).

Pod classes

Flowering began 40 and 42 DAS in Season 1 and 2, respectively. In Season 1 the first pods in each cultivar attained 12 cm length on 56 DAS. All pods of 12 cm or more were tagged on that date. These were regarded as "early pods". Four days later, all new

Table 1. Percentage of the total pod number, contributed by pods of different earliness classes in two seasons and two cultivars. Average values from all harvests.

Pod earliness	Season 1		Season 2	
	Rosecoco	Mwezi Moja	Rosecoco	Mwezi Moja
Early pods	1	4	11	27
Medium pods	16	42	16	20
Late pods	83	54	73	53

pods that were 12 cm in length or more were tagged ("medium pods"). In Season 2 the first pods in each cultivar attained 12 cm 63 DAS and first tagging was done 71 DAS while second tagging was four days later. At harvesting all pods which were not tagged were regarded as "late pods". Table 1 indicates the percent distribution of the total harvested pods over the three classes of pod earliness.

Measurements

At each harvest date, pods were removed from different pod classes separately. In Season 2, 10 pods were randomly picked per class and the number of pods achieving a straw yellow (Y) colour according to Munsell colour chart for plant tissues (Anonymous, 1972) was counted. After hand shelling all pods, seeds with abnormal development and size were discarded and only normal looking seeds were used for further analysis. A sample of 10 seeds per plot from each level was used to measure seed length using a vernier caliper. A sample of 100 seeds per plot was taken at the crop level and a sample of 30 seeds from each of the early, medium and late pods. From each of these samples, fresh weight was assessed and the number of seeds with a red purple (RP) colour pattern at crop level in Season 1 and, at crop and pod levels in Season 2 was counted. Dry weight was determined after splitting all seeds across the cotyledons and drying for 16 hours at 105 °C. In the early pods of Season 1 some harvesting dates did not have enough seeds for reliable analysis.

Statistical analysis

The effect of time of harvest, cultivar and pod class on seed and pod characteristics was tested by standard ANOVA assuming pod class to be an extra split factor within a harvest date. Means were separated by LSD tests. To estimate the maximum dry weight, seed filling rate, moment of maximum dry weight and the moisture content at maximum dry weight, non-linear regression models were fitted using the Genstat 5 (Release 4.1). Maximum dry weight achieved in relation to time was estimated as the y-value of a horizontal line fitted through the last part of the dry weight data against time. The rate of seed filling was the slope of the line through the first part of the data and the moment when maximum dry weight was achieved as the x-axis value of the point at which lines cross. Maximum dry weight in relation to seed moisture content was estimated as the y-value of the horizontal line fitted through the last part of the dry weight data against moisture content. The point at which lines crossed was estimated as the moisture content of the seed when maximum dry weight was achieved for individual plots. The R^2 for the curve fits ranged from 84 - 99%. The derived values were subjected to a standard ANOVA to test cultivar and pod class effects followed by LSD tests to separate the means. Estimates of maximum seed dry weight therefore were derived by three methods: standard ANOVA analysis and two types of non-linear regression.

Results

Seed fresh weight development over time

In both cultivars fresh weight increased sharply in seeds from all pod classes in Season 1 (Fig. 1). In Season 2 fresh weight increased gently in all pod classes in both cultivars (Fig. 2). In Season 1 seeds from early pods achieved maximum fresh weight earliest, seeds from medium and late pods were intermediate, whereas seeds from crop level pods were latest in cv. Rosecoco (Fig. 1). By contrast in cv. Mwezi Moja seeds from all pod classes achieved maximum fresh weight at comparable date (Fig. 1). In Season 2, seeds from early pods achieved maximum fresh weight earliest whereas seeds from medium and late pods achieved maximum fresh weight at moments comparable to seeds from crop level in both cultivars (Fig. 2). The maximum fresh weight achieved was always highest for seeds from early pods, intermediate for seeds from medium pods and lowest for seeds from late and "crop" pods. Fresh weight also started to decrease earlier in seeds from early and medium pods than in late and "crop" pods in both cultivars and seasons.

In Season 1, fresh weight increased more gradually in cv. Rosecoco than in cv. Mwezi Moja. Maximum fresh weight was achieved earlier in seeds from cv. Mwezi Moja than in seeds from cv. Rosecoco in all pod levels (Fig. 1). The fresh weights achieved were higher in cv. Mwezi Moja than in cv. Rosecoco and decreased earlier in cv. Mwezi Moja than in cv. Rosecoco. The increase in Season 2 was comparable in both cultivars with cv. Mwezi Moja again achieving higher maxima earlier than cv. Rosecoco (Fig. 2). Seed fresh weight decreased earlier in cv. Mwezi Moja than in cv. Rosecoco.

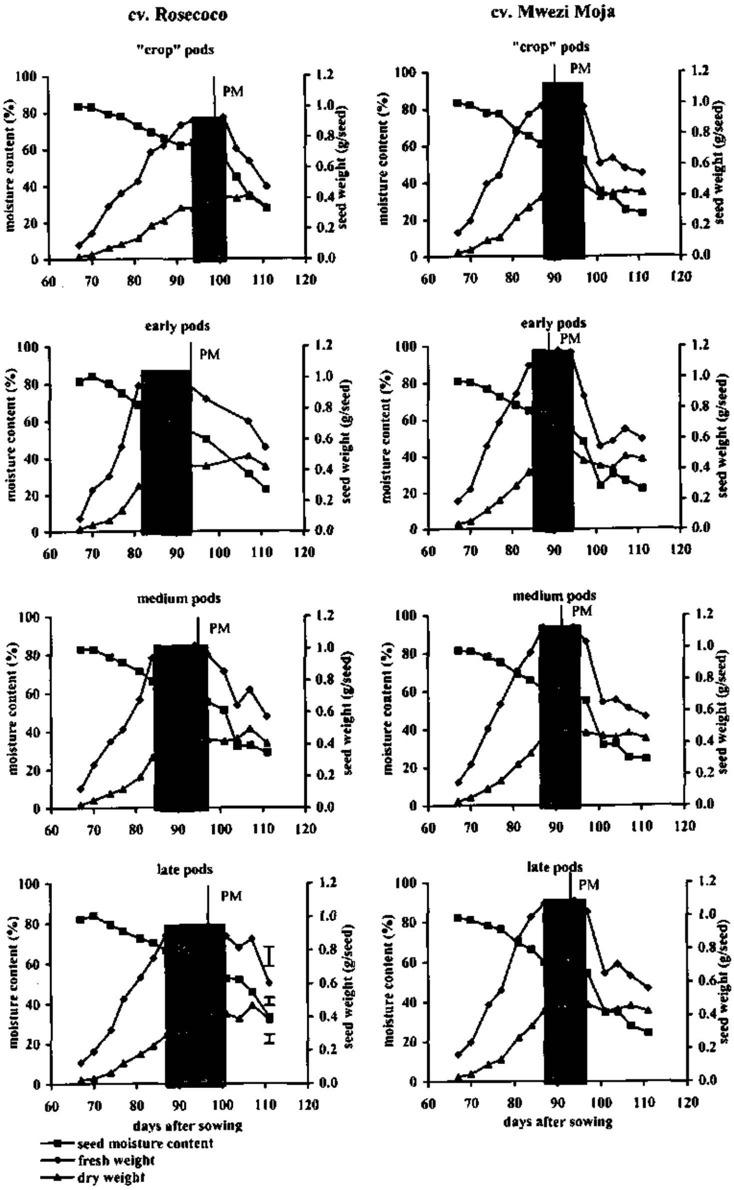

Fig. 1. Seed moisture content, fresh and dry weight development over time for cvs Rosecoco and Mwezi Moja in Season 1. The bars represent LSD ($P<0.05$) for comparisons between the cultivars, between and within the pod class means over time. The shaded area represents the period in time when fresh weight was at maximum. The vertical line represents the moment of physiological maturity (PM), as derived from Table 2.

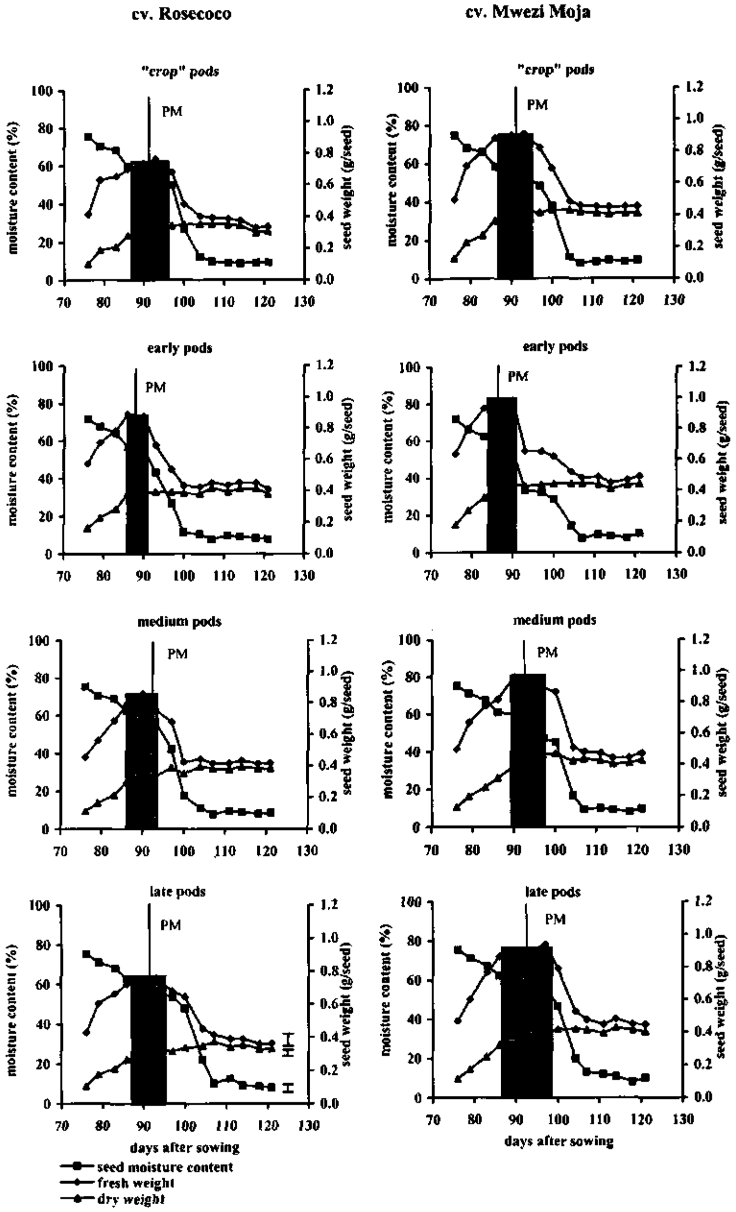

Fig. 2. Seed moisture content, fresh and dry weight development over time for cvs Rosecoco and Mwezi Moja in Season 2. The bars represent LSD ($P<0.05$) for comparisons between the cultivars, between and within the pod class over time except for dry weight where the bars are for comparing pod levels means over time. The shaded area represents the period in time when fresh weight was at maximum. The vertical line represents the moment of physiological maturity (PM), as derived from Table 2.

Seed moisture content development over time

Seed moisture content first declined gradually. This was usually followed by a sharper decline and finally a gentle decline in all pod levels in both cultivars and seasons, until seeds were dry (Figs 1 and 2). In Season 1 the sharper decline was not clear in cv. Rosecoco but in cv. Mwezi Moja the sharper decline in moisture content was at comparable moments in seeds from all pod classes (Fig. 1). In Season 2 the sharper decline in moisture content was earliest in seeds from the early pods whereas in seeds from medium, late and "crop" pods the sharper decline was at comparable moments in cv. Rosecoco (Fig. 2). In cv. Mwezi Moja the sharper decline was earliest in seeds from early pods, intermediate in seeds from late and "crop" pods and latest in seeds from medium pods in Season 2 (Fig. 2). All pod classes in both cultivars were still declining in moisture content at the last date of harvest in Season 1. At that date, seeds from late pods still had higher moisture content than seeds from early and medium pods whereas seeds from "crop" pods were comparable to those from late pods in cv. Rosecoco. In cv. Mwezi Moja the difference was not significant. In Season 2, seeds from all pod classes in both cultivars had comparable moisture content at the final harvest. We defined harvest maturity (HM) as the moment when seed moisture content declined to 20%. HM was not achieved in Season 1 (Fig. 1). HM was achieved earlier in seeds from early and medium pods than in seeds from late and "crop" pods in cv. Rosecoco in Season 2 but at comparable moments in cv. Mwezi Moja (Fig. 2).

In cv. Mwezi Moja compared to cv. Rosecoco the sharper decline in moisture content took place earlier in both seasons. In Season 1, cv. Mwezi Moja had lower moisture content at the end of the experiment than cv. Rosecoco (Fig. 1) whereas both cultivars had comparable moisture content at the end of the experiment in Season 2 (Fig. 2). In Season 2 both cultivars achieved HM at comparable moments (Fig. 2).

Seed dry weight development over time

Conclusions in this section are drawn from the results in Figs 1 and 2, and Tables 2 and 3. Seed filling rate was slower in seeds from later pods in both seasons and cultivars (Fig. 1 and Table 2) but the differences could not be established as significant in Season 1, whereas in Season 2 only seeds from early pods differed from seeds from later pods. Seed filling rate at crop level was comparable to that in medium or late pods (Table 2).

Maximum seed dry weight was achieved earlier in seeds from early pods, intermediate in seeds from medium pods and later in seeds from late and "crop" pods in both cultivars in Season 1 but the differences were not significant (Fig. 1 and Table 2). In Season 2 maximum seed dry weight was achieved earlier in seeds from early pods but seeds from medium, late and "crop" pods achieved maximum dry weight at comparable moments in both cultivars (Fig. 2 and Table 2).

The maximum seed dry weight achieved was higher in seeds from earlier pods (Figs 1, 2 and Tables 2, 3) but the differences could not be assessed as significant when non-linear regression between the moment of achieving maximum dry weight and dry weight was used to establish maximum dry weight (Table 2, P=0.078) in both seasons. When non-linear regression between moisture content and dry weight was used to assess maximum dry weight (Table 3), seeds from early, medium and late pods achieved comparable maximum dry weight in Season 1, whereas in Season 2 dry weight decreased in later pods. Maximum seed dry weight in "crop" pods was estimated to be lower than that of the different pod fractions in Season 1 or comparable to late pods in Season 2 (Table 3).

Table 2. Seed filling rate up to the maximum seed dry weight, maximum seed dry weight and the moment when the maximum dry weight was achieved (physiological maturity) in seeds from different pod classes in two cultivars and two seasons, as derived from a non-linear regression analysis between days after sowing and seed dry weight.

Pod class	Season 1			Season 2		
	Rosecoco	Mwezi Moja	*Mean*	Rosecoco	Mwezi Moja	*Mean*
Seed filling rate (g/day)						
Crop	0.013	0.019	0.016 a[1]	0.017	0.017	0.017 a
Early	0.016	0.020	0.018 a	0.021	0.025	0.023 b
Medium	0.015	0.019	0.017 a	0.016	0.018	0.017 a
Late	0.014	0.017	0.016 a	0.015	0.017	0.016 a
Mean	0.015 a	0.019 b	0.017	0.017 a	0.019 a	0.018
Moment of achieving maximum dry weight (days after sowing)						
Crop	98.9	90.3	94.2 a	90.9	91.1	91.0 a
Early	93.9	88.8	91.4 a	87.3	86.3	86.8 b
Medium	94.7	91.3	93.0 a	93.0	92.7	92.8 a
Late	97.4	93.2	95.3 a	91.4	93.1	92.3 a
Mean	96.0 a	90.9 a	93.5	90.7 a	90.8 a	90.7
Maximum seed dry weight (g/seed)						
Crop	0.389	0.423	0.406 a	0.369	0.394	0.381 a
Early	0.426	0.447	0.436 a	0.394	0.437	0.416 a
Medium	0.426	0.442	0.434 a	0.379	0.429	0.404 a
Late	0.425	0.437	0.431 a	0.339	0.410	0.375 a
Mean	0.417 a	0.437 a	0.427	0.370 a	0.418 b	0.394

[1] Means followed by the same letter in column or row within a season and characteristic are not significantly different ($P \geq 0.05$) according to LSD test.

Table 3. Maximum seed dry weight achieved and the moisture content at which the maximum dry weight was achieved, of seeds from different pod classes in two cultivars and two seasons, as derived from a non-linear analysis between moisture content and seed dry weight.

Pod class	Season 1			Season 2		
	Rosecoco	Mwezi Moja	*Mean*	Rosecoco	Mwezi Moja	*Mean*
Moisture content at achieving maximum dry weight (%)						
Crop	57.8	59.4	58.6 a[1]	59.4	56.4	57.9 a
Early	58.6	60.4	59.5 a	57.7	57.3	57.5 a
Medium	58.1	59.7	58.9 a	56.5	56.5	56.5 a
Late	57.1	59.5	58.3 a	57.1	57.8	57.4 a
Mean	57.9 a	59.8 a	58.8	57.7 a	57.1 a	57.4
Maximum dry weight (g/seed)						
Crop	0.392	0.420	0.406 a	0.334 a	0.415 c	0.374
Early	0.422	0.447	0.434 b	0.394 b	0.437 d	0.416
Medium	0.435	0.449	0.442 b	0.380 b	0.425 cd	0.402
Late	0.415	0.447	0.431 b	0.341 a	0.411 c	0.376
Mean	0.416 a	0.441 a	0.429	0.362	0.422	0.392

[1] Means followed by the same letter in column, row or cultivar × pod class combination within a season and characteristic are not significantly different ($P \geq 0.05$) according to LSD test.

In cv. Mwezi Moja compared to cv. Rosecoco, seed filling rate was significantly faster in Season 1 (Fig. 1, Table 2) but not significantly so in Season 2 (P=0.107) (Fig. 2, Table 2). Cv. Mwezi Moja achieved higher dry weights than cv. Rosecoco (Fig. 1, Tables 2 and 3) but the differences were not significant in Season 1 (P=0.183 in Table 2). The moments of achieving maximum dry weight did not differ significantly between cultivars (Fig. 1 and Table 2) but cv. Mwezi Moja tended to achieve maximum dry weight earlier than cv. Rosecoco in both Season 1 (P=0.081) and 2.

Seed length development over time

Seed length increased to a maximum then declined gradually in both cultivars and seasons (Figs 3 and 4). The increase was always earlier in seeds from early and medium pods than in late and "crop" pods. In Season 1, seeds from all pod classes achieved maximum length at comparable moments in both cultivars (Fig. 3). In Season 2 seeds from early pods achieved maximum seed length earlier than seeds from all pod classes in both cultivars (Fig. 4). The maximum length achieved was highest in earlier

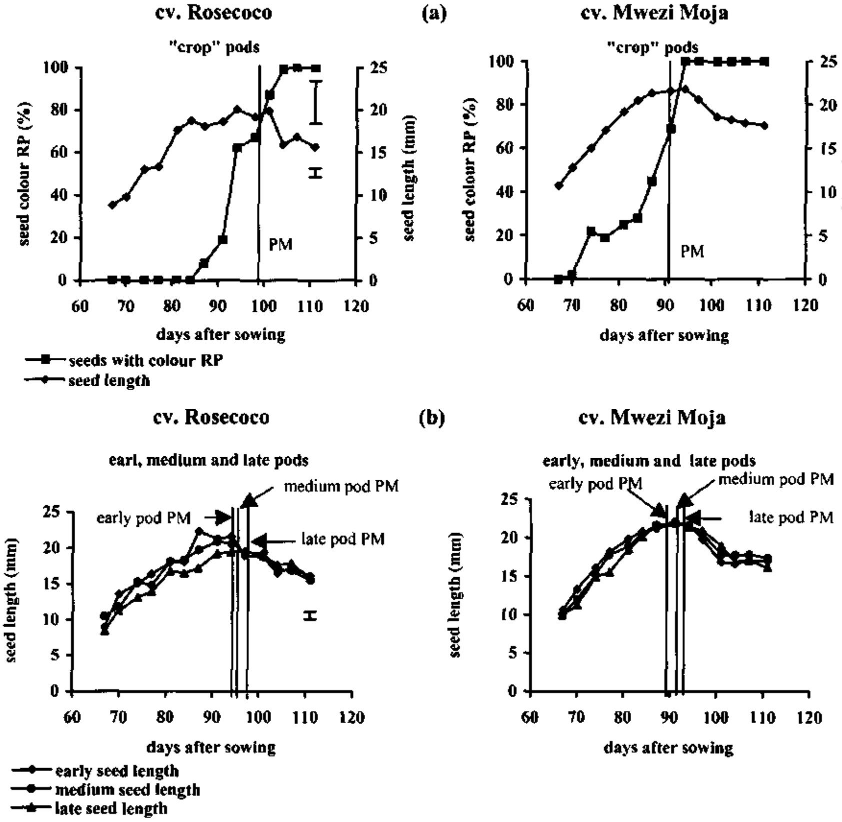

Fig. 3. (a) Percentage of seeds with red purple (RP) colour and seed length development over time in seeds from "crop" pods in Season 1. The bars represent LSD ($P<0.05$) for the comparison of the seed colour means over time and cultivar × time × pod class for the seed length while vertical lines represent the moment of physiological maturity (PM) as derived from Table 2. (b) Seed length development over time in pods of different earliness with bars representing LSD ($P<0.05$) for the comparison of cultivar × time × pod class means in Season 1 for cvs Rosecoco and Mwezi Moja. The vertical lines represent the moment of PM as derived from Table 2.

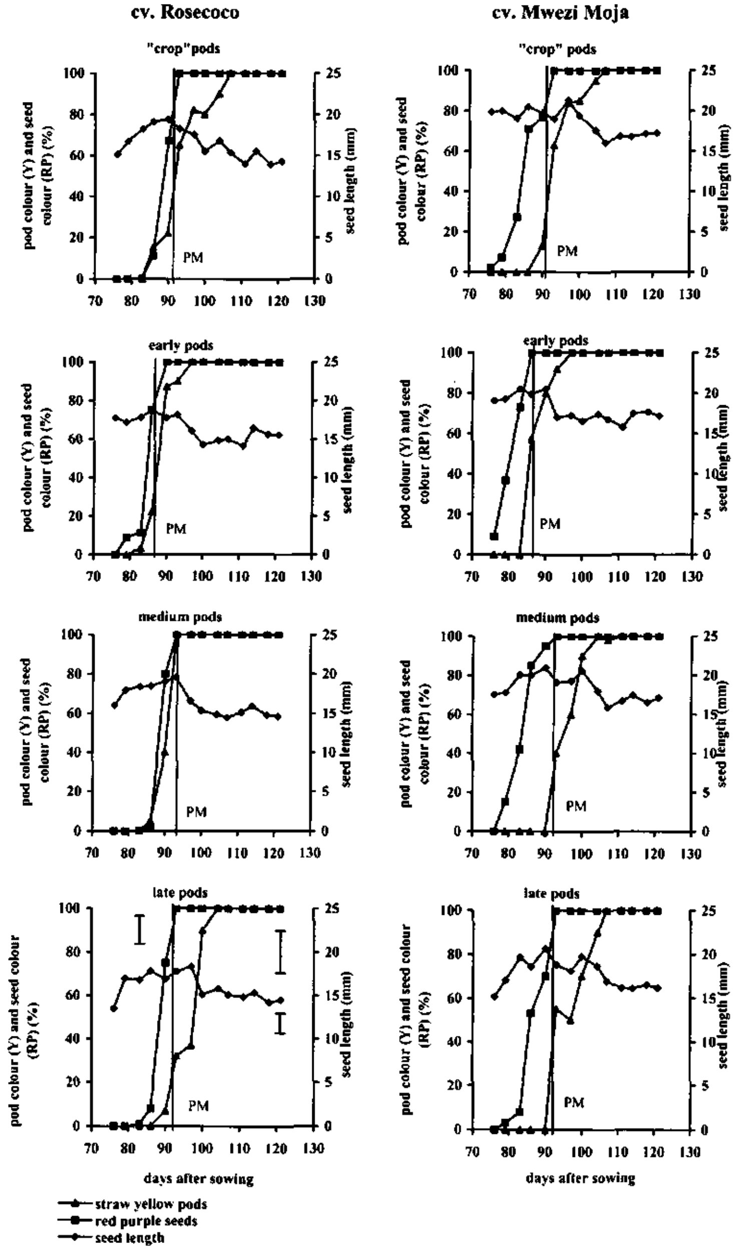

Fig. 4. Development of the percentage of pods with straw yellow colour, percentage of seeds with red purple (RP) colour and seed length over time, with LSD ($P<0.05$) bars for the comparison of cultivar × time × pod class means for pod and seed colour, and seed length in cvs Rosecoco and Mwezi Moja in Season 2. The vertical lines represent the moment of physiological maturity (PM) as derived from Table 2.

pods than in seeds from later pods in cv. Rosecoco whereas seeds from all pod classes in cv. Mwezi Moja achieved maximum seed length at comparable moment in Season 1 (Fig. 3). In Season 2 seeds from pod level classes achieved comparable maximum seed length lower than in seeds from crop level in cv. Rosecoco whereas in cv. Mwezi Moja all pod level classes achieved comparable maximum seed length (Fig. 4). In Season 1 seed length decreased earlier in seeds from early pods than in seeds from all other pod classes in cv. Rosecoco whereas in cv. Mwezi Moja the decrease in seed length was comparable in all pod classes (Fig. 3). In Season 2, the moment when decrease in seed length started was earliest for earlier pods but latest for seeds from late and "crop" pods in both cultivars (Fig. 4).

In cv. Mwezi Moja compared to cv. Rosecoco, seed length increased earlier and achieved a higher maximum in both seasons (Figs 3 and 4). The decrease in seed length was earlier in cv. Mwezi Moja than in cv. Rosecoco but the two cultivars had comparable seed length at the end of the experiments.

Pod and seed colour development over time

Effects of pod earliness on seed colour were only analysed in Season 2. In both cultivars the moment that 100% of the seeds from early pods had achieved their final red purple (RP) colour pattern was earlier than for seeds from medium, late and "crop" pods, which did not differ among each other (Fig. 4).

Pods changed to their final colour later than seeds. In Season 2, majority (> 50%) pods in early and medium pods in cv. Rosecoco achieved final straw yellow colour earlier than late and "crop" pods (Fig. 4). In cv Mwezi Moja majority pods in early pods achieved final straw yellow colour earlier, medium pods were intermediate whereas late and "crop" pods were later (Fig. 4).

The percentage of seeds with their final red purple (RP) colour pattern increased to 100% earlier in cv. Mwezi Moja than in cv. Rosecoco in Season 1 (Fig. 3a). In Season 2, the percentage of seeds with their final purple (RP) colour pattern increased to 100% at comparable moments in both cultivars (Fig. 4). The majority of pods achieved final straw yellow colour earlier in cv. Rosecoco than in cv. Mwezi Moja (Fig. 4).

In Season 1, the moment that 100% of the seeds achieved their final red purple (RP) colour was after the moment when seed dry weight was at maximum (PM) in both cultivars (Fig. 3a). In Season 2 the moment that 100% of the seeds from each pod class achieved their final red purple (RP) colour pattern was just after or at the moment when seed dry weight was at maximum (PM) in both cultivars (Fig. 4).

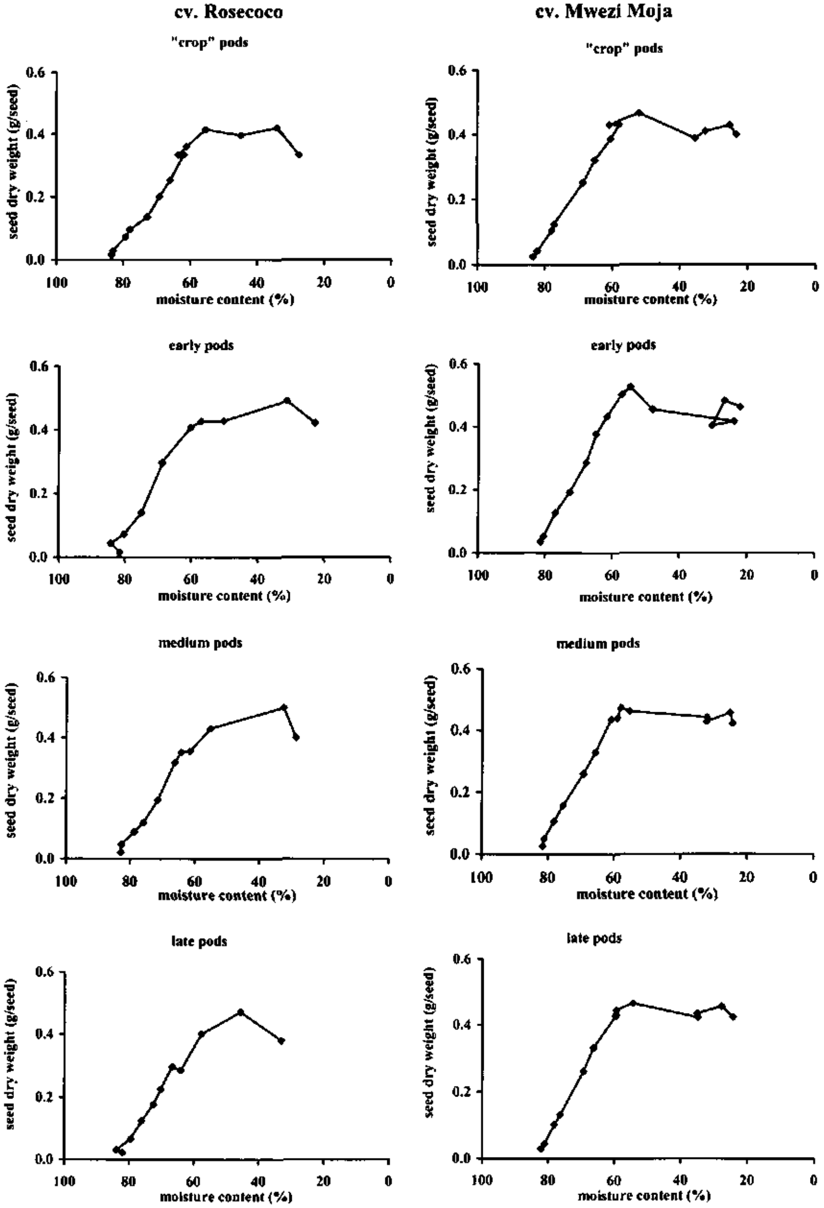

Fig. 5. Relationship between seed dry weight and moisture content in seeds from different pod classes for cvs Rosecoco and Mwezi Moja in Season 1.

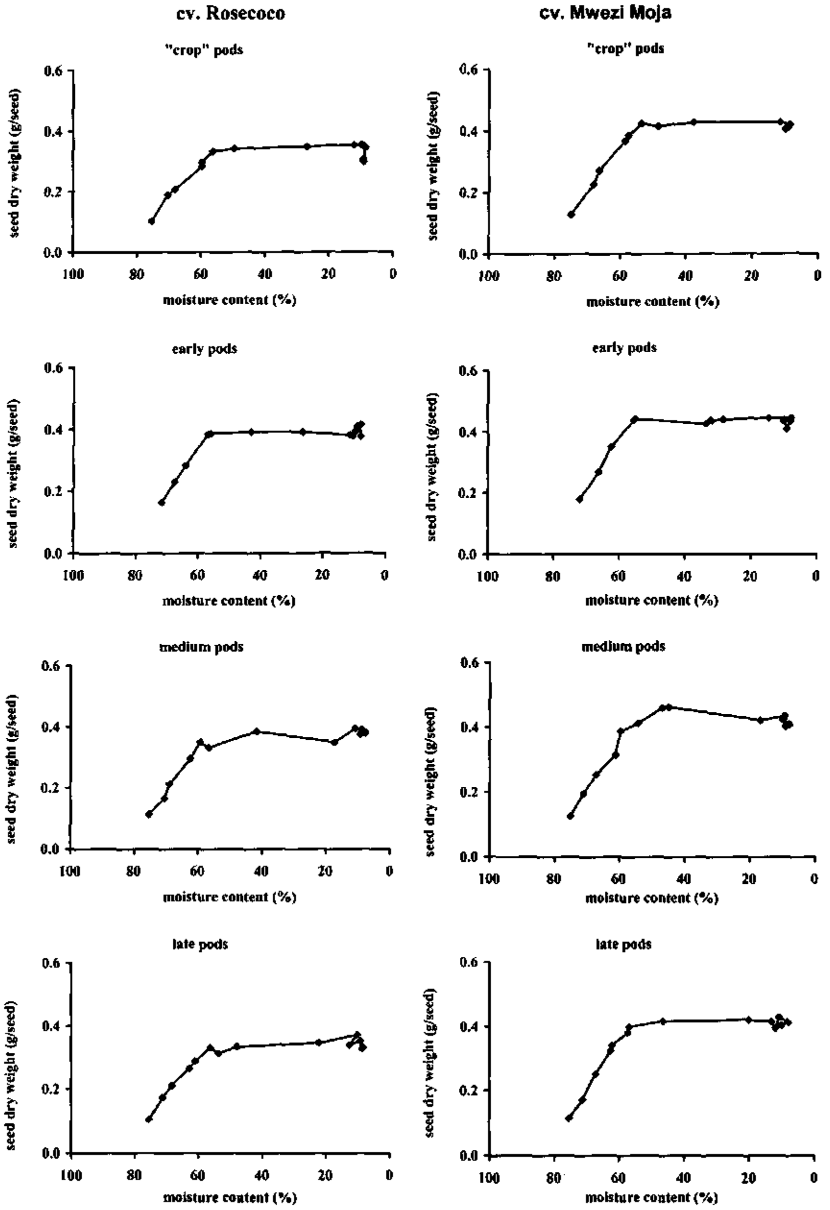

Fig. 6. Relationship between seed dry weight and moisture content in seeds from different pod classes for cvs Rosecoco and Mwezi Moja in Season 2.

Relationship between seed moisture content and seed dry weight

Seed dry weight first increased linearly as moisture content declined, and then achieved a maximum and remained more or less constant till the end of harvesting (Figs 5 and 6). This pattern was clearest in Season 2.

Two lines were fitted by non-linear regression analysis through data points of individual plots to achieve an estimate of moisture content at which maximum dry weight was achieved and the maximum dry weight. In both seasons and cultivars no significant differences could be established in the moisture content at which maximum dry weight was achieved in seeds from different pod classes (Table 3). There were also no differences between cultivars in the moisture content at which maximum dry weight was achieved. Maximum dry weight was achieved at 58.8% moisture content in Season 1 and at 57.4% in Season 2 (Table 3).

Relationship between seed moisture content and seed and pod colour

Effects of pod earliness on seed colour was only analysed in Season 2. In cv. Rosecoco 100% of seeds from all pod classes achieved their final red purple colour pattern at comparable moisture content i.e. 58% (Fig. 7). Also in cv. Mwezi Moja 100% of seeds from early, medium and late pods achieved their final red purple colour pattern at comparable moisture content of 58% whereas "crop" pods achieved 100% of their final red purple colour pattern at moisture content lower than 58% (Fig. 7). In Season 1 100% of seeds from "crop" pods achieved their final red purple colour pattern at moisture content lower than 58% in both cultivars (Fig. 7).

In Season 2, the majority (>50%) of pods in early, medium and "crop" pods, achieved their final straw yellow colour at comparable moisture content (60%) which was higher than in late pods in cv. Rosecoco (Fig. 7). In cv. Mwezi Moja, the majority of pods in early and "crop" pods, achieved their final straw yellow colour at a higher moisture content than in medium and late pods in Season 2 (Fig. 7).

The majority (>50%) of pods at crop level in both cultivars achieved their final straw yellow colour after PM (58% moisture content) (Fig. 7). The majority of pods in early and medium pods in cv. Rosecoco had already achieved their final straw yellow colour at PM whereas the majority of late pods achieved straw yellow colour after PM (Fig. 7). In cv. Mwezi Moja, the majority of pods in early and late pods achieved a straw yellow colour at PM whereas the majority of medium pods is achieved after PM (Fig. 7).

Hundred percent of the early and late pods achieved a straw yellow colour at moisture contents between 58 and 22% which is higher than HM (20%), whereas 100% of the "crop" pods achieved the straw yellow colour after seeds had dried below 20% moisture in cv. Rosecoco (Fig. 7). Also in cv. Mwezi Moja, 100% of the early

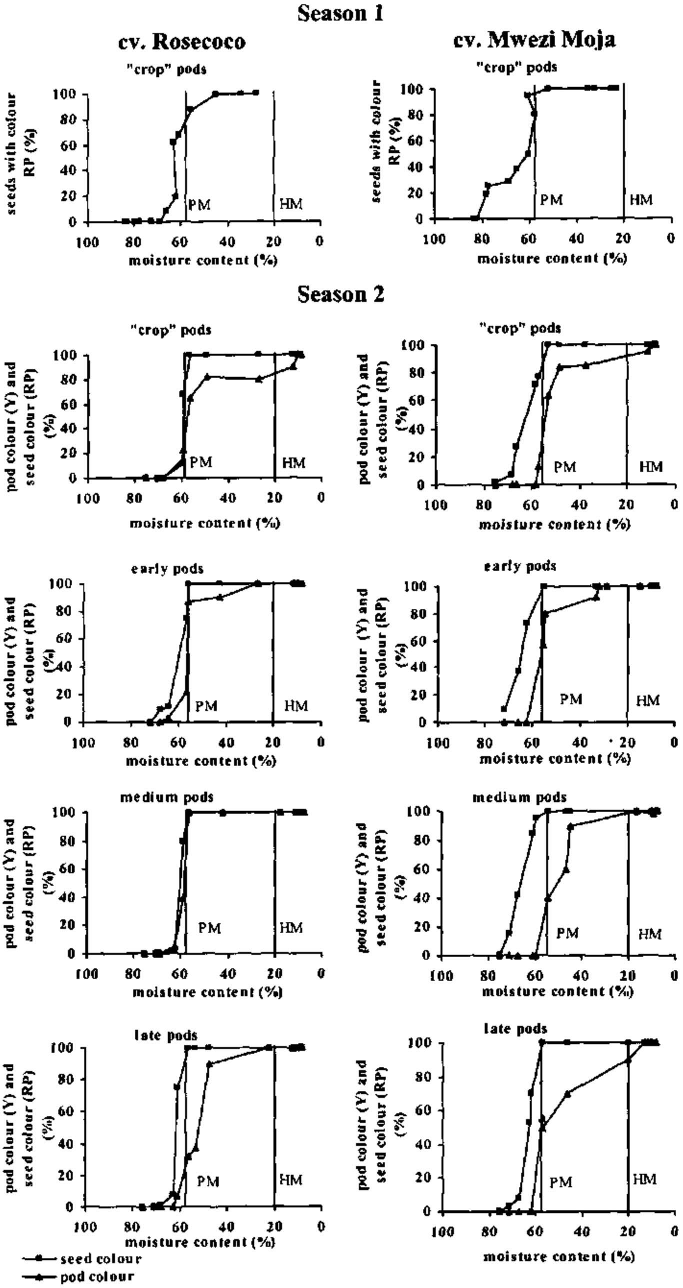

Fig. 7. Relationship between moisture content and percentage of seeds with final red purple colour pattern and straw yellow pod colour for cvs Rosecoco and Mwezi Moja in Season 1 and 2. The vertical lines represent the moments of physiological maturity (PM) (as derived from Table 3) and harvest maturity (HM).

and medium pods achieved a straw yellow colour at a moisture content higher than 20%, but 100% of the late and "crop" pods achieved a straw yellow colour at moisture contents lower than 20% (Fig. 7).

In both cultivars 100% of the seeds achieved the final red purple colour pattern at moisture content lower than 58% in Season 1 (Fig. 7). In Season 2 100% of the seeds achieved the final red purple colour pattern at comparable moisture content which was lower than that at PM. Majority pods in both cultivars achieved straw yellow colour at comparable moisture content slightly lower than 58% in Season 2 (Fig. 7).

Discussion

Seed development in common bean

Our results show that in common bean crops seed fresh weight increased until it remained around a maximum value for a few days, in which period changes in fresh weight were not significant (Figs 1 and 2). The moment of PM was achieved in this time period (Figs 1 and 2). This is comparable to results in lupin (Gorecki et al., 1997) whereas in wheat maximum dry weight is achieved when fresh weight is already declining (Bewley and Black, 1994). At the moment of PM, moisture content in the seed was gradually declining. A sharp decrease occurred only after PM, when fresh weight also was clearly declining (Figs 1 and 2). Seed length more or less started to decline at the moment fresh weight was starting to decline (Figs 1, 2, 3 and 4), as a result of water loss during maturation drying (Egli, 1998). This is in agreement with findings in lupin (Gorecki et al., 1997). The moment 100% of the seeds from each pod class achieved their final red purple colour pattern was just after or at the moment of PM. Our results show that all these general patterns were not only consistently found in common bean crops from both cultivars and in both seasons, but were also found in pods from different earliness within a crop.

At the moment of PM moisture content was 58% (Table 3). This moisture content did not differ significantly over pods from different earliness, over whole crops and pod fractions, or over cultivars. The moisture content at PM only slightly differed over seasons. The moisture content at PM in our experiments was higher than the 52% found by van de Venter et al. (1996) for common bean cv. Teebus, which probably resulted from differences in methodology or large variations in the environment and the cultivar. Our results also confirm that seeds attain maximum dry weight when moisture content is still high (e.g. TeKrony et al., 1980a; van de Venter et al., 1996; Pokojska and Grzelak, 1996; TeKrony and Egli, 1997). The seed has to undergo a period of maturation drying after PM before harvesting and threshing are possible without damage (Egli, 1998). We defined the moment of harvest maturity (HM) to occur at 20% moisture content.

Differences in seed development in pods from different earliness

Whereas the general patterns of development observed and the moisture content at PM were more or less the same for seeds from pods of different earliness, seeds from earlier pods achieved higher maximum fresh weights, tended to have higher maximum dry weights, and had lower moisture contents at the end of Season 1 (Figs 1, 2 and Tables 2, 3).

The timing of development was also different. Seeds from early pods tended to achieve maximum fresh weight and PM (58% moisture content) earlier than seeds from later pods (Figs 1, 2 and Table 2). The latter implies that seeds from early pods remain longer on the plant after PM than seeds from later pods. They therefore may have a longer time available for ageing than seeds from later pods. In soybean, seeds from earlier pods may have a lower viability than seeds from later pods (Illipronti Jr et al., 2000b).

HM (20% moisture content) also was achieved earlier in seeds from early and medium pods than in seeds from late pods in cv. Roscoco, but at the same moment in cv. Mwezi Moja (Fig. 2). Because of this, the period of drying from 58 - 20% moisture content was longer in seeds from earlier pods in cv. Mwezi Moja whereas in cv. Rosecoco no systematic differences were found (Figs 1, 2 and 7). This implies that the moisture content in earlier pods of cv. Mwezi Moja after PM may remain longer at higher levels. How fast moisture content in seeds declines to levels of around 20% may be very important for their final vigour or viability. At a relatively high moisture content the metabolic activity e.g. respiration may not stop immediately (Egli, 1998), and also seed deterioration will be higher. Therefore, the shorter the period between PM and HM, without forced drying, the less deterioration of the seed during in-plant storage may occur.

Our results show that there were also differences between seeds from different pod fractions in the course of the decline in moisture content between 58 and 20%. When seeds after PM first decline gradually in moisture content and only later sharply, moisture content remains longer at a relatively high level, and therefore the possibilities for deterioration "in planta" may also remain high. In Season 1, seeds from early pods in both cultivars were following this pattern (Fig. 1), but this was true only for cv. Mwezi Moja in Season 2, whereas in that season also late pods from both cultivars showed this pattern (Fig. 2). Differences in the course of decline in moisture content in seeds from different crop fractions therefore existed, but were not systematically related to pod earliness.

Differences in seed dry weight in pods from different pod classes

Maximum dry weight varied slightly depending on the method of analysis (Figs 1 and 2, Tables 2 and 3). Because seed dry weight approaches its maximum asymptotically, and because normal sampling variation exists in measurements of seed dry weight with time, it is very difficult to accurately estimate the maximum dry weight (Egli, 1998). Different methods have always resulted in different values of maximum dry weight even within the same cultivar (e.g. Crookston and Hill, 1978; Grabe, 1989; TeKrony and Egli, 1997). The higher dry weight found in seeds from early and medium pods than in late and "crop" pods in both cultivars (Figs 1 and 2, Table 3) is consistent with findings in soybean (Kler and Dhillon, 1983; Illipronti Jr et al., 2000b). The higher seed dry weight in early pods in our results could be associated with a higher seed filling rate (Figs 1 and 2, Table 2). The higher seed-filling rate and weight in seeds from early and medium pods could be attributed to less competition for accumulating insoluble reserves before all seeds are formed (Cocks, 1990). The proportion of early and medium pods was lower than that of late pods in this study (Table 1). Early pods also benefit from a high influx of assimilate when the plant switches from the vegetative to the reproductive phase (Bewley and Black, 1994). Also the increase in the proportion of yellow leaves when seed filling in late pods was going on could have contributed to lower seed weight in seeds from late pods.

The fact that seeds from early pods were heavier may have positive and negative implications for the field performance of the early seeds. Heavier seeds in French bean gave the largest seedlings and highest percentage germination (Doijode, 1984). Similarly soybean seedlings from larger seeds were heavier than those from smaller seeds (Egli et al., 1990). Other reports show that in soybean seed viability was reduced by coat etching and cracking which were associated to heavier seeds (Illipronti Jr et al., 1999). Early seeds take longer than later seeds in the field before crop HM and are exposed to deleterious internal and environmental conditions for longer time than late seeds.

Estimating of the moment of PM and HM by colour markers

In common bean, PM has been defined based on seed moisture content (Kelly, 1988; van de Venter et al., 1996). We identified PM at around 58% seed moisture content (Figs 1, 2, 5 and 6, Table 3), which was not significantly different in pods from different earliness, compared to whole crops, and in different cultivars. The moisture content at PM only differed slightly over seasons.

In both cultivars, seasons and in pods from all levels, the moment that 100% of the seeds achieved their red purple colour pattern was at PM or just after PM (Figs 4 and 7). This makes the moment all seeds within a crop or crop fraction achieve their final red purple colour pattern a very good, but not completely accurate indication of PM. The colour change in pods was less well related to the moment of PM. At PM, 50% of the pods from the crop level had achieved their final straw yellow colour in both cultivars (Figs 4 and 7). In cv. Rosecoco, 50% of the early pods achieved their final straw yellow colour at PM, but 50% of the medium pods had turned to straw yellow well before PM was achieved, whereas PM had passed when 50% of the late pods had turned to straw yellow (Fig. 7). In cv. Mwezi Moja the moment 50% of the pods achieved their final straw yellow colour was at PM for early pods, but after PM for medium and late pods (Figs 4 and 7).

Changes in pod colour occurred after changes in seed colour (Figs 4 and 7). Both are associated with the interruption of vascular connections between pod (and its seed) and the mother plant (Le-Deunff, 1989). The later change in pod colour than in seed colour is consistent with the observation that it is possible to find completely yellow seeds in pods that are not completely yellow in soybean (Egli, 1998). Soybean seeds also reached PM in pods that were not completely yellow (TeKrony et al., 1979; Egli, 1998). Our results also imply that the timing of the succession in changes in seed colour and changes in pod colour was different in pods from different earliness. Later pods changed relatively later in pod colour. The results therefore support the conclusion of Housley et al. (1982) that vegetative characters, e.g. pod or leaf colour are usually more variable – showing greater environmental and cultivar effects – than seed characteristics, and are not reliable as indicators of PM.

HM is the moisture content at which seeds are harvestable. In dry beans it is between 17% (van de Venter et al., 1996) and 20 - 25% (Kelly, 1988). We defined HM as the moment when moisture content declined to 20% (Figs 2 and 7). Pod colour change proved to be not a reliable indicator of this moment for pods from different fractions in a crop and a whole crop. In whole crops from both cultivars, 100% of the pods achieved their final straw yellow colour at moisture contents below 20%. Early and medium pods had already all achieved their final straw yellow colour at moisture contents that were much higher than 20%, for instance >30% in early pods in Rosecoco, whereas late pods achieved their final straw yellow colour at moisture contents lower than 20%.

Relationship between whole crop and pod levels

Crop level seeds are a combination of seeds from pods of different earliness and therefore it should be possible to explain their behaviour with regard to timing and weights achieved by the behaviour of pods of different earliness within the crop. The fact that the bulk of pods at pod level were late pods (Table 1) explains the observations that seeds from the whole crop were usually comparable to seeds from later pods or had an intermediate behaviour. For instance, seeds from crop level achieved maximum fresh weight at a comparable moment as seeds from late pods in both cultivars and seasons (Figs 1 and 2). Higher maximum fresh weights, however, were achieved in some pod fractions than in whole crops, because seeds from late pods were still developing when seeds from earlier pods were at maximum (Figs 1 and 2). It is unclear why the maximum dry weight achieved was lower, though not significantly, in whole crops than in seeds from separate crop fractions in both cultivars and seasons (Figs 1, 2 and Tables 2 and 3). The relationships among seed characteristics with regard to colour were different in whole crops compared to individual fractions of pods with different earliness. This is likely because individual seed characteristics do not all change in the same way with time. This pattern showed very clearly in the relationship between moisture content and pod colour. The early and medium pods had already achieved their final straw yellow colour at moisture contents well above 20%, whereas in whole crops 100% of the pods achieved their final straw yellow colour at moisture contents below 20% (Figs 4 and 7). We therefore conclude that the relations among seed characteristics in the whole crop will differ when compared to the relations among seed characteristics in pods of different earliness.

Conclusions

- In common bean physiological maturity (PM) is achieved in the time period when seed fresh weight is maximum, in pods from different earliness, in whole crops, both cultivars, and both seasons. The decline in seed fresh weight is closely associated with decline in seed length in seeds from all pod classes.
- PM is achieved at 58% seed moisture content in seeds from all pod classes, and cultivars. The season hardly affected the moisture content at PM.
- The moment all seeds within a crop or crop fraction achieve their final red purple colour pattern is a good, though not completely accurate indicator of PM at all pod classes.
- Changes in pod colour can not be used to predict the moment of harvest maturity (HM, 20% seed moisture content) consistently over pods from different classes.
- The moment of HM was earlier in early pods in cv. Rosecoco, but was not different in pod classes in cv. Mwezi Moja.
- Differences occur in the course of decline in moisture content between 58% (PM) and 20% (HM). These differences were not clearly related to pod earliness.
- The moment of PM is earlier in seeds from early pods and therefore early seeds takes longer time after PM until harvest.
- The period of drying from 58 - 20% moisture content was longer in seeds from

earlier pods in cv. Mwezi Moja but not systematically affected by pod earliness in cv. Rosecoco.

- The timing in seed development in the whole crop could be explained from differences in timing in seed development in pods of different earliness.
- Relationships among seed characteristics (e.g. seed moisture content and pod colour) can be different in whole crops compared to pods from different earliness.

Chapter 3

Development of seed viability and vigour in common bean (*Phaseolus vulgaris* L.) crops and different pod fractions within a crop[*]

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[*] Muasya, R.M., W.J.M. Lommen & P.C. Struik, 2001. Development of seed viability and vigour in common bean (*Phaseolus vulgaris* L.) crops and different pod fractions within a crop. Submitted to Field Crops Research.

3. Development of seed viability and vigour in common bean (*Phaseolus vulgaris* L.) crops and different pod fractions within a crop

Abstract

Two field experiments with common bean cultivars Rosecoco and Mwezi Moja aimed at increasing insight into the differences in development with time of viability and vigour of seeds from early, medium, late pods and of seeds from the whole crop ("crop" pods). Viability was determined by tetrazolium test (TZ). Maximum viability was achieved at moisture content of 31 - 37% in seeds from all pod levels, and well beyond physiological maturity (PM) (58% moisture content) but closer to harvest maturity (HM) (20% moisture content). No differences were found in the moment at which maximum viability was achieved in different pod classes in Season 1, however in Season 2, maximum viability was achieved earlier in seeds from early (cv. Rosecoco) or medium (cv. Mwezi Moja) pods than in seeds from late pods. Seeds from whole crop pods did not differ from seeds from pod earliness classes in the maximum percentage viability achieved during the season. Vigour was measured by electrical conductivity divided by the seed weight (EC). Maximum seed vigour (minimum EC) was achieved around the moment of PM in all pod classes. Minimum EC tended to be achieved earlier in seeds from earlier pods than in seeds from later pods. At the moment maximum vigour was achieved, earlier seeds also showed a better vigour than the later seeds, especially in Season 2. The vigour of seeds from selected pods at their optimum moment of harvesting was higher than the vigour of seeds from all pods combined.

Keywords: Common bean, electrical conductivity, harvest maturity, moisture content, physiological maturity, pod earliness, tetrazolium

Introduction

Seed viability is defined as the capability of the seed to germinate and produce a normal seedling (Dornbos Jr, 1995). Vigour is the sum of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence (ISTA, 1995). Viability is quickly gained during seed development, while vigour increases later as the seed filling progresses (Dornbos Jr, 1995). Maximum viability is attained earlier than maximum vigour but both are assumed to be at maximum at physiological maturity (PM) (Harrington, 1972; TeKrony and Egli, 1997). After PM seed vigour declines earlier than viability (Dornbos Jr, 1995).

Detailed knowledge on the course of seed development and maturation drying of common bean seed is essential for understanding how viability and vigour evolve and decline, and for assessing the appropriate date the seed crop or a fraction of this crop could be harvested with minimal loss of seed quality. At PM, moisture content in common bean seed is still high, for example 52% (van de Venter et al., 1996) or 58% (Muasya et al., 2001a). Harvest maturity (HM) is the earliest moment the seed moisture has declined to a level that makes the crop harvestable for its dry seeds (TeKrony and Egli, 1997). In common bean values of the moisture content at HM vary between 17% (van de Venter et al., 1996), 20% (Muasya et al., 2001a) and 20 - 25% (Kelly, 1988). In-plant "storage" of seeds after PM at high moisture content may lead to faster ageing equivalent to post-harvest storage of seeds under unfavourable conditions (Lassim and Chin, 1987). At high moisture content during maturation drying seeds exhibit higher respiration, heating and fungal invasion that reduce seed viability more rapidly than at low moisture content (Copeland and McDonald, 1995). Variation in the internal conditions (moisture content) of seeds after PM therefore may result in differences in viability and vigour level achieved at crop HM.

A crop is a mixture of seeds in different stages and different causes of development (Muasya et al., 2001a). Variation in seed viability and vigour has been reported between individual seeds within a seed lot (Keigley and Mullen, 1986; Adam et al., 1989; Illipronti Jr et al., 2000b). Some of the differences between seeds in a crop will result from different internal conditions and/or different length of the period of maturation drying. For example for seeds from earlier pods in common bean, the time period between pod PM and crop HM is longer than for seeds from later pods (e.g. Muasya et al., 2001a). Longer exposure of early pods to deteriorating conditions during maturation drying of the crop was thought to explain the lower viability reported for soybean seeds from earlier than from later pods (Illipronti Jr et al., 2000b). Differences also occur in the course of decline in moisture content between PM and HM, between seeds within a crop, though the differences are not clearly related to pod earliness (Muasya et al., 2001a). The faster the decline, the shorter the period the seed is strongly exposed to deteriorating conditions through in-plant "storage".

Because in a seed crop the harvested lot is a mixture of seeds in different stages of development, the quality of seeds from pods of different earliness at their optimum moment of harvesting might be higher than the average quality of seeds from all pods combined.

This research aims at increasing insight into the differences in development with time of viability and vigour of common bean seeds in crops and in pods of different earliness within a crop, and into the relationship between seed viability and vigour and seed moisture content. The research also aims at testing the hypothesis that quality of seeds from selected pods was higher than from all pods combined at the moment of maximum quality.

Materials and methods

Experimental set-up

Two experiments with common bean seeds were planted at the Chepkoilel campus farm of Moi University in Eldoret, Kenya in two growing seasons in 1998. The Season 1 crop was planted on 24 April and harvested from 29 June to 13 August, the Season 2 crop was planted on 17 August and harvested between 5 November and 21 December.

The site was located 2180 m asl and had a cambisol (Ferralitic) soil, well drained, non-humic and shallow, with low nutrient availability and moisture storage (Anonymous, 1987), pH 4.6 (Muasya, 1996). Season 1 received a total of 554 mm of rainfall and 1471 °Cd temperature sum above 10 °C, while Season 2 received 317 mm of rainfall and 1769 °Cd temperature sum above 10 °C. At planting each plot of 16 m^2 was fertilized with 0.49 kg calcium ammonium nitrate (26% N), 0.76 kg triple super phosphate (48% P_2O_5) and 0.13 kg muriate of potash (60% K_2O), achieving 80 kg N, 100 kg P and 20 kg K per hectare. The experimental design was a split plot with four blocks. Two cultivars, Rosecoco and Mwezi Moja, were assigned randomly to main plots. Both are determinate cultivars, but Rosecoco shows prolonged flowering while the flowering period of Mwezi Moja is short. Harvesting dates (1-14) were assigned to subplots. The first harvesting dates were 67 and 76 days after sowing (DAS) in the first and second season, respectively. Subsequent harvests took place twice a week until 111 and 121 DAS of the first and second season, respectively. The gross plot size was 4×4 m while the net experimental area was 3.40×2.50 m and included 170 plants spaced at 0.5×0.1 m. Within the net experimental area, an area of 2 m^2 was marked for observations of the whole crop (crop level) while the remaining area of 6.5 m^2 was used for observations on three classes of pods (pod level).

Pod classes

Flowering began 40 and 42 DAS in Season 1 and 2, respectively. In Season 1 the first pods in each cultivar attained 12 cm length on 56 DAS. All pods of 12 cm or more were tagged on that date. These were regarded as "early pods". Four days later, all new pods that were 12 cm in length or more were tagged ("medium pods"). In Season 2 the first pods in each cultivar attained 12 cm 63 DAS and first tagging was done 71 DAS while second tagging was four days later. At harvesting all pods which were not tagged were regarded as "late pods". Table 1 indicates the percent distribution of the total harvested pods over the three classes of pod earliness. In Season 1 early pods did not have enough seeds for reliable assessment of seed viability and were therefore excluded from the analysis.

Table 1. Percentage of the total pod number, contributed by pods of different earliness classes in two seasons and two cultivars. Average values from all harvests.

Pod earliness	Season 1		Season 2	
	Rosecoco	Mwezi Moja	Rosecoco	Mwezi Moja
Early pods	1	4	11	27
Medium pods	16	42	16	20
Late pods	83	54	73	53

Laboratory methods

After shelling all pods, seeds with abnormal development and size were discarded and only normal looking seeds were used for further analysis. A sample of 100 seeds per plot was taken at the crop level and a sample of 30 seeds from each of the early, medium and late pods at pod level to determine fresh weight. Dry weight was determined after splitting all seeds across the cotyledons and drying for 16 hours at 105 °C. Then moisture content was calculated. The rest of the seeds were dried in a continuous flow drier at 30 °C and 50% relative humidity until a moisture content of 14% was achieved. Then they were stored at 2 °C and 75% relative humidity.

Tetrazolium test

Per sub plot samples of 20 seeds from the whole crop and each of the pod classes were soaked in water at room temperature for 24 hours. Thereafter, seeds were sliced longitudinally through the middle of the embryonic axis and soaked in a 0.5% tetrazolium solution at 30 °C for 3 hours, briefly washed in distilled water and examined under hand lens magnification (ISTA, 1995). The staining of the embryo was examined per seed. The number of seeds with sound tissues and weak viable tissues were combined to calculate viability, i.e. the percentage of viable seeds.

Electrical conductivity

Electrical conductivity was determined after equilibrating the seeds from cold storage for 3 days at room temperature (19 - 25 °C). The moisture content then was constant at 12%. From each subplot, samples of 25 seeds were taken from the whole crop and each of the pod classes, weighed and incubated in 125 ml of distilled water at 20 °C for 24 hours. The conductivity per gram of seed weight ($\mu S\ cm^{-1}g^{-1}$) at 12% moisture content for each subsample was then calculated to estimate seed vigour (ISTA, 1995).

Calculations

Maximum viability (%), and the day at which maximum viability was achieved (days after sowing) were calculated per block using a quadratic logistic model based on binomial distribution using Genstat 5 (Release 4.1). Viability was modelled as $y = 1/(1+\exp(-(a+b\times DAS+c\times DAS^2)))$ where a, b, c are parameters estimated by the model and DAS is days after sowing. The moment of maximum viability was calculated as $t = -b/2c$. The maximum viability achieved was calculated by substitution of that moment in the fitted model then transformed to percentage by multiplying the calculated value by 100. The same type of model was also used to derive maximum viability in relation to moisture content of the seeds and the moisture content at which viability was maximum.

Minimum electrical conductivity (EC) ($\mu S\ cm^{-1}\ g^{-1}$) and the moment (days after sowing) at which minimum EC was achieved were determined per block by non-linear regression between time and EC. A weighted model fitting a quadratically decreasing curve to a linear line was fitted using the Genstat 5 (Release 4.1) program. The linear line was forced to have a positive slope or be horizontal. The y-intercept at the meeting point of the decreasing quadratic curve and the linear line was estimated to be the minimum EC achieved during the growing season, and the x-value the day at which the minimum EC was achieved. The same type of model was used to derive the moisture content at which EC was minimum and the EC at this moisture content.

The moment of PM (maximum dry weight) was derived by a non-linear regression model between time and seed dry weight as presented by Muasya et al. (2001a). The moment when maximum dry weight was achieved was estimated as the x-axis value of the point at which a linear increasing curve and a horizontal line crossed. The same type of model was used to derive seed moisture content at PM and the seed weight at this moisture content.

HM was defined as the moment seeds achieved 20% moisture content.

Statistical analysis

The effect of time of harvest, cultivar and pod level on seed viability and vigour were tested by standard ANOVA assuming pod level to be an extra split factor within a harvest date. Means were separated by LSD tests. The calculated moments and moisture contents, maximum viability or minimum EC and the viability or EC at these moments or moisture contents were tested for significance using standard ANOVA and the means separated by LSD test.

Results

Seed viability development over time

The proportion of viable seeds increased with time in all pod classes in both cultivars and seasons (Figs 1 and 2), but only in both pod classes in cv. Rosecoco in Season 1 the viability was still increasing at the end of the harvesting period. In Season 1 no significant differences could be established in the moment at which maximum viability was achieved between seeds from all pod classes studied (medium, late and "crop" pods) (Table 2 and Fig. 1). In Season 2 maximum viability was achieved earlier in seeds from early pods and later in seeds from medium and late pods in cv. Rosecoco (Table 2). In cv. Mwezi Moja seeds from medium pods achieved maximum viability earlier than seeds from late pods (Table 2). In both seasons, seeds from "crop" pods did not differ significantly from seeds from other pods in the moment at which they achieved maximum viability (Table 2). The maximum viability achieved, i.e. 98% in Season 1 and 92% in Season 2, was not different for different pod classes in the respective seasons (Table 2). In Season 1 there was no decline in viability in all pod classes in both cultivars but in Season 2 viability had clearly declined in all pod classes in both cultivars towards the end of the experiment (Figs 1 and 2).

In both seasons, there was no significant difference between cultivars in the moment maximum viability was achieved or in the maximum viability achieved (Table 2).

Seed vigour development over time

In both seasons, electrical conductivity (EC) decreased with time in seeds from all pod classes and cultivars (Figs 1 and 2) until a minimum was achieved. After this, no further significant change was observed. The highest values of EC obtained at the earliest harvests in both cultivars and seasons are not plotted in the graphs (Figs 1 and 2). Minimum EC tended to be achieved earliest in seeds from earlier pods and latest in seeds from late pods (Table 3) but the differences were just beyond significance in Season 1 (P=0.063) and in Season 2 (P=0.066). In both seasons there were no significant differences between seeds from "crop" pods and seeds from early, medium and late pods in the moment minimum EC was achieved (Table 3). The minimum EC achieved did not differ significantly between pod classes in Season 1, but in Season 2 minimum EC was lower in seeds from early pods than in seeds from later or "crop" pods (Table 3).

Relationship between seed moisture content and seed viability

Maximum viability in all pod classes was achieved at 37% and 31% moisture content in Season 1 and Season 2, respectively, which is well beyond PM (58% moisture content) and closer to HM (20% moisture content) (Figs 3, 4 and Table 2). No differences could be established in the moisture content at which seeds from different pod classes achieved the maximum viability (Table 2). Maximum viability achieved in

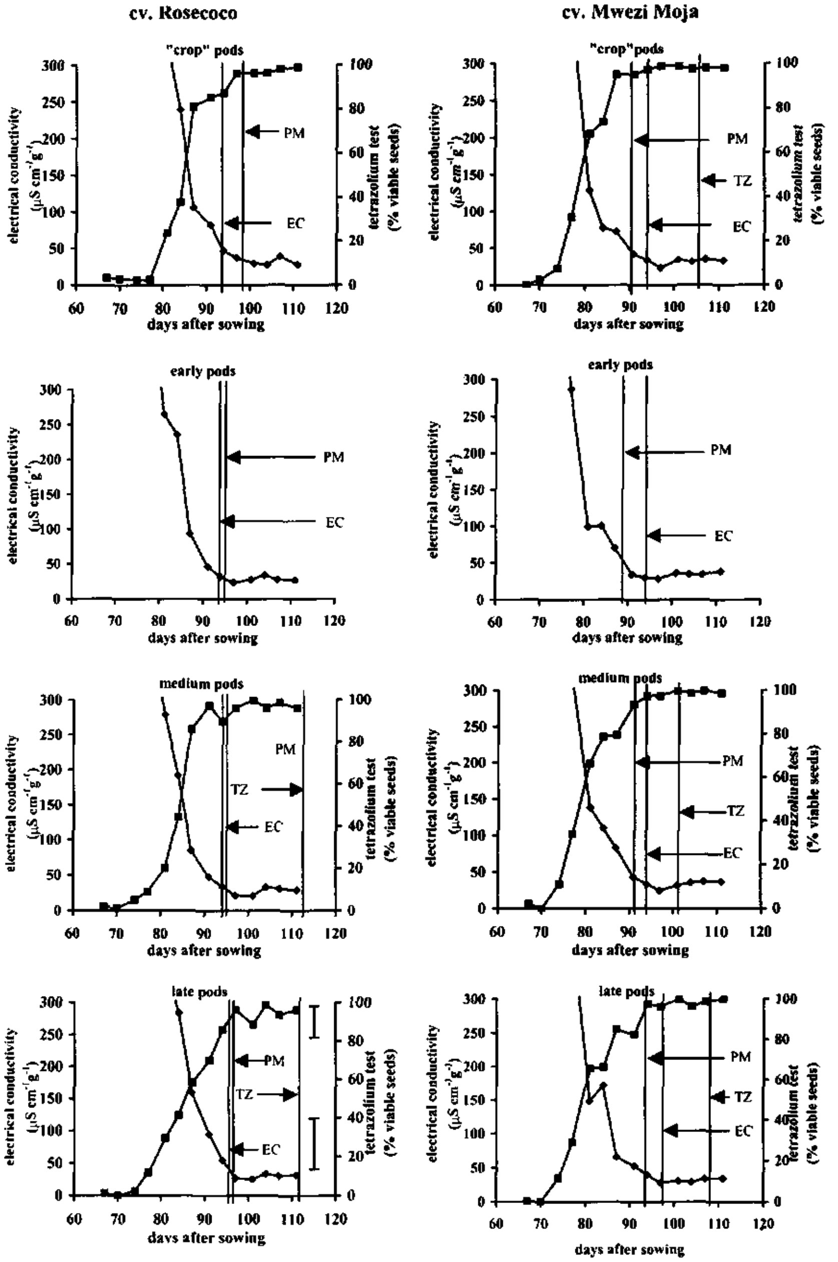

Fig. 1. Seed viability (-■-) and electrical conductivity (-◆-) development over time for cvs Rosecoco and Mwezi Moja in Season 1. The bars represent LSD (P<0.05) for comparisons between the cultivars, between and within the pod class means over time. The vertical lines represent the moments of physiological maturity (PM) as determined by Muasya et al. (2001a) and the moments of maximum viability (TZ) and minimum electrical conductivity (EC), as derived from Tables 2 and 3.

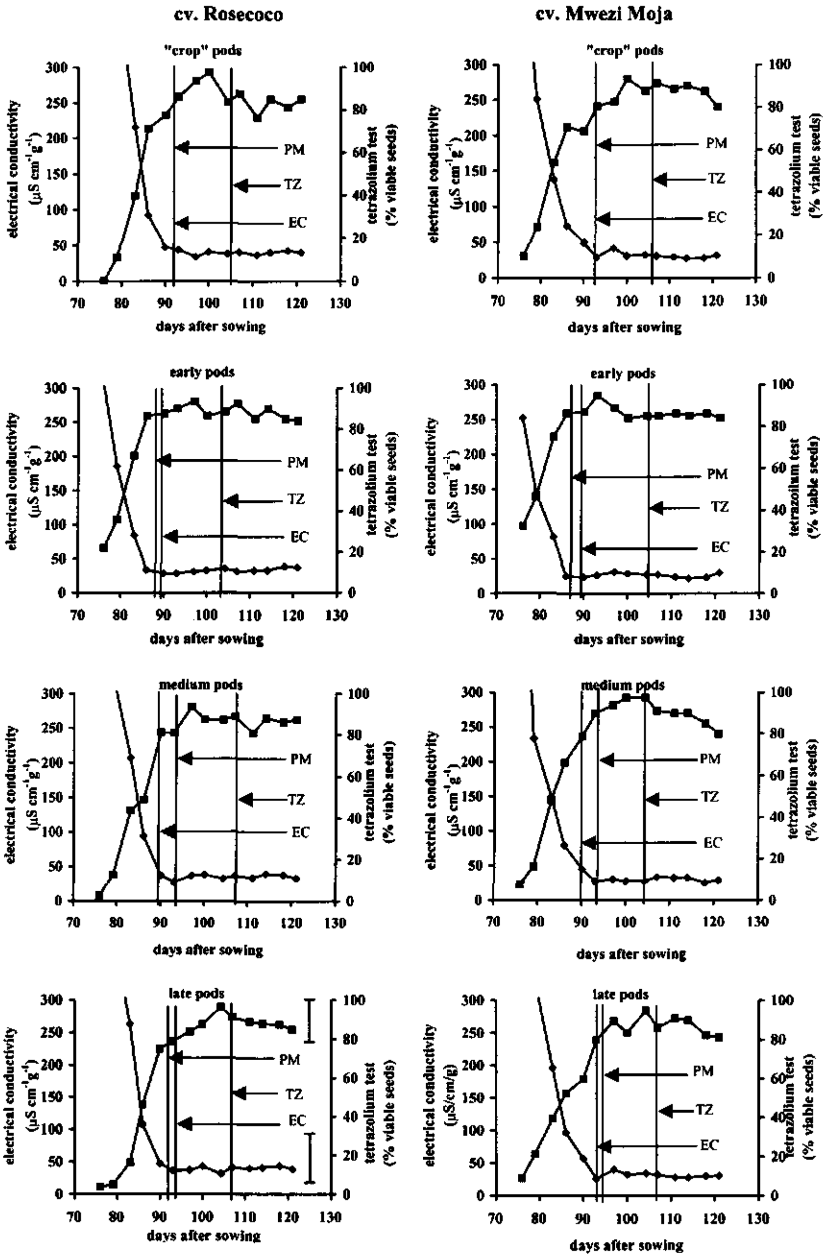

Fig. 2. Seed viability (-■-) and electrical conductivity (-◆-) development over time for cvs Rosecoco and Mwezi Moja in Season 2. The bars represent LSD ($P<0.05$) for comparisons between the cultivars, between and within the pod class means over time. The vertical lines represent the moment of physiological maturity (PM) as determined by Muasya et al. (2001a) and the moments of maximum viability (TZ) and minimum electrical conductivity (EC), as derived from Tables 2 and 3.

Table 2. The moment of achieving maximum viability, maximum viability at that moment, moisture content at achieving maximum viability and maximum viability at that moisture content, in pods of different classes for cvs Rosecoco and Mwezi Moja in two season. Values are derived from fitting a quadratic logistic model based on binomial distribution.

Pod class	Season 1			Season 2		
	Rosecoco	Mwezi Moja	Mean	Rosecoco	Mwezi Moja	Mean
Moment of achieving maximum viability (days after sowing)						
Crop	120.7	106.4	113.6 a[1]	105.2 ab	106.4 ab	105.8
Early	-	-	-	103.0 a	105.2 ab	104.1
Medium	113.5	102.0	107.8 a	107.8 b	103.8 a	105.8
Late	111.6	107.9	109.8 a	107.0 b	107.4 b	107.2
Mean	115.3 a	105.5 a	110.4	105.7	105.7	105.7
Maximum viability at the moment of achieving maximum viability (%)						
Crop	97.1	99.1	98.0 a	92.9	91.5	92.2 a
Early	-	-	-	93.3	89.5	91.4 a
Medium	98.7	98.7	98.7 a	92.1	94.9	93.5 a
Late	96.5	98.6	97.5 a	91.7	91.8	91.7 a
Mean	97.4 a	98.8 a	98.1	92.5 a	91.9 a	91.9
Moisture content at which maximum viability is achieved (%)						
Crop	40.5	32.9	36.7 a	33.4	28.8	31.1 a
Early	-	-	-	31.6	28.6	30.1 a
Medium	34.1	34.5	34.3 a	30.7	32.6	31.7 a
Late	44.9	32.7	38.8 a	31.1	31.8	31.5 a
Mean	39.8 a	33.4 a	36.6	31.7 a	30.4 a	31.1
Maximum viability at the moisture content at which maximum viability was achieved (%)						
Crop	99.0	99.7	99.3 a	98.3	94.8	96.5 a
Early	-	-	-	95.4	96.7	96.0 a
Medium	97.9	99.6	98.7 a	96.1	97.2	96.6 a
Late	98.3	99.4	98.9 a	96.3	95.7	96.0 a
Mean	98.4 a	99.5 b	99.0	96.5 a	96.1 a	96.3

[1] Means followed by the same letter within a characteristic and cultivar and/or pods class are not statistically different ($P \geq 0.05$) according to the LSD test.

relationship to moisture content was not different in different pod classes in both seasons (Table 2).

Seeds from cv. Rosecoco tended to achieve maximum viability at higher moisture contents than seeds from cv. Mwezi Moja but the differences could neither be assessed

Table 3. The moment of achieving minimum electrical conductivity, minimum electrical conductivity at that moment, moisture content at achieving minimum electrical conductivity and the minimum electrical conductivity at that moisture content, in pods of different classes in two seasons. Average values over two cvs. Values are derived from a weighted model fitting quadratically decreasing curve to a linear line in each pod class.

Pod class	Season 1	Season 2
Moment of achieving minimum electrical conductivity (days after sowing)		
Crop	93.0 a[1]	90.8 a
Early	92.7 a	88.5 a
Medium	94.0 a	88.9 a
Late	95.8 a	92.1 a
Minimum electrical conductivity at the moment of achieving minimum electrical conductivity (μS cm^{-1} g^{-1})		
Crop	31.0 a	34.1 b
Early	26.8 a	25.4 a
Medium	26.1 a	31.0 b
Late	28.5 a	32.7 b
Moisture content at which minimum electrical conductivity is achieved is achieved (%)		
Crop	57.7 a	57.8 a
Early	53.3 a	55.7 a
Medium	55.9 a	57.8 a
Late	57.3 a	57.5 a
Minimum electrical conductivity at the moisture content at which minimum electrical conductivity was achieved (μS cm^{-1} g^{-1})		
Crop	28.9 c	35.9 c
Early	22.4 a	26.7 a
Medium	22.6 ab	30.1 ab
Late	27.4 bc	31.4 b

[1] Means followed by the same letter within a characteristic and season are not statistically different ($P \geq 0.05$) according to the LSD test.

as significant in Season 1 (P=0.105) nor in Season 2 (P=0.055) (Table 2). In Season 1 the maximum viability achieved in relationship to moisture content was higher in cv. Mwezi Moja than in cv. Rosecoco but in Season 2 there was no difference between the two cultivars (Table 2).

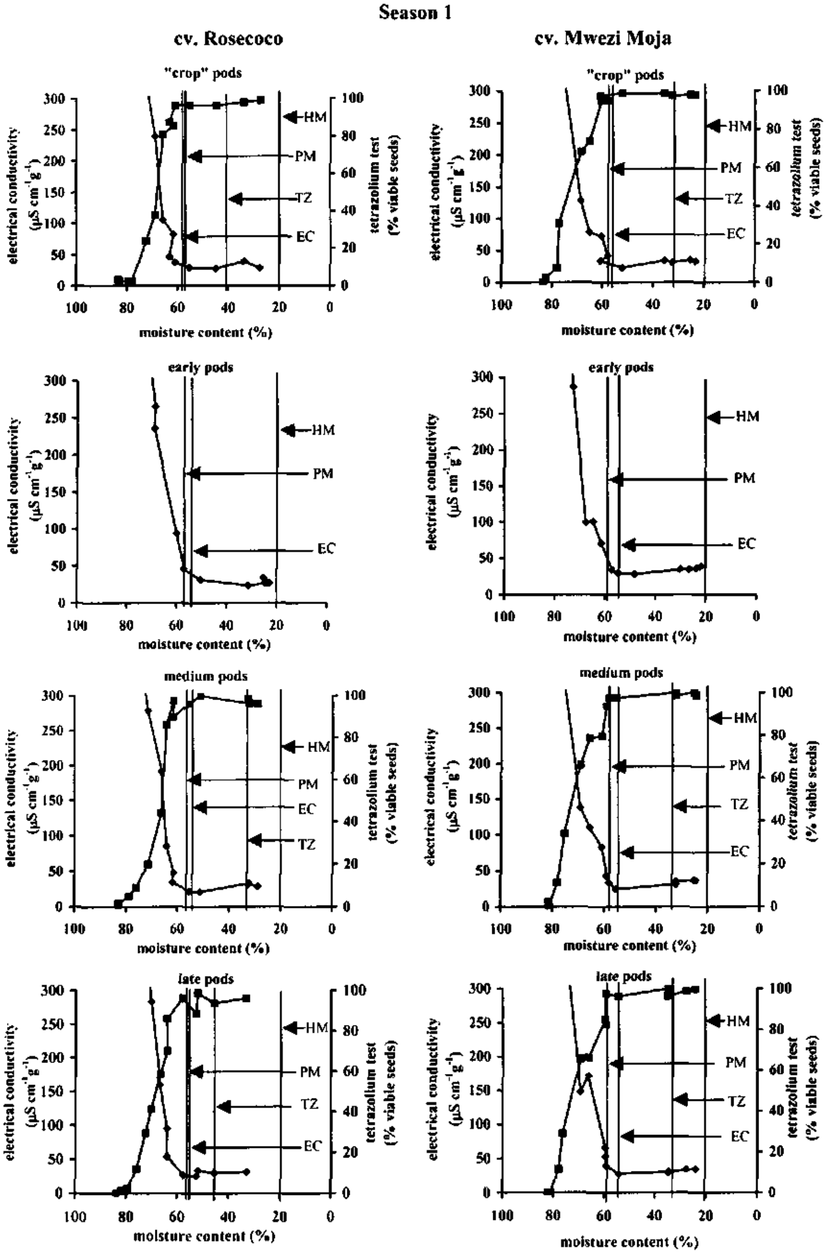

Fig. 3. Relationship between seed moisture content and viability (-■-) and electrical conductivity (-◆-) for cvs Rosecoco and Mwezi Moja in Season 1. The vertical lines represent the moisture contents at physiological maturity (PM) as determined by Muasya et al. (2001a), maximum viability (TZ) and electrical conductivity (EC) as derived from Tables 2 and 3, and harvest maturity (HM) as defined by Muasya et al. (2001a).

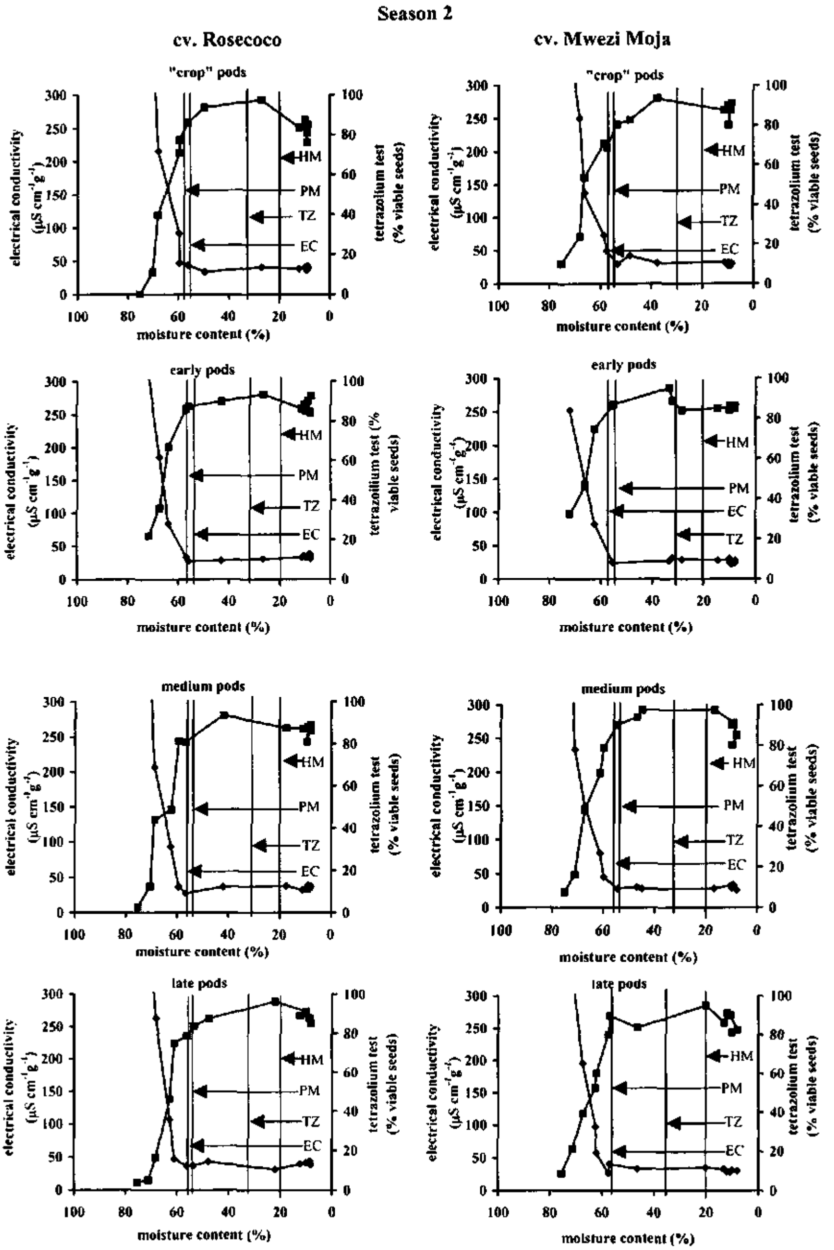

Fig. 4. Relationship between seed moisture content, viability (-■-) and electrical conductivity (-◆-) for cvs Rosecoco and Mwezi Moja in Season 2. The vertical lines represent the moisture content at physiological maturity (PM) as determined by Muasya et al. (2001a), maximum viability (TZ) and minimum electrical conductivity (EC) as derived from Tables 2 and 3, and harvest maturity (HM) as defined by Muasya et al. (2001a).

Relationship between seed moisture content and electrical conductivity

The minimum EC was achieved at moisture contents between 53 and 58% (Table 3) which is close to PM (58% moisture content) (Figs 3 and 4). In Season 2 when seeds stayed in the field well beyond HM there was a tendency for EC to increase but the increase was not significant (Fig. 4). Minimum EC tended to be achieved at slightly lower moisture content in seeds from early pods than in medium, late and "crop" pods, but differences could neither be assessed as being significant (P=0.103) in Season 1 nor in Season 2 (P=0.093) (Table 3). The minimum EC achieved at these moisture contents was lower in seeds from earlier pods whereas the minimum EC achieved in seeds from crop pods was comparable to seeds from late pods (Season 1) or even higher (Season 2) (Table 3).

Discussion

Seed quality development in common bean

In common bean, viability increased with time in seeds from all pod classes in both cultivars and seasons, and even still increased at the end of the harvesting period for seeds from medium and late pod classes in cv. Rosecoco in Season 1 (Figs 1 and 2). The results show that maximum viability was not achieved at PM (58% moisture content) as advocated by Harrington (1972) but rather maximum viability was achieved well after PM and closer to HM (20% moisture content) (Figs 3 and 4). This implies that seed development did not stop at PM but continued during maturation drying. Within a crop, this may partly be caused by some seeds that achieved maximum viability earlier, whereas late developing seeds within the seed lots were still increasing in viability (Figs 1 and 2). This conclusion shows the problems that may be encountered in characterising the end point of dry matter accumulation (PM) during seed development through repetitive dry-weight determinations (TeKrony and Egli, 1997). Such method may lead to estimating PM at a moment when some seeds are still accumulating dry matter (Chamma et al., 1990). Our method of assessing the moment of maximum viability allowed for a sharp logistic increase in an early phase followed by an increase and decrease. Nevertheless, the moment of maximum viability not only was later than PM within the whole crop (crop pods), but also in seeds from pods of different earliness within a crop, indicating that the same phenomenon was observed when differences in development presumably were much smaller. On the basis of our results we therefore concur with reports that the capacity for all the seeds within the seed lot to produce normal seedlings is not acquired by the entire seed population until after PM in soybean and various other grain legumes (Ellis et al., 1987; Zanakis et al., 1994; Pokojska and Grzelak, 1996), nor that this is the case for seeds from pods of comparable earliness within a seed lot.

The findings that minimum EC - as a measure of maximum vigour - was always achieved around PM in both cultivars and seasons (Figs 1 and 2), support reports that the maximum expression of seed vigour is closely related to the occurrence of PM in all crops harvested as dry seeds (TeKrony and Egli, 1997). The weak tendency for EC to increase after HM (Figs 1 and 2) could probably result from gradual seed deterioration as a result of ageing (Dornbos Jr, 1995).

The observation that maximum viability was always achieved after minimum EC (Figs 1 and 2), is contrary to the reports that viability is achieved earlier than vigour (Dornbos Jr, 1995). However, the results that maximum viability was achieved after minimum EC and close to HM are probably explained by the fact that seed viability is strongly maintained during maturation drying (Dornbos Jr, 1995) and that each seed contributes equally to the percentage of viable seeds. Within the seed lot, late seeds may achieve maximum viability late and consequently delay the moment when the bulk of the seeds achieve the maximum. This is confirmed by reports in soybean where maximum viability was reported close to HM (Zanakis et al. 1994). By contrast, later seeds may contribute less to the EC because of their lower seed weight as compared to earlier seeds (cf. Muasya et al., 2001a; Illipronti Jr et al., 2000b). It is unlikely that the later achievement of maximum viability than of maximum vigour was merely caused by the experimental techniques used to establish the respective moments.

Differences in seed quality development in pods from different pod earliness

Variation in maturity, seed viability and vigour has been reported between individual seeds within a seed lot (Keigley and Mullen, 1986; Adam et al., 1989). This paper shows that even in determinate common bean cultivars, differences in pod earliness can be one of the sources of this variation. Maximum viability was achieved at the same moment in seeds from different pod earliness classes in Season 1, but in Season 2 maximum viability was achieved earlier in seeds from early pods than from medium and late pods in cv. Rosecoco and in seeds from early and medium pods than from late pods in cv. Mwezi Moja (Table 2). One of the reasons for Season 1 showing no clear differences was the high coefficient of variation in the data in that season (not shown). In both cultivars the moment of minimum EC tended to be earlier in seeds from earlier pods than in seeds from late pods in both seasons (Table 3). Differences in achieving the moment of maximum viability or vigour were small because also the differences in earliness between pods were small.

There was no significant difference in the maximum viability achieved between seeds from different pod classes in both seasons (Table 2), but in Season 2 minimum

EC was lower in seeds from early pods than in seeds from medium and late pods (Table 3). It is unknown whether the latter is a real virtue of these seeds, or whether this has to be attributed to their heavier weight compared to seeds from later pods (Muasya et al., 2001a). Seed weights were especially different among seeds from different earliness in Season 2. Because EC is expressed per seed weight, the seed weight, may have influenced the minimum EC calculated. Seeds from early pods showed a higher seed-filling rate (Muasya et al., 2001a). Smaller seeds are usually formed later when most of the pods have been formed and are likely to face higher competition for accumulating insoluble reserves (Cocks, 1990). However, even if the lower EC is of early seeds results from their higher seed weight, this still may indicate a higher vigour. In French bean heavier seeds gave the largest seedlings (Doijode, 1984) and in soybean seedlings from large seeds were heavier than those from small seeds (Egli et al., 1990).

Relationship between seed quality at whole crop and pod levels

Seeds from whole crops are made up of seeds from individual pods of different earliness and therefore the quality of the whole crops could be explained by the quality of pods of different earliness within the crop. The fact that the bulk of pods at pod level were later pods (Table 1) explains the observations that seeds from the whole crop were usually comparable in timing to seeds from later pods or had an intermediate behaviour. The moment maximum viability and minimum EC was achieved did not differ between seeds from the whole crop and those from the pod level in both seasons (Tables 2 and 3). Also maximum viability achieved between seeds from the whole crop and those from pod level was not significantly different in both seasons (Table 2). This is likely because our results did not show significant viability differences in pods of different earliness within the crop, and because viability is strongly maintained during maturation drying (Dornbos Jr, 1995). Minimum EC was higher in seeds from whole crops than in seeds from pod level in both seasons, especially when calculated in relation to the change in moisture content and when compared to the least variable fractions of seeds from early and medium pods. This is what was expected when seeds from pods of different earliness achieve minimum EC at different moments and thereafter again start to increase in EC. Even though the increase in EC could not be assessed as significant, the differences in the minimum achieved were clear. We therefore conclude that the vigour of seeds from selected pods at their optimum moment of harvesting is higher than the vigour of seeds from all pods combined.

Conclusions

- Maximum seed viability was achieved well beyond PM and closer to HM, at 31 - 37% seed moisture content.
- Maximum vigour (minimum EC) was achieved around PM, at 57 - 58% seed moisture content.
- Maximum viability was achieved after minimum EC in all pod classes, cultivars and seasons.
- Seeds from earlier pods tended to achieve maximum quality earlier than those from later pods.
- Maximum viability achieved was not different in seeds from pods of different earliness.
- Maximum vigour achieved – as measured by EC – was higher in seeds from earlier pods than from later pods.
- Maximum viability achieved by seeds from pods of different earliness within a crop was not different from that achieved by the whole crop.
- Maximum vigour achieved by seeds from pods of different earliness within a crop was higher than that achieved by the whole crop.

Chapter 4

Seed variation in common bean (*Phaseolus vulgaris* L.) crops and in different pod fractions within a crop[*]

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[*] Muasya, R.M., W.J.M. Lommen & P.C. Struik, 2001. Seed variation in common bean (*Phaseolus vulgaris* L.) crops and in different pod fractions within a crop.

4. Seed variation in common bean (*Phaseolus vulgaris* L.) crops and in different pod fractions within a crop

Abstract

Development over time of the variation in seed characteristics may be relevant for the precise timing of the seed harvest and may affect quality characteristics of the resulting seed lot. This study focuses on variation in characteristics that both reflect the physiological behaviour of individual seeds (seed fresh and dry weight, moisture content) within a crop and are associated with their harvestability (moisture content) and final quality as propagules (electrical conductivity). Two field experiments with common bean cultivars Rosecoco and Mwezi Moja were therefore carried out to analyse the trend over time in variation in weight, moisture content and electrical conductivity among individual seeds from early, medium and late pods, and among seeds from the whole crop ("crop" pods) in different seasons. Variation among individual seeds was expressed as the population standard deviation of seeds from pods differing in earliness and pods of the whole crop.

Seed variation in weight, moisture content and electrical conductivity was not only different between cultivars but also varied within the crop. Seed variation in seed weight and moisture content initially increased as seed filling progressed and became stable around maturity (PM). Seed fresh weight and moisture content variation declined with time thereafter but dry weight variation remained high. The highest seed variation in moisture content was observed after PM but before harvest maturity (HM). Minimum electrical conductivity variation was achieved around PM in seeds from all pod classes in both cultivars and seasons. Maximum seed variation in weight and moisture content was earlier and lower in seeds from early pods than in seeds from medium, late or "crop" pods. Electrical conductivity variation was lower in seeds from early pods than in seeds from medium, late and "crop" pods, at least for one season. Seed variation in weight, moisture content and electrical conductivity was higher in seeds from whole crop than in the seeds from pods of the different classes of earliness.

In both seasons and cultivars timely harvesting of all seeds is difficult without selection for earliness. The variation in behaviour observed among individual seeds is therefore a significant factor in the quality of a seed lot.

Keywords: Common bean, electrical conductivity, moisture content, pod earliness, seed variation, standard deviation

Introduction

The quality of a seed lot results from the combined qualities of the individual seeds within a seed lot. Within a seed crop, the development of individual seeds, however, is not uniform. Some seeds may reach physiological maturity (PM, defined as the stage at which maximum dry weight is obtained) before seed filling in other seeds is completed (Chamma et al., 1990; TeKrony and Egli, 1997). This is associated with variation among seeds in time of seed filling, seed filling rate and duration of seed filling. These and other factors sometimes result in significant variation in seed fresh weight (Nanda et al., 1996). After PM moisture content declines, but, again, the exact timing and the rate of this decline vary within a common bean crop. The period from PM to harvest maturity (HM, defined as the moment when the dry matter content reaches a value that allows the seed to be harvested without seed damage), also indicated as the phase of maturation drying, may vary in timing and in duration among individual seeds within the seed lot. Development over time of the variation in seed characteristics is therefore relevant for the precise timing of the seed harvest and influences the quality characteristics of the resulting seed lot. On the other hand, time of harvest affects not only the quality of the whole lot, but also the variation in quality within the lot, unless pods are selectively harvested based on their earliness.

Some causes of these variations in individual seed characteristics are obvious. For example in common bean, seeds from earlier pods tend to be heavier than seeds from later pods (Muasya et al., 2001a). Seeds from earlier pods also achieve the moment of PM earlier than seeds from later pods. When harvest date is fixed for the entire crop, the period between PM and harvest is longer for seeds from earlier pods than for seeds from later pods in common bean crops (Muasya et al., 2001a). This longer time is partly required to reach HM in all seeds: moisture content in seeds from earlier pods in the common bean cv. Mwezi Moja takes longer to decline from 58% (PM) to 20% (HM) than moisture content in seeds from later pods (Muasya et al., 2001a). During this longer period, seeds of early pods may be exposed longer to seed quality decline, resulting in inferior seed quality if the maximum quality of early seeds is similar to that of later seeds.

Maximum seed viability and vigour also vary within the common bean crop: early/heavier seeds show better maximum vigour than late/lighter seeds when timing of harvest is optimised (Muasya et al., 2001b). In contrast, higher viability has been reported in soybean seeds from later pods than in seeds from earlier pods at harvest when temperature during seed production was high (Illipronti Jr et al., 2000b).

Quantitative information on the pattern of variation within a crop during seed development in common bean crops is not available so far, but it can be surmised that the within-crop variation in seed characteristics changes over time (Copeland and McDonald, 1995). The pattern of this change may be related to developmental events specific for certain phenological changes. For instance, the variation in seed dry weight may achieve a constant level at the moment of PM as a result of the end of seed filling. At that moment variation in seed fresh weight or moisture content may still be changing because of variation in maturation drying after PM. Variation in dry matter content may then show a well defined pattern with clear peaks during certain physiological phases. Variation in electrical conductivity however may become temporarily stable at PM until seed deterioration starts.

This research aims at increasing insight into the trends in variation in weight, moisture content and electrical conductivity of individual seeds, during development of common bean crops and in pods differing in earliness within the crop, and into the association between the variation in seed characteristics and developmental events. Variation for seeds from entire common bean crops and variation for seeds from pods differing in earliness within the crop are also compared to assess whether variation at crop level is higher than variation within pod classes.

Materials and methods

Experimental set-up

Two experiments with common bean seeds were planted at the Chepkoilel campus farm of Moi University in Eldoret, Kenya in two growing seasons in 1998. The Season 1 crop was planted on 24 April and harvested from 29 June to 13 August, the Season 2 crop was planted on 17 August and harvested between 5 November and 21 December.

The site was located 2180 m asl and had a cambisol (Ferralitic) soil, well drained, non-humic and shallow, with low nutrient availability and moisture storage (Anonymus, 1987) and pH 4.6 (Muasya, 1996). Season 1 received a total of 554 mm of rainfall and 1471 °Cd temperature sum while Season 2 received 317 mm of rainfall and 1769 °Cd temperature sum. At planting, each plot of 16 m^2 was fertilized with 0.49 kg calcium ammonium nitrate (26% N), 0.76 kg triple super phosphate (48% P_2O_5) and 0.13 kg muriate of potash (60% K_2O), achieving 80 kg N, 100 kg P and 20 kg K per hectare. The experimental design was a split plot with four blocks. Two cultivars, Rosecoco and Mwezi Moja, were assigned randomly to main plots. Both are determinate cultivars, but Rosecoco shows prolonged flowering while the flowering period of Mwezi Moja is short, suggesting less variation in time of podding. Harvesting dates (1-14) were assigned to subplots. The first harvesting dates were 67 and 76 days after sowing (DAS) in the first and second season, respectively. Subsequent harvests took place twice a week until 111 and 121 DAS of the first and second season, respectively. The gross plot size was 4×4 m while the net experimental area was 3.40×2.50 m and included 170 plants spaced at 0.5×0.1 m. Within the net experimental area, an area of 2 m^2 was marked for observations of the whole crop (crop level) while the remaining area of 6.5 m^2 was used for observations on three classes of pods (pod level).

Table 1. Percentage of the total pod number, contributed by pods of different earliness classes in two seasons and two cultivars. Average values from all harvests.

Pod earliness	Season 1		Season 2	
	Rosecoco	Mwezi Moja	Rosecoco	Mwezi Moja
Early pods	1	4	11	27
Medium pods	16	42	16	20
Late pods	83	54	73	53

Pod classes

Flowering began 40 and 42 DAS in Season 1 and 2, respectively. In Season 1, the first pods in each cultivar attained 12 cm length on 56 DAS. All pods of 12 cm or more were tagged on that date. These were regarded as "early pods". Four days later, all new pods that were 12 cm in length or more were tagged ("medium pods"). In Season 2, the first pods in each cultivar attained 12 cm 63 DAS and first tagging was done 71 DAS, while second tagging was four days later. At harvesting all pods which were not tagged were regarded as "late pods". Table 1 indicates the percent distribution of the total harvested pods over the three classes of pod earliness. In Season 1, early pods did not have enough seeds for reliable assessment of different seed characteristics and were therefore excluded from the analysis.

Measurements

At each harvest date, pods were removed from different pod classes separately and hand shelled. Seeds with abnormal development and size were discarded and only normal looking seeds were used for further analysis. Per block, a sample of 30 seeds per plot was taken at the crop level and a sample of 20 seeds from each of the early, medium and late pods. The fresh weight of each seed was determined. The dry weight of each seed was determined after splitting the seed across the cotyledons and drying for 16 hours at 105 °C. Individual seed moisture content was then calculated. The remaining seeds per plot and pod level were dried in a continuous flow drier at 30 °C and 50% relative humidity until a moisture content of 14% was achieved. Then they were stored at 2 °C and 75% relative humidity.

The moisture contents plotted along the x-axes of Figs 5 and 6 are based on the analysis of the whole samples and not on the average of the moisture content of individual seeds.

HM was defined as the moment seeds achieved 20% moisture content.

Electrical conductivity was determined after equilibrating the seeds from cold storage at room temperature (19 - 25 °C) for 3 days. The moisture content was then constant at 12%. From each subplot, a sample of 30 seeds was taken from the whole crop and a sample of 20 seeds from each of the pod classes. Each seed from these samples was weighed and incubated in 50 ml of distilled water at 20 °C for 24 hours. The conductivity per gram of seed weight (µS cm^{-1} g^{-1}) at 12% moisture content was then calculated for each seed to estimate seed vigour (ISTA, 1995).

The moment of PM (maximum dry weight) was derived by a non-linear regression model between time and seed dry weight as presented by Muasya et al. (2001a). The moment when maximum dry weight was achieved was estimated as the x-axis value of the point at which a linear increasing line and a horizontal line crossed. The same type of model was used to derive seed moisture content at PM. Data for these assessments came from a sample of 100 seeds per plot at crop level and a sample of 30 seeds from each of the early, medium and late pods.

Statistical analysis

The population standard deviation (SD) was used as a characteristic to express variation between individual seeds. The SD of the seed fresh weight, dry weight, moisture content and electrical conductivity for each sample were calculated per plot. The relative standard deviation (RSD) was assessed by dividing all the SD means of a seed parameter within a pod class and cultivar by the highest mean SD value obtained in time and multiplying by 100. The effects of cultivar, time of harvest and pod class on seed variation were tested by standard ANOVA assuming pod class to be an extra split factor within a harvest date. Means were separated by LSD tests. Conclusions on the electrical conductivity SD were based on square root transformations of the electrical conductivity SD values. In Season 1, sampling started earlier than in Season 2. Data from the first two observations in medium, late and "crop" pods in Season 1 were not included for statistical analysis because of extreme values.

Results

The significances of SD values of different seeds parameters reported in both seasons are shown in Table 2. The trends of seed weight, moisture content and electrical conductivity standard deviations during seed development were clearer in Season 2 than in Season 1 (Figs 1 and 2).

Significant two-way interactions between cultivar and harvest time or harvest time and pod level showed that the development of SD with time usually depended on the pod level and/or the cultivar (Table 2). Only in two out of eight comparisons, three-way interactions were significant (Table 2). Graphical representation of the development of SD with time or with decreasing seed moisture content, however, has been

Table 2. Sources of variation and the significance of differences in SD for seed fresh weight, dry weight, moisture content and electrical conductivity in Season 1 and Season 2.

Source of variation	Seed development parameters							
	Fresh weight (SD)		Dry weight (SD)		Moisture content (SD)		Electrical conductivity[2] (SD)	
	Season		Season		Season		Season	
	1	2	1	2	1	2	1	2
Harvest date (T)	**[1]	***	***	***	***	***	***	***
Pod level (P)	*	***	NS	***	***	***	***	***
Cultivar (C)	**	*	*	*	*	NS	**	*
T × P	NS	***	NS	*	*	***	NS	***
T × C	NS	***	NS	NS	**	***	NS	NS
P × C	*	*	***	***	NS	*	NS	NS
T × P × C	*	NS	NS	NS	NS	**	NS	NS

[1] NS, *, ** and *** indicate statistically non-significant, significant at $P<0.05$, $P<0.01$, and $P<0.001$, respectively.

[2] Data on electrical conductivity were square root transformed before analysis.

done for all combinations of pod level and cultivars separately. Although this might suggest that three-way interaction occurred for all characteristics in all seasons, it increased the uniformity in the different presentations, and allowed studying the development of SD in relation to PM and HM, which are achieved at different moments for seeds from different pod levels and cultivars. Nevertheless, conclusions on trends in time and effects of pod level and cultivar were based on the relevant interactions from the ANOVA-analysis (Table 2).

Seed fresh weight variation over time

The significant three-way interaction showed that the development of fresh weight SD over time depended on the pod level in a different way in the two cultivars in Season 1, whereas in Season 2 two-way interactions between time and pod level and time and cultivar showed that the development over time of fresh weight SD depended on the pod level in a similar way in both cultivars and depended on the cultivar in a similar way in all pod levels (Table 2).

In Season 1, seeds from all pod classes and both cultivars presented showed a slight increase in fresh weight standard deviation (SD) until 80 - 90 days after sowing with a slight decrease after about 100 days after sowing and some erratic behaviour in

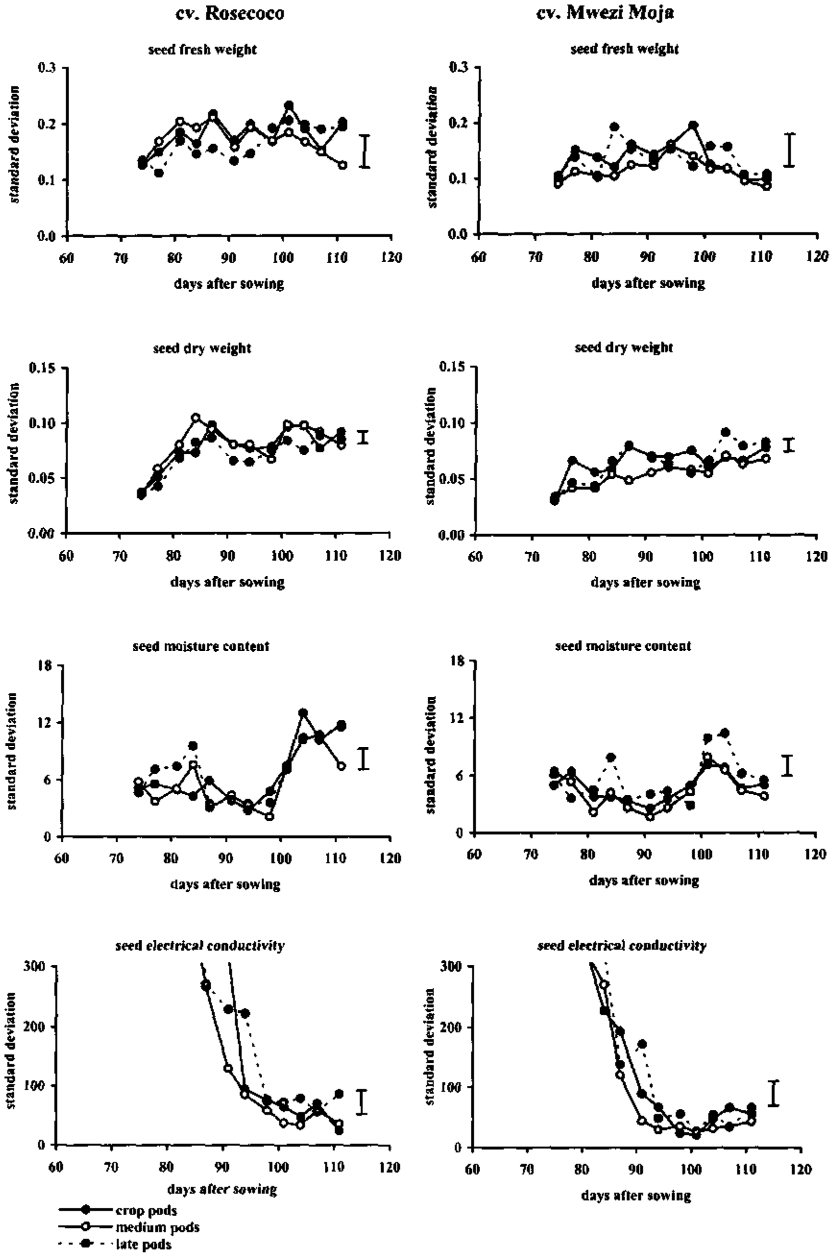

Fig. 1. Seed fresh weight, dry weight, moisture content and electrical conductivity standard deviations over time for seeds from medium, late and "crop" pods in cvs Rosecoco and Mwezi Moja in Season 1. The bars represent LSD (*P*<0.05) for comparisons between and within pod class means over time.

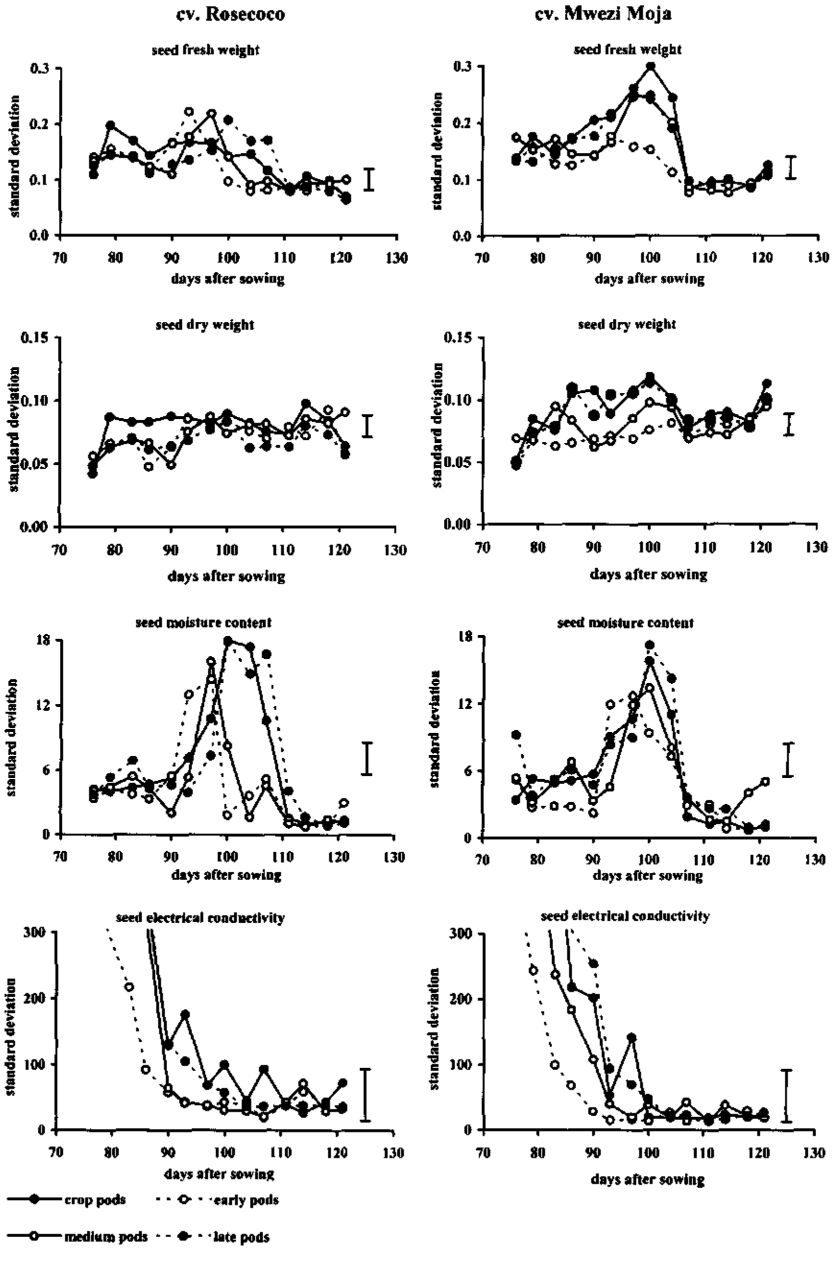

Fig. 2. Seed fresh weight, dry weight, moisture content and electrical conductivity standard deviations over time for seeds from early, medium, late and "crop" pods in cvs Rosecoco and Mwezi Moja in Season 2. The bars represent LSD ($P<0.05$) for comparisons between and within pod class means over time.

between in both cultivars (Fig. 1). Fresh weight SD was lower in cv. Mwezi Moja than in cv. Rosecoco. Seeds from medium and "crop" pods showed higher fresh weight SD than seeds from late pods during seed filling but fresh weight SD tended to increase in seeds from the "crop" pods towards the end of the experiment in cv. Rosecoco. In cv. Mwezi Moja seeds from medium pods showed lower fresh weight SD than seeds from late pods. Seeds from "crop" pods were intermediate in cv. Mwezi Moja (Fig. 1).

In Season 2, seeds from all pod classes first showed a temporary increase in fresh weight SD (Fig. 2). Maximum fresh weight SD was higher in cv. Mwezi Moja than in cv. Rosecoco. The increase in fresh weight SD was earlier in cv. Rosecoco than in cv. Mwezi Moja. The increase over time was earlier in seeds from early pods, at intermediate moment in seeds from medium pods and later in seeds from late and "crop" pods (Fig. 2).

We consider the following trends as most important: the SD in seed fresh weight increased, and then slightly declined before increasing again and reaching a peak. The peak was earlier for early pods than for other pod classes or for the whole crop. In cv. Mwezi Moja, the peak was also considerably lower for early pods.

Seed dry weight variation over time

Development of dry weight SD over time was not affected by pod level in Season 1. The main effect of harvest date was significant, but its interactions with cultivar or pod level were not. However, the SD of seed dry weight was affected by the pod level in Season 2, as shown by a significant two-way interaction between harvest date and pod level (Table 2).

In Season 1, seed dry weight SD increased over time in both cultivars in all pod classes in the early phases of seed filling with a temporary decline in the middle part and stabilized at a high level at the end of the experiment (Fig. 1).

In Season 2, an increase in seed dry weight SD was observed early during seed filling in seeds from all pod classes in both cultivars (Fig. 2). The increase levelled off later in all pod classes. Whenever there were differences between pod levels, seeds from early pods showed lowest dry weight SD, seeds from medium and late pods were comparable and seeds from "crop" pods showed seed dry weight SD comparable to seeds from late pods (Fig. 2).

We consider the following trends as most important: the SD in seed dry weight increased, and then slightly declined in some cases until it reached a stable and rather high level at around 90-100 days after sowing. The final value was lower and reached earlier for early pods than for other pod classes or for the whole crop.

Seed moisture content variation over time

Development of moisture content SD with time in Season 1 was affected by the pod level similarly in both cultivars (as shown by the significant two-way interaction between harvest date and pod level, but no three-way interaction), whereas in Season 2 the development of moisture content SD was affected by the pod level differently in both cultivars (as shown by significant three-way interaction) (Table 2).

In Season 1, seed moisture content SD initially increased slightly and declined reaching the lowest level between 90 and 100 days after sowing but increased to a peak before declining at the end of the experiment in all pod classes in both cultivars (Fig. 1). The moisture content SD was lower in cv. Mwezi Moja than in cv. Rosecoco. The increase after the lowest level of moisture content SD was at comparable moment in seeds from all pods. Moisture content SD in seeds from "crop" pods increased earlier than in seeds from pod level classes studied in Season 1 (Fig. 1). Seed moisture content SD was higher in seeds from late and "crop" pods than in medium pods (Fig. 1).

In Season 2, seed moisture content SD was much higher between 90 and 108 days after sowing than before or after that period (Fig. 2). The increase of moisture content SD was earlier in seeds from early pods, intermediate in seeds from medium pods and later in seeds from late pods, whereas moisture content SD in seeds from "crop" pods increased to the peak in between medium and late pods in cv. Rosecoco. In cv. Mwezi Moja, moisture content SD in seeds from early pods increased to the peak earlier than those from the other pod classes (Fig. 2).

We consider the following trends as most important: the SD in seed dry matter content initially slightly increased, then slightly declined, then rose to a sharp peak before strongly declining again. The sharp peak was reached earlier for early pods than for other pod classes or for the whole crop.

Seed electrical conductivity variation over time

The effect of time on electrical conductivity SD was similar in all pod levels and cultivars in Season 1, whereas in Season 2 the pod level affected the development of electrical conductivity SD with time (Table 2).

In Season 1, electrical conductivity SD declined to a minimum and remained constant thereafter in seeds from all pod classes (Fig. 1). Electrical conductivity SD was lower in cv. Mwezi Moja than in cv. Rosecoco and lower in seeds from medium pods than in seeds from late pods. In seeds from "crop" pods electrical conductivity SD was usually between the electrical conductivity SDs of seeds from medium and late pods (Fig. 1).

In Season 2, electrical conductivity SD declined to a minimum and remained constant thereafter in seeds from all pod classes (Fig. 2). Electrical conductivity was lower in cv. Mwezi Moja than in cv. Rosecoco. Minimum electrical conductivity SD was achieved earlier in seeds from early pods, at intermediate moment in seeds from medium pods and later in seeds from late pods. Seeds from "crop" pods achieved minimum electrical conductivity SD at a moment comparable to seeds from late pods. Minimum electrical conductivity SD was comparable in seeds from all pod classes in Season 2 (Fig. 2).

We consider the following trends as most important: the SD in seed electrical conductivity declined during seed filling, reaching a minimum between 95 and 110 days, and remained more or less constant thereafter. The minimum was reached earlier for earlier pods than for later pods.

Relationships between standard deviations of different seed parameters

Crop pods

In Season 1, seed fresh weight relative standard deviation (RSD) and dry weight RSD increased during early seed filling. These RSDs then stabilized in cv. Rosecoco, whereas in cv. Mwezi Moja fresh weight RSD decreased towards the end of the experiment (Fig. 3). Moisture content RSD showed two peaks, an early and a late one in both cultivars but this was most pronounced in Mwezi Moja (Fig. 3).

In Season 2, fresh and dry weight RSD increased during seed filling, stabilized and then slowly declined in cv. Rosecoco (Fig. 4). In cv. Mwezi Moja, fresh and dry weight RSD took more time to reach maximum with fresh weight RSD dropping much more than dry weight RSD at the end of the experiment. Moisture content RSD started to increase considerably around PM, was maximum at 100 days after sowing in both cultivars, and finally decreased strongly (Fig. 4).

In both seasons and cultivars, electrical conductivity RSD declined over time reaching a minimum around or after PM (Figs 3 and 4).

Early pods

Early pods are only reported for Season 2. Fresh and dry weight RSD were low at PM increasing to a peak thereafter (Fig. 4). Fresh and dry weight RSD declined after the peak with fresh weight RSD declining stronger than dry weight RSD in cv. Rosecoco. In cv. Mwezi Moja fresh weight RSD showed a low level at PM and increased to a peak thereafter and then declined. Dry weight RSD showed a gradual increase after PM and no further decline was observed in cv. Mwezi Moja. Moisture content RSD was low at PM and increased showing a peak around 90-100 days after sowing before declining in both cultivars. Electrical conductivity RSD declined to a minimum just after PM and remained constant thereafter in both cultivars (Fig. 4).

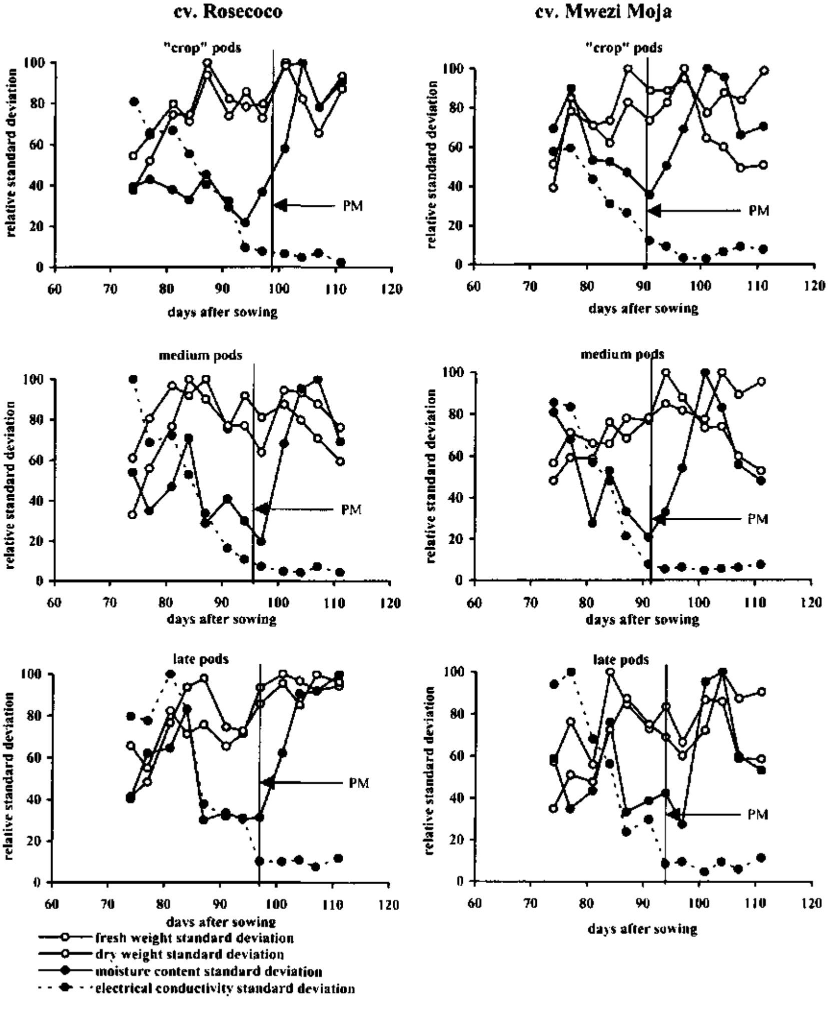

Fig. 3. Seed fresh weight, dry weight, moisture content and electrical conductivity relative standard deviations over time for seeds from medium, late and "crop" pods in cvs Rosecoco and Mwezi Moja in Season 1. The vertical lines represent the moment of physiological maturity (PM) as determined by Muasya et al. (2001a).

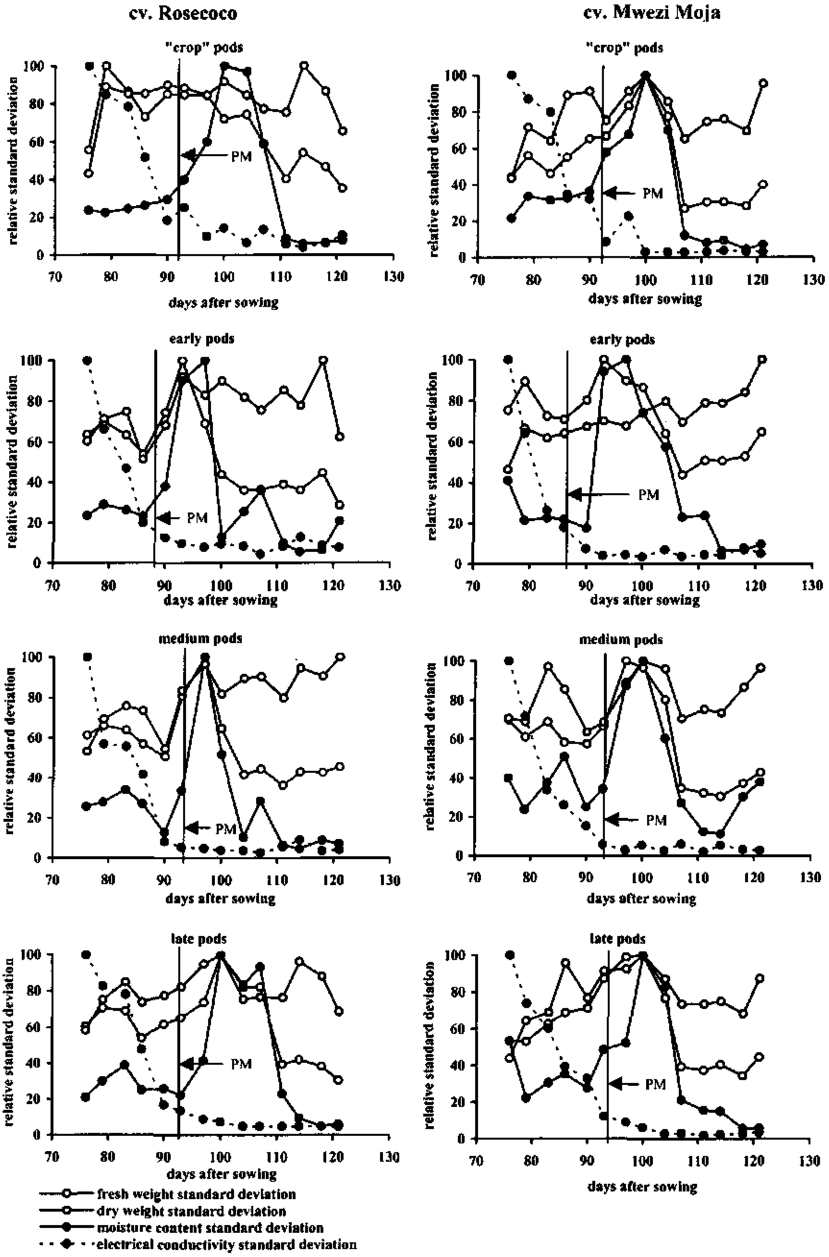

Fig. 4. Seed fresh weight, dry weight, moisture content and electrical conductivity relative standard deviations over time for seeds from early, medium, late and "crop" pods in cvs Rosecoco and Mwezi Moja in Season 2. The vertical lines represent the moment of physiological maturity (PM) as determined by Muasya et al. (2001a).

Medium pods

In Season 1, fresh and dry weight RSD increased during seed filling to a peak and then declined in cv. Rosecoco, whereas in cv. Mwezi Moja fresh weight RSD increased longer before it declined after PM and dry weight RSD even continued to increase after PM. Moisture content RSD was lowest at PM but increased thereafter to a peak then declined in both cultivars (Fig. 3).

In Season 2, fresh and dry weight RSD increased around PM to a peak with fresh weight RSD declining more than dry weight RSD in both cultivars. The patterns of moisture content RSD and electrical conductivity RSD in both cultivars were comparable to those in seeds from early pods (Fig. 4).

Late pods

In Season 1, fresh weight, dry weight and moisture content RSD increased to a peak but the decrease in these RSDs was not obvious after PM in cv. Rosecoco, whereas in cv. Mwezi Moja fresh weight and moisture content RSD strongly declined after the peak. In both cultivars the pattern of electrical conductivity RSD in late pods was comparable to that for seeds from medium pods except that in seeds from late pods the minimum was achieved later than in seeds from medium pods (Fig. 3).

In Season 2, fresh weight, dry weight, moisture content and electrical conductivity RSD trends were comparable to seeds from early and medium pods but in seeds from late pods, the trends were later than in seeds from early and medium pods in both cultivars (Fig. 4).

Changes in relative standard deviations of different seed parameters between PM and HM

In both seasons and cultivars, seed fresh weight, dry weight and electrical conductivity RSD underwent drastic changes before PM (Figs 5 and 6). Electrical conductivity RSD were lower at PM than during seed filling. Changes after PM are relevant for the timing of harvest. After PM, fresh weight and dry weight RSD tended to decrease or stabilize, whereas the seed moisture content RSD showed a clear peak before HM (20% moisture content) in both cultivars and seasons (Figs 5 and 6). No changes were observed in both cultivars and seasons for electrical conductivity RSD between PM and HM (Figs 5 and 6). After HM dry weight RSD tended to remain high but fresh weight and moisture content RSD tended to decline with decrease in seed moisture content (Fig. 6).

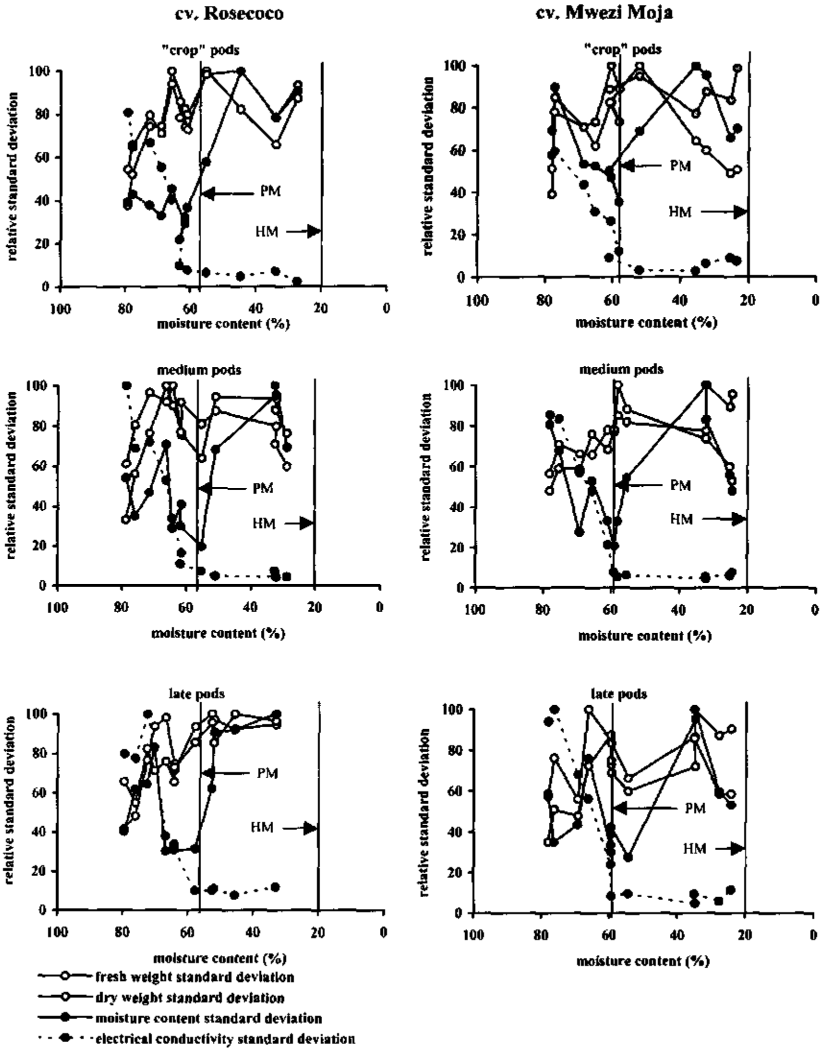

Fig. 5. Relationship between seed fresh weight, dry weight, moisture content and electrical conductivity relative standard deviations and seed moisture content for seeds from medium, late and "crop" pods in cvs Rosecoco and Mwezi Moja in Season 1. The vertical lines represent the moisture contents at physiological maturity (PM) as determined by Muasya et al. (2001a), and harvest maturity (HM) as defined by Muasya et al. (2001a).

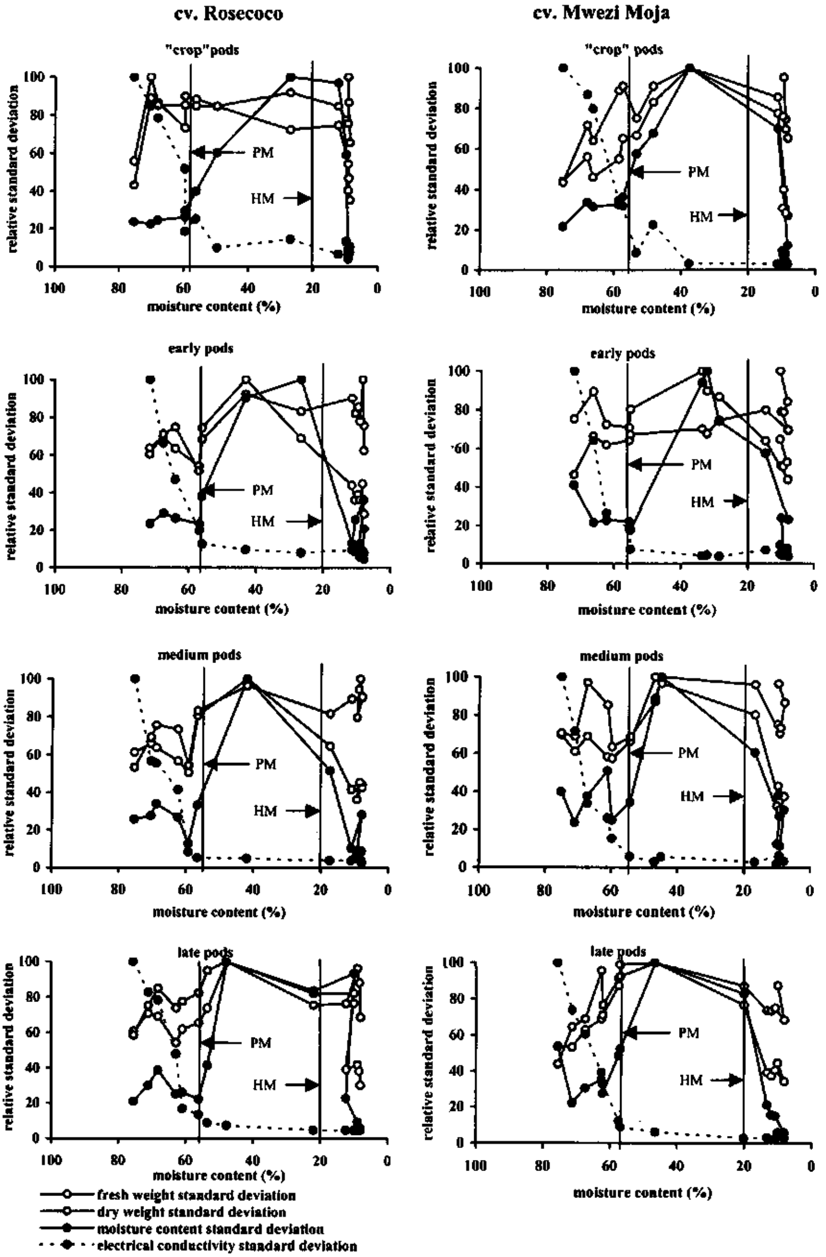

Fig. 6. Relationship between seed fresh weight, dry weight, moisture content and electrical conductivity relative standard deviations and seed moisture content for seeds from early, medium, late and "crop" pods in cvs Rosecoco and Mwezi Moja in Season 2. The vertical lines represent the moisture contents at physiological maturity (PM) as determined by Muasya et al. (2001a), and harvest maturity (HM) as defined by Muasya et al. (2001a).

Discussion

Variation in trends in seed development in common bean

In seeds from all pod classes in both cultivars and seasons, seed fresh weight and dry weight SD showed a tendency to increase during seed filling (Figs 1 and 2). Fresh weight SD tended to increase until after seed filling before declining, suggesting not only variation in seed filling, but also in timing of water loss. Seed dry weight SD showed a tendency to level off at the end of seed filling (Figs 1 and 2). Our results showed a cultivar effect for the change in fresh weight SD, especially in Season 2: the peak in seed fresh weight SD was much more pronounced in Mwezi Moja. The increase in dry weight SD did not differ between cultivars in both seasons (Figs 1 and 2). Fresh and dry weight SD maximum levels also varied among cultivars in our experiments. For instance in Season 1 maximum fresh weight SD was higher in cv. Rosecoco than in cv. Mwezi Moja but in Season 2 maximum fresh weight SD was higher in cv. Mwezi Moja than in cv. Rosecoco (Figs 1 and 2). Our results underline the finding that the onset of seed filling, the seed filling rate and the duration of seed filling vary within the crop (Muasya et al., 2001a). Chamma et al. (1990) also reported that seed filling may not be completed at once in all seeds within a seed lot at the end of seed filling. These differences in timing within the crop in combination with sampling errors could also have lead to the erratic behaviour in fresh and dry weight SD shown by both cultivars in both seasons at the end of seed filling (Figs 1 and 2).

Similarly, large variation was found for the onset and rate of water accumulation in the seed and maturation drying. Seed moisture content SD therefore was very variable and at the same time strongly influenced by pod level, cultivar and date of sampling. It increased slightly during the early stages of seed filling (Figs 1 and 2) which could be attributed to variable (rates of) increase or decrease in seed moisture amount in the seed as dry matter accumulation progressed during seed filling. According to our results, moisture content SD was lowest at maximum dry weight and towards the end of the experiment in both cultivars and seasons (Figs 1 and 2). At maximum dry weight there likely were smaller changes in dry weight and therefore little variation in seed moisture content but immediately after this moment, moisture content SD increased to maximum in response to variation in timing and rate of the rapid loss of water in the seed (Figs 1 and 2). So, the highest variation in seed moisture content was after PM and before HM (Figs 5 and 6) and we attribute this to variation in rapid seed water loss during maturation drying. This conclusion is consistent in all pod classes and over the cultivars and seasons. Seeds usually lose water rapidly after PM because the seed is no longer attached to the vascular system of the plant and no longer receives water to replace that lost to the environment (Egli, 1998). Moisture content

SD was still declining at HM and only stabilized thereafter in seeds from all pod classes in Season 2 (Figs 5 and 6). Our results imply that even when common bean seeds are harvested at 20% moisture content, seeds are still variable due to the high moisture content SD and could still undergo further deterioration unless seeds are dried further immediately after harvest to reduce the moisture content to a low level in all seeds.

Electrical conductivity SD decreased over time in seeds from all pod classes in both cultivars and seasons, and already became very low before PM (Figs 3-6). Seeds harvested during early seed filling were immature and had a high electrical conductivity value per gram of seed (cf. Siddique and Goodwin, 1983). The decline in electrical conductivity SD during seed filling may have resulted from the decrease in absolute level of electrical conductivity (EC) and from the phenomenon that seeds gradually all achieved very low and stable EC levels when approaching PM, thus reducing seed-to-seed variation. These observations on EC SD are consistent with reports that maximum seed vigour (minimum electrical conductivity) is achieved around PM (Harrington, 1972; TeKrony and Egli, 1997; Muasya et al., 2001b). Although the period after PM is followed by changes within the seeds due to seed moisture variation, electrical conductivity SD remained constant after PM (Figs 3-6). This is logical because EC in bulk seed samples also remained constant after PM (Muasya et al., 2001b).

Variation in seed development among pods differing in pod earliness

Seeds from earlier pods increased earlier in fresh weight and dry weight SD than seeds from later pods (Figs 1 and 2). The onset of seed filling in seeds from earlier pods is usually earlier than in seeds from later pods and, therefore, variation in dry weight could correspond to the moment when seeds begin to increase in weight in response to dry matter and moisture accumulation. Seeds from earlier pods tended to show lower maximum fresh weight and dry weight SD than seeds from later pods (Figs 1 and 2). Because earlier pods may be more uniform in dry matter accumulation than later pods, fresh and dry weight variation could be lower in seeds from earlier pods than in seeds from later pods. The method of tagging (see section Materials and methods) most likely induced the smallest variation in time of reaching 12 cm pod length in the fraction of the medium pods.

The initial increase in moisture content SD was at comparable moments in seeds from medium and late pods in Season 1 but in Season 2 the increase was earlier in seeds from early pods than in seeds from late pods (Figs 1 and 2). The results that moisture content SD was lower at PM than during seed filling or after PM was consistent over all pod levels in both cultivars and seasons (Figs 3 and 4). The timing of this moment was different for different pod levels. Seeds from earlier pods were earlier than seeds from later pods (Figs 1 and 2) which could be due to differences in seed development within the crop. In Season 1 the increase in moisture content SD after PM was comparable in seeds from medium and late pods (Fig. 3). In Season 2 seeds from early pods showed earlier increase in moisture content SD than seeds from late pods in cv. Rosecoco whereas in cv. Mwezi Moja the increase was comparable in seeds from all pod classes (Fig. 4).

Differences occur in the course of decline in moisture content between PM and HM (Muasya et al., 2001a) and although these differences were not related to pod earliness, our results show that the increase of variation in moisture content after PM was related to pod earliness. In cv. Mwezi Moja in Season 1 and in both cultivars in Season 2 moisture content SD after PM was higher in seeds from late pods than in seeds from early or medium pods (Figs 3-6). Also the maximum moisture content SD was achieved earlier in seeds from early pods than in seeds from late pods in Season 2 (Figs 3-6). The late pods are made up of the majority of the pods in the crop (Table 1). Some of the seeds from late pods are fully developed at PM while others are still increasing in dry weight. Consequently, variation in moisture content within the seed lot increases longer and to a higher level in seeds from late pods than in seeds from early pods.

Minimum electrical conductivity SD seemed to be reached later and showed lower values in seeds from earlier pods than in seeds from later pods (Figs 1 and 2). We attribute these findings to the fact that more late than earlier seeds may still have been immature and thus may still have a higher EC value, thus contributing to variation at the moment they were harvested (Siddique and Goodwin, 1983). The largest contrast in minimum EC SD, however, was found between early pods and crop pods (see below).

Seed variation at whole crops and pod levels

Seeds from whole crops are made up of seeds from individual pods of different earliness and therefore the variation of the whole crops could be explained by the variation of pods of different earliness within the crop. The fact that the bulk of pods at pod level were later pods (Table 1) explains the observations that seeds from the whole crop were usually comparable to seeds from later pods or had an intermediate behaviour. In general, seeds from "crop" pods were comparable to seeds from late pods in dry weight SD, moisture content SD and electrical conductivity SD in both cultivars and seasons (Figs 1-6). The whole crop is made up of seeds at different stages of development (Muasya et al., 2001a). The combined variation of seeds from pods differing in earliness makes up the crop. Seeds from late pods which form the majority of the crop, develop later, are usually lighter, and are harvested at higher moisture content than seeds from early pods (Muasya et al., 2001a). When these later seeds are combined with earlier seeds, the seed lot becomes more variable than when seeds from pods of different earliness are considered separately. We therefore conclude that the EC of seeds from the whole crop will differ from the EC of seeds when pods differing in earliness are considered separately due to high variability in the seeds from the whole crop.

Conclusions

- Seed variation in fresh weight, dry weight and moisture content initially increased during seed filling.
- At the moment of maximum dry weight (PM) seed variation in fresh weight and moisture content was lower than earlier during seed filling.
- Regardless of cultivar and season, seed fresh weight variation and moisture content variation were highest immediately after PM and before HM.
- Seed dry weight variation increased during seed filling but stabilized after PM.
- Electrical conductivity variation was minimum at PM and remained more or less constant thereafter.
- The peak in variation in seed fresh weight and moisture content was earlier and lower in seeds from early pods than in seeds from medium, late and "crop" pods.
- Seed variation in weight, moisture content and electrical conductivity was higher in seeds from whole crop than in seeds from pods differing in earliness.

Chapter 5

Variation parameters describing the distribution patterns of individual seed electrical conductivity in common bean (*Phaseolus vulgaris* L.) seed lots*

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* Muasya, R.M., W.J.M. Lommen, E.O. Auma & P.C. Struik, 2001. Variation parameters describing the distribution patterns of individual seed electrical conductivity in common bean (*Phaseolus vulgaris* L.) seed lots. Submitted to Seed Science and Technology.

5. Variation parameters describing the distribution patterns of individual seed electrical conductivity in common bean (*Phaseolus vulgaris* L.) seed lots

Abstract

Twenty-four different seed lots of common bean (*Phaseolus vulgaris* L.) cultivars Rosecoco and Mwezi Moja were produced under different conditions to gain knowledge on the kind of distributions found in individual seed electrical conductivity (EC, $\mu S\ cm^{-1}\ g^{-1}$). Several variation parameters that quantify variation in the distribution patterns were assessed. Distributions were usually skewed to higher values. Variation quantified by mean - median, range 0 - 100%, variance and standard deviation was high when there were extremely high values deviating from the bulk of the seeds. In cv. Rosecoco, seed lots with these outliers did not necessarily have large variation in the bulk of the seeds. The latter was estimated by the ranges 0 - 75% and 25 - 75%. Commonly used parameters to quantify variation like coefficient of variation, skewness and kurtosis were not suitable to quantify variation in seed lots from different origin, because they render lower values when more seeds with very high values occur in seed lots. Ln transformation of the EC data before calculation of the variation parameters reduced skewness of the distribution pattern, but did not give rise to more suitable parameters describing variation in EC. Different parameters quantified variation in individual seed EC within a seed lot very differently.

Introduction

Seed lots are made up of individual seeds which together determine the quality of the whole seed lot. Individual seed quality measurements in a seed lot are useful for several reasons. For example Steere et al. (1981) used individual seed measurements to determine the distribution of seed leakage current within a soybean seed lot. In another example Levengood et al. (1975) used individual seeds to demonstrate a basic association between high current levels and poor seed quality. We are interested in variation in seed quality in order to link homogeneity with quality of the seed lot.

To be able to quantify variation in seed lots, a parameter is needed that describes that variation properly. Different variation parameters describe different attributes of a distribution pattern. For example standard deviation and coefficient of variation are commonly used parameters to quantify variation (Steel and Torrie, 1980). Hacisalihoglu et al. (1999) used standard deviation to quantify germination uniformity in lettuce. Illipronti Jr et al. (2000a) used coefficient of variation to quantify uniformity in seedling performance in different soybean seed lots. Range of values is usually also regarded as a measure of variation. However, some ranges are based on extreme values which may be eccentric and also depend on the size of the sample (Mead et al. 1993). Tomas et al. (1992) used ranges to measure the variation in time for 10 - 90% (T90 - 10) of the lettuce radicles to emerge and Jalink et al. (1998) used the range 25 - 75% (T75 - 25) to measure uniformity of germination in cabbage. Skewness is a measure of symmetry in distributions, kurtosis is considered to be a measure of bimodality in symmetric distributions (Wyszomirski, 1992). Wyszomirski (1992) used skewness and kurtosis to detect and display size bimodality of evenly aged plant populations. Huhn (1993) used skewness and kurtosis to evaluate variability of harvest index and grain/straw – ratio in winter oilseed rape. For non-normal distributions, logarithmic transformations are usually used to make distributions less asymmetric and reduce variance (Wyszomirski, 1992).

This research aims at gaining knowledge into the kind of distributions found in individual seed electrical conductivity in different seed lots of common bean crops and select variation parameters that quantify variation in the distribution of the individual seed electrical conductivity in different seed lots.

Materials and methods

Experimental site and set-up

Twenty four different seed lots of two cultivars of common bean (*Phaseolus vulgaris* L.) were produced using three sowing dates in each of two seasons at two locations (Kitui and Eldoret) in Kenya. In each site and season the experimental design was a split plot with four blocks. The two cultivars, Rosecoco and Mwezi Moja, were assigned to main plots. Both are determinate cultivars, but Rosecoco shows prolonged flowering while the flowering period of Mwezi Moja is short. The three sowing dates were assigned to subplots.

At planting, each plot of 16 m^2 was fertilized with 0.49 kg calcium ammonium nitrate (26% N), 0.76 kg triple super phosphate (48% P_2O_5) and 0.13 kg muriate of potash (60% K_2O), achieving 80 kg N, 100 kg P and 20 kg K per hectare. The gross plot size was 4×4 m^2 while the net experimental area was 3.40×2.50 m^2. Two seeds per hill were planted at a spacing of 0.5×0.1 m^2. At full emergence the seedlings were thinned out leaving a single seedling per hill. At harvest maturity, defined as when pods had changed colour from green yellow to straw yellow, seeds were harvested from an area consisting of 40 plants and individual seed electrical conductivity determined.

Measurements

After harvesting and shelling the pods, 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 a moisture content of 14% was achieved, then stored at 2 °C and 75% relative humidity for an average of three months.

Electrical conductivity test

Individual seed electrical conductivity was determined after equilibrating the seeds from cold storage for 3 days at room temperature (19 ÷ 25 °C). The moisture content was then constant at 12%. To establish individual seed electrical conductivity four samples of 20 seeds each were taken and each seed was weighed and individually incubated in 50 ml of distilled water at 20 °C for 24 hours. A control sample of equivalent amount of water was included with the samples. Conductivity ($\mu S\ cm^{-1}$) was measured using a Fieldlab – LF conductivity meter and LF 513T electrode dip – type cell (Schott Gerate Glass Company, Mainz, Germany). The conductivity per gram of seed weight ($\mu S\ cm^{-1}\ g^{-1}$) at 12% moisture content in 50 ml of water was then calculated (ISTA, 1995).

Calculation of variation parameters

To characterize variation within cultivars, variation parameters shown in Table 1 were calculated from the combined data of four samples of 20 individual seed electrical conductivity (EC) data. Variation parameters were calculated both from the original individual seed EC and from ln transformed individual seed EC data using Genstat 5 (Release 4.1). Variation in these parameters over seed lots was calculated per cultivar as coefficient of variation (%). To establish correlations between different parameters, correlation coefficients were calculated per cultivar.

Results

Variation of parameters over seed lots

All parameters calculated showed variation over seed lots in both cultivars (Table 2). Seed lots in both cultivars showed higher variation in parameters mean - median, 0 - 100%, variance, SD, skewness and kurtosis than in parameters 25 - 75% and 0 - 75% (Table 2). In the parameters calculated from the ln transformed data, variation over the seed lots was higher in mean - median, variance, skewness and kurtosis than in 0 - 100%, 25 - 75%, 0 - 75%, and CV% in both cultivars (Table 2).

Ln transformation reduced the variation over seed lots in range 0 - 100, variance, SD and CV%, but increased the variation in skewness and kurtosis (Table 2). Nevertheless, variation was still present in all parameters derived from ln transformed EC values (Table 2).

Table 1. Variation parameters, how they are derived and how they measure variation within a population as described by Snedecor and Cochran (1967), Mead et al. (1993) and Steel and Torrie (1980).

Variation parameter	**Units**	**Formula**	**Description**
Mean - median	$\mu S\ cm^{-1}g^{-1}$	μ - median, where μ is the mean $(\Sigma X_i)/n$ where n is the number of seeds	This is the difference between the average value and the position of 50% of the values in the population.
Range 0 - 100%	$\mu S\ cm^{-1}g^{-1}$	Maximum - minimum	This parameter tells how wide is the difference between the maximum and minimum values recorded.
Range 25 - 75%	$\mu S\ cm^{-1}g^{-1}$	Upper quartile - lower quartile	This parameter tells the difference between 25 and 75% of the population thus excluding the extreme parts of the population.
Range 0 - 75%	$\mu S\ cm^{-1}g^{-1}$	Upper quartile - minimum	This parameter tells the difference between the minimum value and 75% of the observations in a population thus excluding the highest extremes of the population.
Variance	$[\mu S\ cm^{-1}g^{-1}]^2$	$\sigma^2 = [\Sigma(X_i - \mu)^2]/n$	This is a measure of average squared deviations from the mean.
Standard deviation (SD)	$\mu S\ cm^{-1}g^{-1}$	$\sigma = \sqrt{\sigma^2}$	This is the square root of the variance. This parameter measures the spread of the individual measurements from the mean in the same units as the observations.
Coefficient of variation (CV%)	%	$(\sigma/\mu)\times100$	This parameter measures variation as a ratio of standard deviation divided by the mean expressed in percentage. It takes a constant value when the standard deviation is directly proportional to the mean.
Skewness		$[\Sigma(X_i - \mu)^3/n]/\sigma^3$	This parameter describes the tendency of a distribution to have a pronounced tail to the right (positive) or left (negative). Skewness is 0 in normal distributions.
Kurtosis		$[\Sigma(X_i - \mu)^4/n]/\sigma^4$	This parameter characterizes the relative peakedness or flatness of a distribution compared with the normal distribution. For normal distributions, kurtosis is 3. If kurtosis exceeds 3 there is usually excess of values near the mean and far from it resulting in depletion of flanks of the distribution curve, a high peak and/or wide tails. Kurtosis less than 3 results from curves that have flatter top than the normal and/or less wide tails.

Table 2. Variation in parameters describing variation of individual seed electrical conductivity measurements ($\mu S\ cm^{-1}\ g^{-1}$) and ln transformed individual seed electrical conductivity measurements over seed lots from cvs Rosecoco and Mwezi Moja (n=12 for each cultivar).

	Parameters describing variation																	
	Untransformed measurements									Ln transformed measurements								
	Mean - median	0 - 100	25 - 75	0 - 75	Variance	SD	CV%	Skewness	Kurtosis	Mean - median (ln)	0 - 100 (ln)	25 - 75 (ln)	0 - 75 (ln)	Variance (ln)	SD (ln)	CV% (ln)	Skewness (ln)	Kurtosis (ln)
Rosecoco																		
Minimum	1.97	92.66	22.22	38.98	405	20.12	31.36	0.58	-0.18	-0.02	1.37	0.31	0.57	0.10	0.32	7.54	-0.38	-0.63
Maximum	76.93	3151	34.43	71.50	151919	390	255	8.67	73.77	0.12	4.71	0.70	1.69	0.51	0.72	15.79	5.56	38.62
CV%	**115.6**	**125.9**	**15.6**	**16.8**	**207.9**	**117.1**	**79.2**	**64.4**	**93.3**	**115.2**	**33.1**	**25.8**	**30.5**	**56.4**	**27.5**	**27.7**	**128.8**	**153.5**
Mwezi Moja																		
Minimum	-0.32	121	13.99	31.95	431	20.75	24.48	0.93	2.02	-0.02	1.32	0.21	0.57	0.05	0.23	5.22	-0.49	-0.21
Maximum	119	1679	94.33	135	90606	301	144	8.28	69.32	0.26	3.81	0.78	1.64	0.64	0.80	16.54	3.76	18.01
CV%	**118.6**	**66.5**	**62.5**	**42.8**	**144.8**	**73.6**	**36.8**	**53.3**	**91.9**	**153.0**	**24.4**	**47.1**	**33.8**	**57.2**	**29.9**	**28.6**	**76.3**	**107.6**

Range 0 - 100 = maximum - minimum, 25 - 75 = Upper - lower quartile, 0 - 75 = Upper quartile - minimum, SD = standard deviation, CV% = coefficient of variation.

Relationships between variation parameters and individual seed EC distribution patterns

Individual seed EC within seed lots from different sites, seasons, sowing dates and cultivars was usually not normally distributed. Five typical examples of individual seed EC frequency distributions presented in Fig. 1 show that seed lots usually showed a tail extending towards high values.

In some seed lots, seeds with extremely high values occurred, for example D and E (Fig. 1). This resulted in high values for the range 0 - 100%. The high values for outliers also had a large impact on the value of SD, variance and mean - median. Sometimes the bulk of the seeds showed a relatively small variation e.g. seed lot D as compared to B and C but because of outliers variation as described by mean - median, the range 0 - 100%, variance and SD was rated much higher (Fig. 1). Consistently in both cultivars, mean - median, the range 0 - 100%, variance and SD indeed were mutually correlated (Tables 3 and 4).

Seed lots with small variation in the bulk of the seeds e.g. A and D showed lower values for ranges 25 - 75% than those seed lots having wide bases (Fig. 1). The ranges 25 - 75% and 0 - 75% were not consistently correlated over cultivars to the other variation parameters or mutually (Tables 3 and 4).

In seed lots showing high values for outliers CV% usually was also high, e.g. seed lots D and E compared to A and C (Fig. 1). However, in some of the seed lots many high values increased the mean as well as SD, e.g. seed lot E compared to seed lot D (Fig. 1). This resulted in a lower CV% than expected when the outliers were many (Fig. 1). CV% was not correlated to 25 - 75% or 0 - 75% in both cultivars. CV% was not systematically correlated over cultivars to mean - median and variance, but was systematically correlated to 0 - 100%, SD, skewness and kurtosis (Tables 3 and 4).

Skewness usually increased in seed lots in which the asymmetric tail extended towards high values, e.g. B compared to A or C compared to A (Fig. 1). However, in seed lots showing many outliers, e.g. seed lot E, relatively low skewness values were observed (Fig. 1). Over cultivars, skewness was only systematically correlated to CV% and kurtosis but not to the other variation parameters calculated (Tables 3 and 4).

Kurtosis was always high in seed lots showing high values for outliers, e.g. B, D and E compared to C and in seed lots with sharp peaks, e.g. A and D compared to C and E (Fig. 1). Kurtosis was not correlated to mean - median, 25 - 75%, 0 - 75% and variance but was correlated to CV% and skewness in both cultivars (Tables 3 and 4).

Ln transformation of individual seed EC resulted in lower coefficients of variation and lower skewness values in all seed lots (Fig. 1). Seed lots with small variation, e.g. seed lot A, had their distribution patterns shifted to the left (Fig. 1). Seed lots with high values for outliers still showed outliers after ln transformation, e.g. Fig. 1B, D and E.

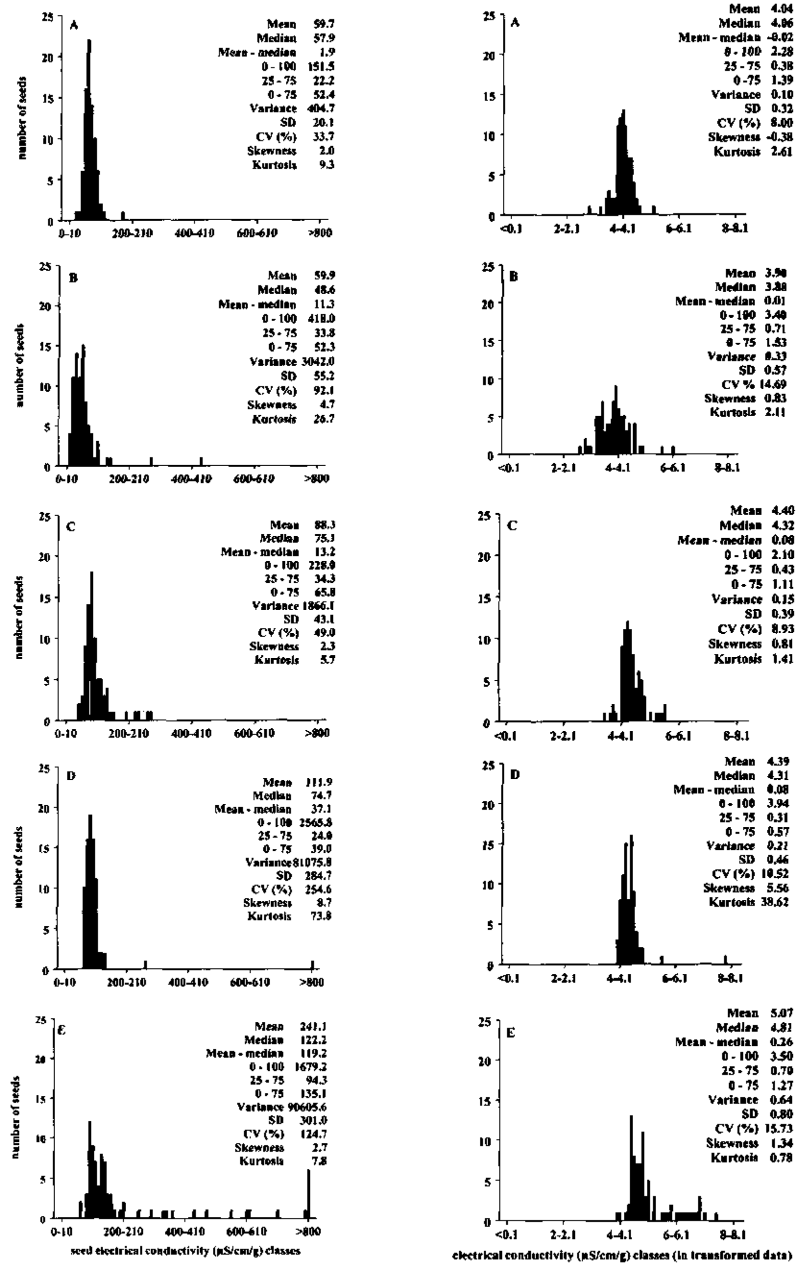

Fig. 1. Individual seed electrical conductivity (µS cm^{-1} g^{-1}) distribution patterns of five seed lots (A - E) for untransformed (left side) and ln transformed (right side) data.

Table 3. Correlation coefficients between different parameters describing variation in individual seed electrical conductivity values (µS cm^{-1} g^{-1}) and ln transformed electrical conductivity values in different seed lots (n = 12) from cv. Rosecoco.

	Mean-median	0 - 100	25 - 75	0 - 75	Variance	SD	CV%	Skewness	Kurtosis	Mean-median (ln)	0 -100 (ln)	25 - 75 (ln)	0 - 75 (ln)	Variance (ln)	SD (ln)	CV% (ln)	Skewness (ln)
0 - 100	0.938***[1]																
25 - 75	0.218NS	-0.035NS															
0 - 75	0.219NS	-0.048NS	0.346NS														
Variance	0.974***	0.961***	0.084NS	0.133NS													
SD	0.964***	0.996***	0.030NS	0.016NS	0.978***												
CV%	0.871***	0.970***	-0.053NS	-0.177NS	0.880***	0.956***											
Skewness	0.570NS	0.743**	-0.293NS	-0.270NS	0.551NS	0.699*	0.832***										
Kurtosis	0.540NS	0.745**	-0.349NS	-0.379NS	0.545NS	0.695*	0.831***	0.989***									
Mean-median (ln)	0.771**	0.661*	0.451NS	0.198NS	0.672*	0.695*	0.632*	0.361NS	0.322NS								
0 – 100 (ln)	0.748**	0.784**	-0.079NS	0.100NS	0.702*	0.776**	0.843***	0.832***	0.768**	0.439NS							
25 – 75 (ln)	-0.181NS	-0.331NS	0.616*	0.029NS	-0.273NS	-0.296NS	-0.228NS	-0.298NS	-0.354NS	-0.151NS	-0.008NS						
0 – 75 (ln)	-0.201NS	-0.375NS	0.089NS	0.589*	-0.267NS	-0.340NS	-0.357NS	-0.312NS	-0.418NS	-0.323NS	0.136NS	0.534NS					
Variance (ln)	0.770**	0.620*	0.416NS	0.354NS	0.667*	0.661*	0.639*	0.424NS	0.337NS	0.554NS	0.810***	0.385NS	0.347NS				
SD (ln)	0.744**	0.608*	0.395NS	0.319NS	0.631*	0.644*	0.649*	0.469NS	0.378NS	0.563NS	0.834***	0.390NS	0.346NS	0.994***			
CV (ln)	0.603*	0.472NS	0.391NS	0.263NS	0.486NS	0.505NS	0.545NS	0.401NS	0.307NS	0.437NS	0.786**	0.535NS	0.459NS	0.965***	0.979***		
Skewness (ln)	0.630*	0.814***	-0.149NS	-0.432NS	0.635*	0.775**	0.887***	0.881***	0.909***	0.584*	0.646*	-0.373NS	-0.627*	0.311NS	0.349NS	0.252NS	
Kurtosis (ln)	0.445NS	0.706**	-0.409NS	-0.548NS	0.509NS	0.648*	0.781**	0.812**	0.865***	0.377NS	0.521NS	-0.506NS	-0.634*	0.077NS	0.117NS	0.039NS	0.940***

[1] NS, *, ** and *** indicate statistically non-significant ($P \geq 0.05$), significant at $0.01 \leq P < 0.05$, $0.001 \leq P < 0.01$ and $P < 0.001$, respectively.

Range 0 - 100 = maximum - minimum, 25 - 75 = Upper - lower quartile, 0 - 75 = Upper quartile - minimum, SD = standard deviation, CV% = coefficient of variation.

Table 4. Correlation coefficients between different parameters describing variation in individual seed electrical conductivity values (µS $cm^{-1}g^{-1}$) and ln transformed electrical conductivity values in different seed lots (n = 12) from cv. Mwezi Moja.

	Mean-median	0 - 100	25 - 75	0 - 75	Variance	SD	CV%	Skewness	Kurtosis	Mean-median (ln)	0 - 100 (ln)	25 - 75 (ln)	0 - 75 (ln)	Variance (ln)	SD (ln)	CV% (ln)	Skewness (ln)
0 - 100	0.747**[1]																
25 - 75	0.911***	0.599*															
0 - 75	0.928***	0.701*	0.967***														
Variance	0.966***	0.819***	0.875***	0.926***													
SD	0.945***	0.899***	0.807**	0.882***	0.956***												
CV%	0.503NS	0.815**	0.398NS	0.429NS	0.502NS	0.681*											
Skewness	-0.160NS	0.462NS	-0.297NS	-0.218NS	-0.101NS	0.101NS	0.669*										
Kurtosis	-0.235NS	0.413NS	-0.307NS	-0.227NS	-0.152NS	0.023NS	0.578*	0.968***									
Mean-median (ln)	0.873***	0.677*	0.705*	0.703*	0.765**	0.819***	0.600*	0.046NS	-0.074NS								
0 -100 (ln)	0.327NS	0.659*	0.370NS	0.338NS	0.339NS	0.462NS	0.887***	0.642*	0.592*	0.379NS							
25 - 75 (ln)	0.332NS	0.096NS	0.649*	0.482NS	0.274NS	0.192NS	0.224NS	-0.267NS	-0.221NS	0.189NS	0.497NS						
0 - 75 (ln)	0.046NS	-0.038NS	0.412NS	0.272NS	0.036NS	-0.040NS	0.140NS	-0.157NS	-0.075NS	-0.102NS	0.494NS	0.931***					
Variance (ln)	0.826***	0.553NS	0.927***	0.839***	0.756**	0.726**	0.534NS	-0.183NS	-0.242NS	0.704*	0.575NS	0.777**	0.556NS				
SD (ln)	0.761**	0.527NS	0.868***	0.770**	0.680*	0.681*	0.588*	-0.111NS	-0.178NS	0.660*	0.648*	0.796**	0.597*	0.984***			
CV (ln)	0.486NS	0.294NS	0.682*	0.528NS	0.399NS	0.393NS	0.499NS	-0.085NS	-0.130NS	0.422NS	0.685*	0.912***	0.791**	0.884***	0.934***		
Skewness (ln)	0.082NS	0.477NS	-0.268NS	-0.133NS	0.117NS	0.324NS	0.560NS	0.707**	0.560NS	0.248NS	0.279NS	-0.633*	-0.652*	-0.213NS	-0.165NS	-0.302NS	
Kurtosis (ln)	-0.235NS	0.341NS	-0.474NS	-0.306NS	-0.118NS	0.070NS	0.400NS	0.793**	0.746**	-0.163NS	0.219NS	-0.667*	-0.538NS	-0.473NS	-0.422NS	-0.482NS	0.870***

[1] NS, *, ** and *** indicate statistically non-significant ($P \geq 0.05$), significant at $0.01 \leq P < 0.05$, $0.001 \leq P < 0.01$ and $P < 0.001$, respectively. Range 0 - 100 = maximum - minimum, 25 - 75 = Upper - lower quartile, 0 - 75 = Upper quartile - minimum, SD = standard deviation, CV% = coefficient of variation.

Variation calculated before transformation in both cultivars was correlated to variation calculated after transformation for the parameters mean - median, the range 0 - 100%, variance and SD (Tables 3 and 4). Mean - median of the ln transformed data was not significantly correlated to 0 - 100% of the ln transformed data (Tables 3 and 4).

Variation as measured by ranges before transformation was in both cultivars correlated to variation as measured by ranges after transformation (Tables 3 and 4). However, transformation was more efficient in reducing differences between high EC values than between low EC values. Consequently, seed lots with low ranges in EC values before transformation in which individual seeds had low EC values, after transformation could show wider ranges than seed lots having initially wider ranges but with individual seeds having higher EC values, e.g. ranges 0 - 100% or 0 - 75% of seed lot A compared to seed lot C (Fig. 1).

Seed lots showing high values for skewness and kurtosis in the untransformed data still showed higher values after ln transformation compared to other seed lots (Fig. 1, Tables 3 and 4), but CV%s calculated before and after transformation were not correlated in both cultivars. Skewness and kurtosis of the ln transformed data were mutually correlated in both cultivars (Tables 3 and 4).

Discussion

Suitability of different variation parameters to describe individual seed EC distribution patterns

Mean - median, the range 0 - 100%, variance and SD were mutually correlated in both cultivars (Tables 3 and 4), partly because all these parameters were sensitive to outliers. Variation quantified by these parameters was high when there were extremely high values deviating from the bulk of the seeds (Fig. 1). High values for outliers increased the range 0 - 100% directly, but also shifted the position of the mean towards high values away from the bulk of the seeds. This increased the difference mean - median in distributions skewed to the right, which were most common (Fig. 1). Variance and SD both are calculated from deviations of individual values from the mean. These deviations will be larger when the mean increases, whereas also the extremely high value(s) strongly contribute. Comparing the four parameters mutually, variation quantified using 0 - 100 was high even when a seed lot had only one seed with a high value, e.g. seed lot D (Fig. 1). This makes the range 0 - 100% less suitable for describing the overall variation within a seed lot. Variance and SD take all values into account, but also these parameters are extremely sensitive to one extreme value. While variance measures spread in the units of squared observations, SD measures spread in the same units as the mean (Mead et al., 1993). This makes SD a more useful parameter to describe variation within a seed lot than variance. The difference mean - median seemed especially appropriate to describe variation when many seeds showed much higher values than the bulk of the seeds.

The ranges 25 - 75% and 0 - 75% were not in both cultivars correlated to the parameters mean - median, 0 - 100, variance and SD (Tables 3 and 4). The ranges 25 - 75% and 0 - 75% quantified variation in the bulk of the seeds, excluding extremes, and thus were not sensitive to a limited number of outliers. The lack of significant correlations between the two groups of parameters in cv. Rosecoco implies that seed lots with outliers in this cultivar not necessarily also had a large variation in the bulk of the seeds. While variation established by ranges 25 - 75% and 0 - 75% could be low, the variation in the same seed lot as established by mean - median, 0 - 100%, variance and SD could be high because of few deviating seeds with high EC values. The range 0 – 75% is likely a more useful variation parameter than the range 25 - 75%, because it comprises more seeds in its evaluation and because seeds with extreme values were found in the 75 - 100% range and not in the 0 - 25% range. Surprisingly, 25 - 75% and 0 - 75% were not consistently correlated to each other over cultivars, suggesting the distribution of seeds in the high quality ranges was not affected in the same way by weather conditions in both cultivars.

CV%, skewness and kurtosis were mutually correlated in both cultivars. CV% is the ratio of SD and mean expressed as percentage, and is constant if SD is directly proportional to the mean (e.g. Mead et al., 1993). As a ratio, CV% is usually regarded as a stable measure of variation (Steel and Torrie, 1980). It was expected that seed lots with many high values, e.g. seed lot E which was regarded to be the most variable seed lot, would also show high CV%. This was found sometimes, e.g. E compared to A. However, our results show that many high values increased the mean as well as SD. This resulted in a lower CV% in seed lots with many outliers than in seed lots with only one outlier, e.g. seed lot E compared to D (Fig. 1). CV% therefore was regarded to be not a good parameter for quantifying individual seed variation in EC in different seed lots. Skewness increased as the tail extended towards high values, e.g. when seed lot A was compared to C or B (Fig. 1). Like CV%, skewness also was relatively low in seed lots with many high values, e.g. E (Fig. 1). Consequently skewness was also not regarded a good parameter describing variation. Kurtosis was high in seed lots showing low values for EC in the bulk of seeds, e.g. seed lot B and D compared to C and E but, comparably to CV% and skewness, increased when the number of outliers increased (Fig. 1). Although useful as a measure of bimodality in symmetric distributions around its mean in non-normal situations (Snedecor and Cochran, 1967; Wyszomirski, 1992; Singh et al., 1999), kurtosis also was regarded not to be a suitable parameter for quantifying individual seed variation in EC in seed lots from different origin.

In log-normal distributions, ln transformations are used to stabilize the variance (Snedecor and Cochran, 1967) and produce distributions which approach normality (Robbins and Sjulin, 1986). Our study showed that ln transformation indeed reduced skewness of individual seed EC distribution patterns (Fig. 1). As a result variance over the seed lots was more homogeneous (Table 2), though seeds usually were not normally distributed as shown by kurtosis values of less than 3, e.g. seed lot A, B, C and E, or kurtosis values higher than 3, e.g. seed lot D (Fig. 1). The gain achieved by transforming the data, however, was questionable and did not outweigh the disadvantages. Except for CV%, parameters derived from transformed and untransformed data were correlated (Tables 3 and 4). Moreover, high quality seed lots were at clear disadvantage after ln transformation of the data compared to seed lots in which the quality of the individual seeds was poorer (having higher EC). With the same range in EC values in untransformed data, a seed lot with high quality seeds after transformation will show a higher range than a seed lot with poor quality seeds. This for instance showed when seed lots A and C were compared: After transformation, seed lot A showed wider ranges than seed lot C. We therefore conclude that ln transformation of data will not give rise to more suitable parameters describing individual seed variation in EC than using untransformed data.

Implications

The results imply that different parameters quantify variation in individual seed EC within a seed lot differently. Parameters taking into account outliers may classify variation in a seed lot to be high, whereas parameters taking into account the bulk of the seeds may classify variation in the same seed lot to be low. Generally, parameters commonly used in other research, like CV%, skewness, and kurtosis, were not suitable to quantify variation in EC in seed lots from different origin. This does not rule out that these parameters could be useful describing variation resulting from other sources, e.g. the development of variation in crops with time. Whether variation as quantified by different parameters is indeed related to bulk quality of the seeds will be the topic of a future paper (Muasya et al., 2001e).

Conclusions

- Electrical conductivity (μS cm^{-1} g^{-1}) of individual seeds in seed lots usually was not normally distributed, but showed a skewness towards high values.
- The parameters range 0 - 100%, difference mean - median, variance and SD, characterizing seed to seed variation in EC, were mutually correlated and all were

high when there were one of more seeds with very high values (outliers).

- The ranges 0 - 75% and 25 - 75% characterized individual seed variation in EC in the bulk of the seeds.
- Different variation parameters quantified variation in individual seed EC within a seed lot differently.
- While individual seed variation in EC established by ranges 25 - 75% and 0 - 75% could be relatively low, the variation in the same seed lot of cv. Rosecoco as established by mean - median, 0 - 100%, variance or SD could be high due to few deviating seeds with high EC values.
- CV% was not a good parameter for quantifying individual seed variation in EC within different seed lots, because as a ratio of SD and mean it was lower in seed lots with many outliers than in seed lots with only one outlier.
- Skewness and kurtosis were found not to be good parameters for quantifying individual seed variation in EC, because seed lots having many outliers could be classified as having a lower skewness/kurtosis than seed lots having only one outlier.
- Ln transformation of EC data did not give rise to more suitable parameters describing individual seed variation in EC than using untransformed data.

Chapter 6

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

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[*] Muasya, R.M., W.J.M. Lommen, E.O. Auma & P.C. Struik, 2001. Relationship between individual seed quality variation and bulk seed quality in common bean (*Phaseolus vulgaris* L.) seed lots. Submitted to Seed Science and Technology.

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

Abstract

Twenty-four different seed lots of common bean (*Phaseolus vulgaris* L.) cultivars Rosecoco and Mwezi Moja contrasting in uniformity of flowering were produced at two locations in different periods to test whether a large variation between individual seeds at harvest in lots from different production conditions is also associated with a poor quality of the entire seed lot. Variation between individual seed electrical conductivity (EC) ($\mu S\ cm^{-1}\ g^{-1}$) was quantified using the parameters mean - median, standard deviation (SD), coefficient of variation (CV%) and the range 0 - 75%. Bulk seed lot quality was determined using EC and percentage viable seeds. Over all 24 seed lots, low variation in individual seed EC as quantified by the parameters mean - median, SD and the range 0 - 75%, was found to be associated with good quality as measured by low bulk EC and high percentage viable seeds at physiological maturity (PM). At harvest maturity (HM) most seed lots showed bulk EC values comparable to each other and a smaller variation obtained over seed lots. Consequently associations found were clearer at PM than at HM. No relationship was found between variation in individual seed EC measured by CV% and bulk quality over seed lots. Adding site to the regression model significantly improved the associations both at PM and HM indicating that the relationships between bulk quality and variation was different at the two sites. Both at PM and HM the association between bulk quality and individual seed quality variation did not improve significantly when cultivars were considered in the model separately without sites. This implies that there was no difference in the relationship between bulk quality and individual seed variation between the two cultivars.

Introduction

Seed development within a crop is not uniform. Longer periods of flowering or seed formation may lead to production of seeds varying in age within the plants and within the crop (Gavras, 1989; Padrit et al., 1996). This could lead to differences in the moments when individual seeds achieve physiological maturity (PM). In common bean, seeds from earlier pods achieve PM earlier than seeds from later pods whereas they have a longer time period between PM and harvest when the whole crop is harvested at crop harvest maturity (HM) (Muasya et al., 2001a). In common bean, seeds from earlier pods tended to achieve maximum seed quality earlier than those from later pods within a crop (Muasya et al., 2001b). Seeds from pods differing in age also undergo different courses of moisture content decline between PM and harvest: seeds from earlier pods take longer time for moisture content to decline between PM and harvest (Muasya et al., 2001a) and may be exposed longer to deteriorating conditions during maturation drying. In soybean, longer exposure of early pods to deteriorating conditions was thought to explain the lower viability in seeds from earlier than later pods (Illipronti Jr et al., 2000b). Consequently, differences in development of seeds within a crop could lead to a seed lot in which individual seeds differ in age and moisture content.

The quality of a seed lot is the resultant of the combination of quality characteristics of individual seeds within the seed lot. Large differences in quality between individual seeds could be accompanied by low bulk quality of the seed lot. For instance, the differences in time of germination of individual seeds within a seed lot increase when the quality of that seed lot decreases due to ageing (e.g. Siddique and Goodwin, 1983; Hosnedl and Horakova, 1998). Also after harvest, individual seed differences in quality within seed lots have been reported in various crops (e.g. Levengood et al., 1975; Steere et al., 1981; Siddique and Goodwin, 1983; Moore et al., 1988). We assume that also over different seed lots a relationship exists between the magnitude of the variation in individual seed performance and the final bulk seed quality. This relationship has never been quantified.

In order to describe individual seed quality variation, parameters are needed that quantify the variation properly. Muasya et al. (2001d) evaluated several parameters by which individual seed variation in electrical conductivity, expressed per gram of seed, could be described. These parameters that can be used to determine whether indeed variation in individual seeds is related to bulk seed quality.

This research aims at testing whether higher variation between individual seeds at physiological and harvest maturity resulting from different set of conditions, is associated with poorer bulk quality of the seed lot.

Materials and methods

Experimental site and set-up

Twenty four seed lots of two cultivars of common bean (*Phaseolus vulgaris* L.) were produced under the same cultural conditions at three sowing dates in each of two seasons at two locations (Kitui and Eldoret) in Kenya. Kitui site was situated in a semi arid lowland area. Rainfall during the growing periods varied from 117 - 845 mm average temperature from 21.4 - 26.1 °C. Eldoret site was situated in the highland areas. Rainfall during the growing period varied from 287 - 546 mm, average temperatures from 13.0 - 14.2 °C. In each site and season the experimental design was a split plot with four blocks. The two cultivars, Rosecoco and Mwezi Moja, were assigned at random to main plots. Both are determinate cultivars, but Rosecoco has prolonged flowering while the flowering period of Mwezi Moja is short. The three sowing dates were assigned to subplots.

At planting, each plot of 16 m^2 was fertilized with 0.49 kg calcium ammonium nitrate (26% N), 0.76 kg triple super phosphate (48% P_2O_5) and 0.13 kg muriate of potash (60% K_2O), achieving 80 kg N, 100 kg P and 20 kg K per hectare. The gross plot size was 4×4 m while the net experimental area was 3.40×2.50 m. Two seeds per hill were planted at a hill spacing of 0.5×0.1 m. At full emergence the seedlings were thinned, leaving a single seedling per hill. Within the net experimental area, two separate areas consisting of 40 plants were marked. At physiological maturity seeds were harvested from one of the areas and individual seed electrical conductivity, bulk electrical conductivity and percentage viable seeds were determined. The same tests were repeated at harvest maturity on seeds harvested from the second area of 40 plants. Seeds at physiological maturity were harvested when pods had changed colour from green to green yellow and seeds had achieved their final red purple colour while at harvest maturity seeds were harvested when pods had changed colour from green yellow to straw yellow.

After harvesting and shelling the pods, 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 a moisture content of 14% was achieved. Then they were stored at 2 °C and 75% relative humidity for an average of three months.

Electrical conductivity tests

Electrical conductivity was determined after equilibrating the seeds from cold storage for 3 days at room temperature (19 - 25 °C). The moisture content was then constant at 12%. To establish bulk electrical conductivity four replicate samples (one from each block) of 50 seeds were weighed and incubated in 250 ml of distilled water at 20 °C for 24 hours. A control sample of equivalent quantity of water was included with the samples. Conductivity ($\mu S\ cm^{-1}$) was measured using a Fieldlab – LF conductivity metre and LF 513T electrode dip – type cell (Schott Gerate Glass Company, Mainz, Germany). The conductivity per gram of seed weight ($\mu S\ cm^{-1}\ g^{-1}$) at 12% moisture content in 250 ml of water was then calculated (ISTA, 1995). To establish individual seed electrical conductivity four samples of 20 seeds each were taken and combined into one sample of 80 seeds. Each seed was weighed and individually incubated in 50 ml of distilled water at 20 °C for 24 hours using the same method as for the establishment of bulk EC. The conductivity per gram of seed weight ($\mu S\ cm^{-1}\ g^{-1}$) at 12% moisture content in 50 ml of water was then calculated (ISTA, 1995).

Tetrazolium test

Four replicate samples of 20 seeds (one from each block) were taken from the cold storage and kept at room temperature (19 - 25 °C) for a day before soaking in water at room temperature for 24 hours. Thereafter, seeds were sliced longitudinally through the middle of the embryonic axis and soaked in a 0.5% tetrazolium (2,3,5-triphenyltetrazolium chloride) solution at 30 °C for 3 hours, briefly washed in distilled water and examined under hand lens magnification (ISTA, 1995). The staining of the embryo was examined per seed. Seeds with sound tissues and weak viable tissues were combined to calculate viability, i.e. the percentage of viable seeds.

Calculations and statistical analysis

The following parameters were used for describing individual seed variation within common bean seed lots:

- Mean - median. This is the difference between the average value and the position of 50% of the values in the population.
- Standard deviation (SD). SD is the square root of the variance calculated as $\sigma = \sqrt{(\sum (X_i - \mu)^2/n)}$, where μ is the mean $(\sum X_i)/n$ and n is the number of seeds.
- Coefficient of variation (CV%). CV% measures variation as a ratio of standard deviation divided by the mean expressed as percentage $CV\% = (\sigma/\mu)\times 100$.
- The range 0 - 75%. This is the difference between the minimum value and 75% of the observations in a population.

To show the association between individual seed variation and bulk seed quality over seed lots and to evaluate whether these associations depended on the site and cultivar, simple and multiple linear regression analysis 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), whereas cultivars and sites were added as factors to the regression model. Percentage variance (adjusted R^2) over all seeds lots and adjusted R^2 after adding cultivar, site, and site and cultivar as a factors to the regression model were calculated using Genstat 5 (Release 4.1). In case adding a factor to the model significantly increased R^2 linear regression was carried out for each level of a factor and the significance of the regression coefficient determined.

Results

At physiological maturity (PM), clear associations existed between bulk seed quality (bulk EC and percentage viable seeds) and individual seed variation in EC quantified by mean - median, SD and the range 0 - 75%, as shown by significant R^2 over all seed lots (Table 1). A higher variation in individual seed EC measured by these parameters was associated with a poorer quality, i.e. a higher EC and lower viability (Fig. 1). Adding site as a factor to the regression model, significantly improved the proportion of variance accounted for by the relationships (Table 1) showing that the relationship between bulk quality and individual seed variation was different at the two sites. There was no significant increase in R^2 after adding cultivar as a factor in the regression model (Table 1) showing there was no difference in the relationship between bulk quality and individual seed variation between the two cultivars. There was no linear association between bulk quality and variation in individual seed EC when the latter was measured as CV% (Table 1).

Table 1. Coefficient of determination (adjusted R^2) of linear curves fitting bulk electrical conductivity ($\mu S\ cm^{-1}\ g^{-1}$) and percentage viable seeds (y) at physiological maturity to different variation parameters describing individual seed variation (x) before and after adding cultivar and site as factors to the regression model (n=24). The most suitable models are underlined.

	R^2				Significance of net change after adding			
Variation parameter	over all seed lots	after adding cv as a factor to the model	after adding site as a factor to the model	after adding site and cv as factors to the model	cv as a factor	site as a factor	cv as a factor after site	site as a factor after cv
Bulk electrical conductivity								
Mean – median	0.441***[1]	0.408**	0.691***	0.649***	NS	**	NS	*
SD[2]	0.416***	0.380**	0.658***	0.638***	NS	**	NS	*
CV%	-[3]	-	0.005NS	0.069NS	NS	NS	NS	NS
Range 0 - 75%	0.537***	0.514***	0.656***	0.648***	NS	*	NS	NS
Percentage viable seeds								
Mean - median	0.312**	0.255**	0.624***	0.682***	NS	***	NS	**
SD	0.278**	0.209NS	0.579***	0.666**	NS	**	NS	**
CV%	-	-	0.106NS	0.303NS	NS	NS	NS	*
Range 0 - 75%	0.426***	0.401**	0.628***	0.694***	NS	**	NS	**

[1] NS,*, ** and *** indicate statistically non-significant $P \geq 0.05$, statistically significant at $0.01 \leq P < 0.05$, $0.001 \leq P < 0.01$ and $P < 0.001$, respectively.

[2] SD = standard deviation, CV% = coefficient of variation, cv - cultivar.

[3] - Residual variance exceeded variance of response variate.

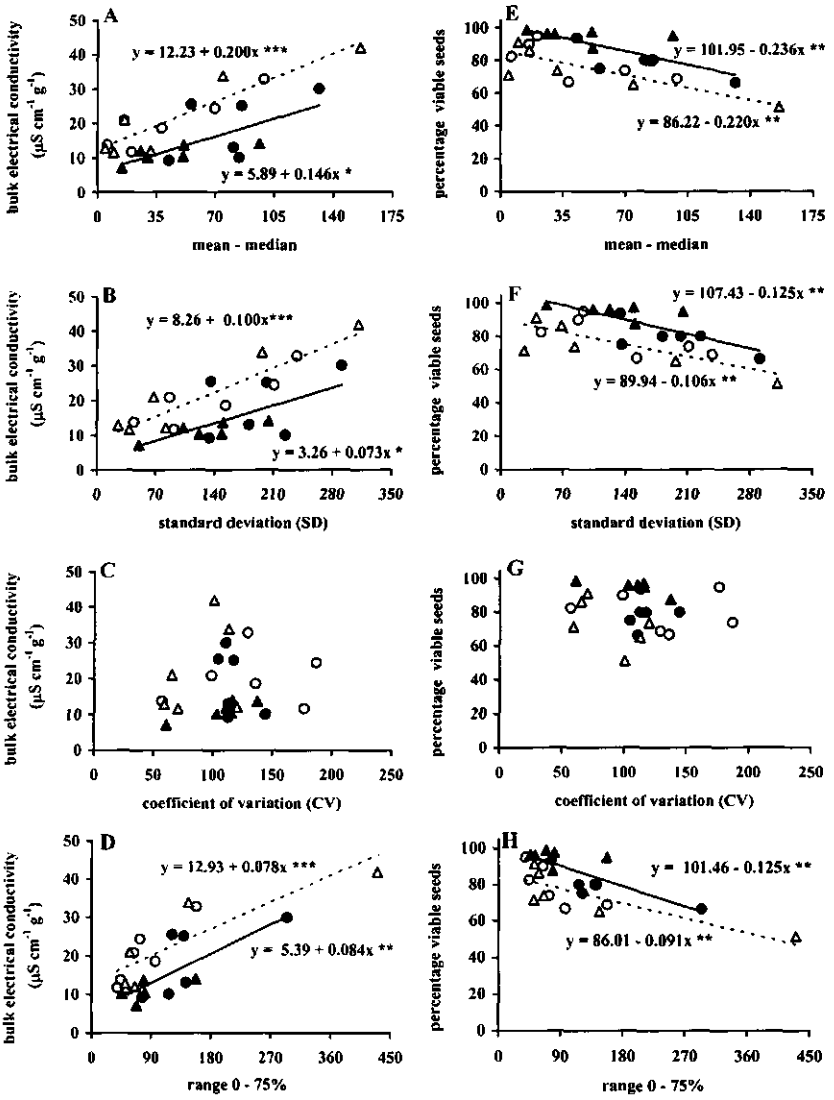

Fig. 1. Relationship between parameters describing individual seed quality variation at physiological maturity and bulk quality as measured by electrical conductivity and percentage viable seeds for cv. Rosecoco in Eldoret (●), cv. Mwezi Moja in Eldoret (▲), cv. Rosecoco in Kitui (○) and cv. Mwezi Moja in Kitui (△). The curves represent the most suitable models as shown by R^2 in Table 1. NS, *, ** and *** indicate statistically non-significant $P\geq0.05$ or statistically significant regression coefficients at $0.01\leq P<0.05$, $0.001\leq P<0.01$ and $P<0.001$, respectively.

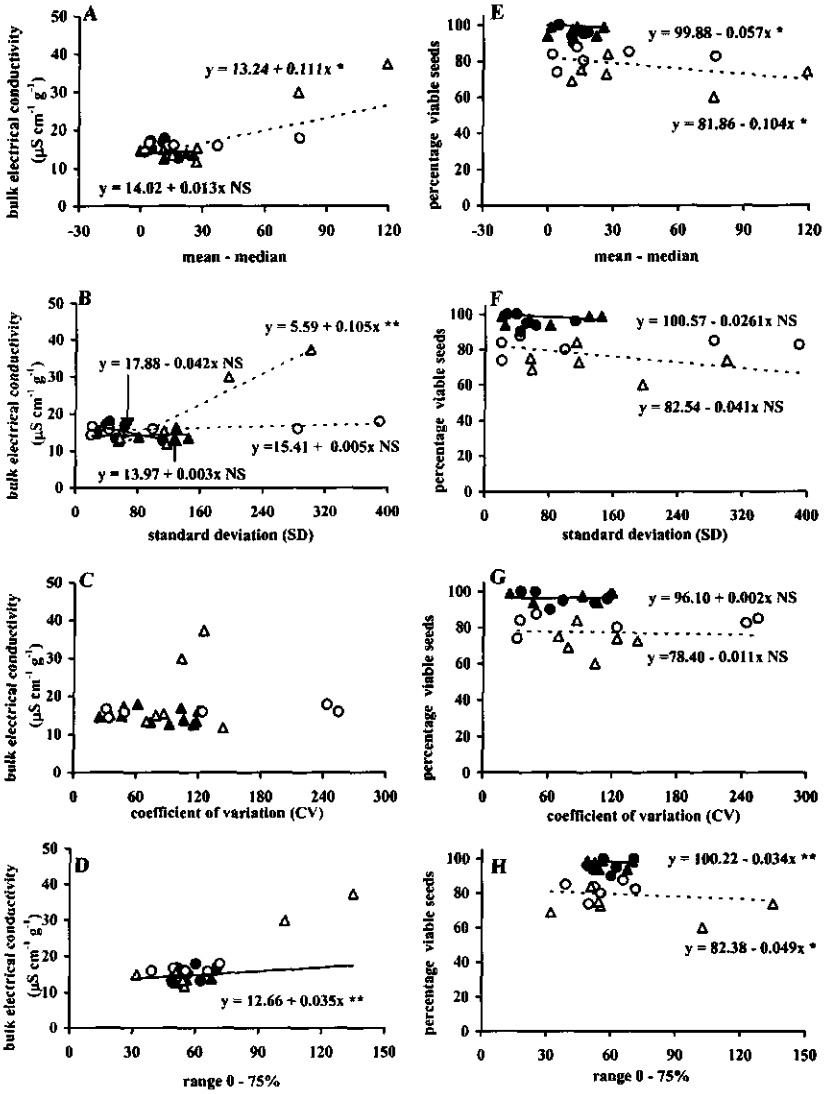

Fig. 2. Relationship between parameters describing individual seed quality variation at harvest maturity and bulk quality as measured by electrical conductivity and percentage viable seeds for cv. Rosecoco in Eldoret (●), cv. Mwezi Moja in Eldoret (▲), cv. Rosecoco in Kitui (o) and cv. Mwezi Moja in Kitui ( ). The curves represent the most suitable models as shown by R^2 in Table 2. NS, *, ** and *** indicate statistically non-significant $P \geq 0.05$ or statistically significant regression coefficients at $0.01 \leq P < 0.05$, $0.001 \leq P < 0.01$ and $P < 0.001$, respectively.

At harvest maturity (HM), bulk electrical conductivity of most seed lots was very comparable (Fig. 2) and the variation in bulk EC obtained over seed lots consequently was less than at PM. For most seed lots variation parameters quantifying variation between individual seeds were also lower than at PM. Nevertheless over all seed lots a higher variation as measured by mean - median, SD, and the range 0 - 75% was associated with a lower bulk EC (Table 2 and Fig. 2). When site was added as a factor to the regression models, the proportion of variance (R^2) accounted for by these relationships increased, except for 0 - 75% (Table 2). Regression analysis for the individual sites showed that a positive association between bulk EC and variation measured as mean - median only was significant in Kitui whereas the positive association between bulk EC and SD was only significant in cv Mwezi Moja in Kitui (Table 2, Fig. 2).

At HM, no significant associations were found between variation and percentage

Table 2. Coefficient of determination (R^2) of linear curves fitting bulk electrical conductivity and percentage viable seeds (y) at harvest maturity to different variation parameters (x) before and after adding cultivar and/or site as a factor to the regression model (n=24). The most suitable models are underlined.

	R^2				Significance of net change after adding			
Variation parameter	over all seed lots	after adding cv as a factor to the model	after adding site as a factor to the model	after adding site and cv as factors to the model	cv as a factor	site as a factor	cv as a factor after site	site as a factor after cv
Bulk electrical conductivity								
Mean - median	0.179*[1]	0.286*	0.426**	0.491**	NS	*	NS	*
SD[2]	0.179*	0.314*	0.406**	0.631***	NS	*	*	**
CV%	-[3]	-	-	0.233NS	NS	NS	NS	*
Range 0 - 75%	0.277*	0.325*	0.407**	0.294NS	NS	NS	NS	NS
Percentage viable seeds								
Mean - median	-	-	0.801***	0.875***	NS	***	*	***
SD	-	-	0.761***	0.862***	NS	***	*	***
CV%	-	-	0.690***	0.839***	NS	***	**	***
Range 0 - 75%	0.130NS	0.090NS	0.829***	0.869***	NS	***	NS	***

[1] NS, *, ** and *** indicate statistically non-significant $P \geq 0.05$, significant at $0.01 \leq P < 0.05$ $0.001 \leq P < 0.01$ and $P < 0.001$, respectively.

[2] SD = standard deviation, CV% = coefficient of variation, cv - cultivar.

[3] - Residual variance exceeded variance of response variate.

viable seeds measured as TZ, over all seed lots (Table 2). Adding site to the regression model, however significantly increased the association between percentage viable seeds and variation (Table 2 and Fig. 2). Analysis of the association per site showed that in both Kitui and Eldoret higher variation as quantified by mean - median and the range 0 - 75% was associated with lower percentage viable seeds at HM (Fig. 2).

Discussion

Associations were found between bulk quality and variation as quantified by mean - median, SD and the range 0 - 75% at PM (Fig. 1). At HM associations were found between bulk EC and mean - median, SD and the range 0 - 75%, and between percentage viable seeds and mean - median and the range 0 - 75 (Table 2 and Fig. 2). These all showed clearly that higher individual seed variation was associated with poorer bulk quality as indicated by higher bulk EC and lower percentage of viable seeds (Figs 1 and 2). This is consistent with the decrease in, for instance, germination uniformity found in seed lots that deteriorate in quality because of ageing (Hosnedl and Horakova, 1998). Our results show that a lack of uniformity is also associated with poorer quality over seed lots grown under different conditions. This association, however, was not found when variation between individual seeds was measured as CV% (Figs 1 and 2). This is consistent with the finding that CV% was not found to be a good parameter for quantifying individual seed variation (Muasya et al., 2001d) because CV% quantified variation to be lower in seed lots with many seeds having extremely high EC values than in seed lots with few seeds having high EC values. Differences also existed among the other variation parameters in the way they were related to bulk quality over the seed lots. At HM two seed lots showed high variation as quantified by mean - median and SD (open circles) (Fig. 2 A, B) whereas bulk EC was indicating good quality similar to other seed lots having lower individual seed variation. These two seed lots did not show high variation when variation was quantified by the range 0 - 75% (Fig. 2D). The main reason is that high mean - median and SD were caused by some seeds having higher leakage than the other seeds but a relatively low weight, resulting in extremely very high values for leakage/weight (EC) (data not shown). These seeds will not contribute considerably to the bulk EC value. These seeds will also not be taken into account in the range 0 - 75%. Variation quantified by mean - median and SD was found to be higher when there are values within a population showing extremely high values deviating from the bulk of the population, the range 0 – 75% measured variation in the bulk of the population (Muasya et al., 2001d). By contrast, in percentage of viable seeds as measured by tetrazolium test, individual seeds contribute equally to the bulk quality.

The fact that adding site to the regression model usually significantly increased the percentage variance accounted for at PM and HM (Tables 1 and 2) shows that the association between bulk quality and variation was different at the two sites (Figs 1 and 2). Bulk quality was better at Eldoret than in Kitui at comparable level of variation. This is probably partly related to the prevailing weather conditions leading to different individual seed quality. In Kitui average daily temperatures were higher than in Eldoret and rainfall was always unreliable during the period the seed lots were produced as shown by Muasya et al. (2001f). Drought and high temperature stress during seed filling reduced germination and vigour of soybean (Dornbos Jr and Mullen, 1991). However, at both sites quality was better when variation was lower except for EC at HM because almost no variation existed in EC of the seed lots harvested.

In general associations between bulk seed quality and individual seed variation were clearer at PM than HM. This is explained by the fact that at HM most of the seed lots were within a narrower range of bulk quality and also showed a narrower range of variation than at PM (Figs 1 and 2). The reasons for this narrower range in bulk and individual seed quality at HM compared to PM are unknown, but probably are related to the phenomenon that quality around PM is still increasing considerably (Muasya et al., 2001b) whereas around HM it has become more stable.

Conclusions

- At harvest maturity, associations between quality and individual seed variation generally were less clear because seed lots varied less in bulk quality and individual seed variation than at PM.
- At physiological maturity, lower bulk quality measured by bulk EC and percentage viable seeds was found to be linearly related to higher variation in individual seed EC (μS cm^{-1} g^{-1}) when the latter was quantified by the parameters mean - median, SD or 0 - 75%. There was no linear association between bulk quality and variation in individual seed EC measured by CV% over seed lots.
- The associations between bulk quality and individual seed variation were different for seed lots produced at different sites, indicating that both the degree of variation and the level of individual seed quality can determine bulk quality.
- The association between bulk quality and individual seed variation usually were not different for the two cultivars tested.

Chapter 7

Effects of growth conditions on seed quality, crop uniformity and individual seed variation in common bean (*Phaseolus vulgaris* L.) in Kenya[*]

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[*] Muasya, R.M., W.J.M. Lommen, E.O. Auma & P.C. Struik, 2001. Effects of growth conditions on seed quality, crop uniformity and individual seed variation in common bean (*Phaseolus vulgaris* L.) in Kenya.

7. Effects of growth conditions on seed quality, crop uniformity and individual seed variation in common bean (*Phaseolus vulgaris* L.) in Kenya

Abstract

Twenty-four common bean crops were grown from two cultivars, at different sowing dates in two sites in Kenya. We evaluated whether weather conditions affect the seed quality mainly through effects on the maximum quality achievable at physiological maturity of seed crops, through enhancing deterioration of quality during maturation drying of the seeds between physiological maturity and harvest maturity and/or through changing the uniformity of the crop, consequently influencing the variation between individual seeds at harvest. Higher temperature and less rainfall both could reduce seed quality, but over the range of conditions studied, high temperatures were more detrimental to seed quality than limited rainfall. The two cultivars used differed in susceptibility to high temperature. High temperature and less rainfall seemed to reduce seed quality mainly through reducing the maximum quality attainable during the course of crop development. Maximum quality, however, was not always achieved at physiological maturity: the percentage viable seeds sometimes increased thereafter, especially at relatively low temperatures. Variation in weather conditions during the production of different seed lots did not lead to lower quality of the seed lots through increasing the variation within the crop, as measured by length of flowering or pod set periods or plant-to-plant variation in number of flowers. Production conditions leading to low seed yields or light seeds also resulted in low percentage viable seeds. Fairly high yield or heavy seed, however, did not guarantee high percentage viable seeds.

Introduction

During the growth of field crops, maximum seed quality is generally regarded to be obtained at physiological maturity (PM), i.e. the end of seed filling (Egli, 1998). The crop is harvested at harvest maturity (HM), when seeds have dried to a moisture content at which harvesting is possible without considerable damage. Within crop differences during growth and development could lead to individual seed variation at PM and HM. For example in soybean all flowers on a plant are not pollinated at the same time, so individual seeds start growing at different times and can attain PM at different times (TeKrony and Egli, 1997; Egli, 1998). Long flowering and podding duration may lead to large differences in the moment individual seeds reach PM and may eventually lead to large quality differences within a seed lot at PM (Chamma et al., 1990). In common bean the moment of PM was earlier in seeds from earlier pods (Muasya et al., 2001a). Also seeds from earlier pods tended to achieve maximum quality earlier than seeds from later pods in common beans (Muasya et al., 2001b). In soybean seed quality was better when seeds were obtained from the top of the plant than from lower branches (Adam et al., 1989). Illipronti Jr et al. (2000a) reported higher viability for soybean seeds from later pods than from earlier pods when seeds were grown at high temperature. Muasya et al. (2001e) showed that a higher individual seed variation was associated with a poorer bulk quality.

Weather conditions are well known to have pronounced effects on seed quality. Drought during vegetative growth of a soybean crop caused large reductions in vegetative plant size (20 - 50%) and reduced leaf area which eventually affected the process of seed filling leading to seeds of low weight (Meckel et al., 1984). The effects of temperature during vegetative stage on subsequent seed growth and quality are not clear. However, drought and high temperature stress during soybean seed filling led to lack of uniformity of seeds at harvest and reduced germination and vigour of the seed lots produced (Dornbos Jr and Mullen, 1991). High rainfall caused embryo damage in navy bean due to uptake of water by the seed when maturation drying was in progress (Tu et al., 1988). High temperatures during maturation drying lowered seed viability and vigour through accelerated ageing in soybean (Miles, 1985; Spears et al., 1997). In common bean, germination and seedling vigour deteriorated faster at higher temperatures (30/25 °C or 33/28 °C) than at lower temperatures (18/13 °C or 21/16 °C) during maturation drying (Siddique and Goodwin, 1980). The length of the time a seed is exposed to adverse weather conditions during maturation drying, may also affect its quality at harvest. Because individual seeds attain PM at different times and the course of moisture decline between PM to HM in common bean is also different for different seeds (Muasya et al., 2001a), this could lead to bulk seeds varying in age and quality at harvest.

Weather conditions may affect final quality of the seed lot harvested in different ways: through effects on the maximum quality obtained at PM, through deterioration in quality between PM and HM and/or through creating larger variation between individual seeds because of larger variation within a crop. This research aims at understanding the relationship between weather conditions, within crop variation, and individual seed quality variation and bulk quality at PM and HM.

Materials and methods

Experimental sites, set-up and crop and seed management

Twenty four different seed lots of two cultivars of common bean (*Phaseolus vulgaris* L.) were produced using three sowing dates in each of two seasons at two locations (Kitui and Eldoret) in Kenya (Table 1). In each site and season the experimental

Table 1. Sowing and harvesting dates, and temperature and rainfall during different growing periods of common bean crops during different seasons in Kitui and Eldoret sites in Kenya.

Sowing dates	Kitui		Eldoret	
	Season 1	Season 2	Season 1	Season 2
Sowing date 1				
Sowing	5 Nov. 1998	11 Mar. 1999	1 Jul. 1998	11 Mar. '99
Rainfall (mm)	845	382	535	364/321
Temperature sum (°Cd)	2122/2094 [2]	1911	1369	1497/1403
Harvesting	28/27 Jan. '99	3/2 June, '99	12 Oct. 1998	24/17 June '99
Duration (DAS[1])	84/83	84/83	98	113/106
Sowing date 2				
Sowing	19 Nov. 1998	25 Mar. 1999	21 Jul. 1998	26 Apr. 1999
Rainfall (mm)	420	264	486	636/503
Temperature sum (°Cd)	2318/2296	1872	1334	1554/1460
Harvesting	17/16 Feb. '99	17/16 Jun. '99	27 Oct. 1998	17/10 Aug. '99
Duration (DAS[1])	90/89	84/83	98	113/106
Sowing date 3				
Sowing	8 Dec. 1998	9 Apr. 1999	20 Aug. 1998	15 May 1999
Rainfall (mm)	117	144	287	547/544
Temperature sum (°Cd)	2135/2112	1778	1359	1888/1802
Harvesting	27/26 Feb. '99	30/29 Jun. '99	25 Nov. 1998	30/24 Sep. '99
Duration (DAS[1])	81/80	82/81	97	138/132

[1] DAS – days after sowing

[2] When two data are supplied these are for cvs. Rosecoco/Mwezi Moja, respectively.

design was a split plot with four blocks. The two cultivars, Rosecoco and Mwezi Moja, were assigned to main plots. Both are determinate cultivars, but Rosecoco shows prolonged flowering while the flowering period of Mwezi Moja is short. Sowing dates were assigned to subplots. Temperature and rainfall were recorded throughout the growing periods.

At planting, each plot of 16 m^2 was fertilized with 0.49 kg calcium ammonium nitrate (26% N), 0.76 kg triple super phosphate (48% P_2O_5) and 0.13 kg muriate of potash (60% K_2O), achieving 80 kg N, 100 kg P and 20 kg K per hectare. The gross plot size was 4×4 m while the net experimental area was 3.40×2.50 m. Two seeds per hill were planted at a spacing of 0.5×0.1 m. At full emergence the seedlings were thinned out leaving a single seedling per hill. Within the net experimental area, a line of 20 plants was marked for the study on the pattern of flower and pod development over time. Within the net experimental area, two separate areas consisting of 40 plants each were used for quality determinations. At physiological maturity all pods were harvested from one of the areas. Seeds were removed by hand and only normal looking seeds were selected and further dried at 30 °C in a continuous flow dryer until a moisture content of 14% was achieved. Thereafter, seeds were stored at 2 °C and 75% RH for an average of three months. Then samples were taken for determination of electrical conductivity and the percentage viable seeds. The same procedure was repeated at harvest maturity on seeds harvested from the second area of 40 plants. Within the net experimental area a separate area of 2 m^2 was used for yield and moisture content determinations.

Developmental periods

The period of vegetative mass accumulation was the period from seedling emergence (7 days after sowing) till there was no further increase in ground cover by the leaves. To get the pattern of flower and pod development over time, all the flowers in the line of 20 plants were counted every two days starting from the first day of flower opening. The total of all the flowers was calculated at the end of the flowering period. Flowering duration was the time period in days from the first until the last flower was observed. The number of pods at reaching 12 cm length was recorded every two days. The duration of pod set was estimated as the period from the day first pods of 12 cm length were recorded until the day there was no further increase in pod number. Seed filling duration was estimated as the period from the day at which first pods were 12 cm until and including the day of physiological maturity. Maturation drying was the period from the day of physiological maturity until and including the day of harvest maturity. Physiological maturity was estimated based on dry weight accumulation but also by change of pod colour from green to green yellow and seed colour from green yellow to 100% red purple colour. Harvest maturity was determined by the moment all pods had changed colour from green yellow to straw yellow. At this moment all seeds had achieved the red purple colour pattern. Colours were assessed using munsell colour charts (Anonymous, 1972).

Electrical conductivity test

Electrical conductivity was determined after equilibrating the seeds from cold storage for 3 days at room temperature (19 - 25 °C). The moisture content was then constant at 12%. To establish bulk electrical conductivity at PM and HM, four replicate samples (one from each block) of 50 seeds were weighed and incubated in 250 ml of distilled water at 20 °C for 24 hours. A control sample of equivalent amount of distilled water was included. To establish individual seed electrical conductivity at PM and HM four replicate samples of 20 seeds were combined and each seed was weighed and individually incubated in 50 ml of distilled water at 20 °C for 24 hours. A control sample of equivalent amount of distilled water was included. Conductivity (μS cm^{-1}) was measured using a Fieldlab – LF conductivity meter and an LF 513T electrode dip – type cell. The conductivity per gram of seed weight (μS cm^{-1} g^{-1}) at 12% moisture content in 50 and 20 ml of water for respectively bulk and individual seeds at PM and HM was then calculated to estimate vigour (ISTA, 1995).

Tetrazolium test

Four replicate samples of 20 seeds (one from each block) were taken from the cold storage and kept at room temperature (19 - 25 °C) for a day before soaking in water at room temperature for 24 hours. Thereafter, seeds were sliced longitudinally through the middle of the embryonic axis and soaked in a 0.5% tetrazolium (2,3,5-Triphenyltetrazolium chloride) solution at 30 °C for 3 hours, briefly washed in distilled water and examined under hand lens magnification (ISTA, 1995). The staining of the embryo was examined per seed. Seeds with sound tissues and weak viable tissues were combined to calculate viability, i.e. the percentage of viable seeds.

Moisture content and yield determination

Moisture content at physiological maturity was established from four replicate samples of 20 seeds in Eldoret and 10 seeds in Kitui which were individually cut across the cotyledons and dried at 105 °C for 16 hours. Moisture content at harvest maturity was established from 4 replicate samples of 20 seeds, following the same procedure as at physiological maturity. Total dry seed yield at harvest maturity was established from the total fresh weight of seeds harvested from the 2 m^2 and the moisture content of the seeds. Individual seed weight was established from the dry seed yield and the total number of seeds harvested per 2 m^2.

Calculation of variation parameters

To characterize individual seed quality variation within seed lots, the following variation parameters were calculated from the combined data of four samples of 20 individual seed electrical conductivity (EC) data at PM and HM.

- Range 0 - 75%. This is the range from the minimum value to the value of 75% of the observations in the combined sample. It estimates variation in the bulk of the seeds, excluding the highest extremes in the distributions which generally are skewed towards higher values (Muasya et al., 2001d).
- Standard deviation (SD). This is the square root of the variance (σ^2). SD = $\sigma = \sqrt{(\Sigma(X_i-\mu)^2/n)}$, where μ is the mean $(\Sigma X_i)/n$ and n is the number of seed lots. This

parameter measures the spread in the individual measurements from the mean in the same units as the observations.

- Coefficient of variation (CV%). This parameter measures variation as a ratio of SD divided by the mean, expressed as percentage. CV% = $(\sigma/\mu)\times100$.

To quantify variation in development within the crop, the total length of the flowering and pod set periods were calculated from the combined data of four lines of 20 plants, and the following parameters estimating plant-to-plant variation in number of flowers in these plants were calculated:

- Range 25 - 75%. This is the range in values between 25% to the value of 75% of the observations in the combined sample. It estimates variation in the bulk of the plants, excluding the lowest and highest extremes.
- Standard deviation (SD). See above.
- Coefficient of variation (CV%). See above.

Statistical analysis

Single and multiple linear regression analyses were carried out using Genstat 5 (Release 4.1) to show associations between weather data and the average seed quality, parameters quantifying individual seed variation in EC, parameters quantifying variation in development within crops and average seed yield and size over the 24 seed crops. Weather data were allocated as explanatory variates (x), the other parameters as response variates (y). The significance of associations was established using the adjusted R^2. First linear associations were established over all seed lots. Thereafter, site and cultivar were added individually and combined as factors to the regression model. When addition of these factors significantly ($P<0.05$) increased the R^2, regression curves were also calculated for the individual combinations of site and/or cultivar. A similar procedure was used to establish associations between parameters quantifying variation in development within crops (x) and seed quality or parameters quantifying individual seed variation in EC.

Linear correlation coefficients were calculated to establish associations between different seed quality parameters of the seed lots, using seed lot means.

T-tests on seed lot averages were used to determine whether quality was different between sites, cultivars and/or harvest moments. T-tests on replicate plot results were used to test whether quality of individual seed lots significantly changed between physiological and harvest maturity.

Results

Seed moisture content at physiological and harvest maturity

Phenological assessment of the moment of physiological maturity by change of the pod colours from green to green yellow, usually lead to seeds harvested around the targeted moisture content of 58%, but some obvious exceptions occurred in which seed moisture content was already reduced down to 35% (Fig. 1A; negative skewness). Phenological assessment of harvest maturity by the change in pod colour of all pods from green yellow to straw yellow proved to be even more difficult. Seeds had already achieved the red purple colour well before harvest maturity. Almost half of the seed lots had much higher moisture content than the expected 20%.

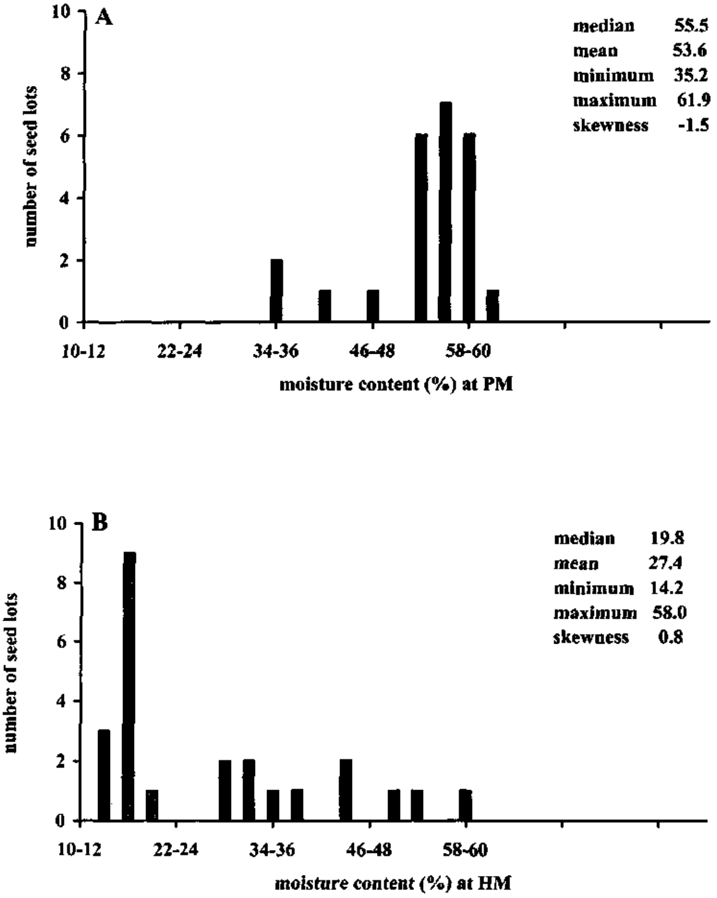

Fig. 1. Frequency distributions showing the number of seed lots having specific moisture contents at the moments of physiological maturity (A) and harvest maturity (B) as determined by change in colour of pods and seeds. Total number of seed lots: 24.

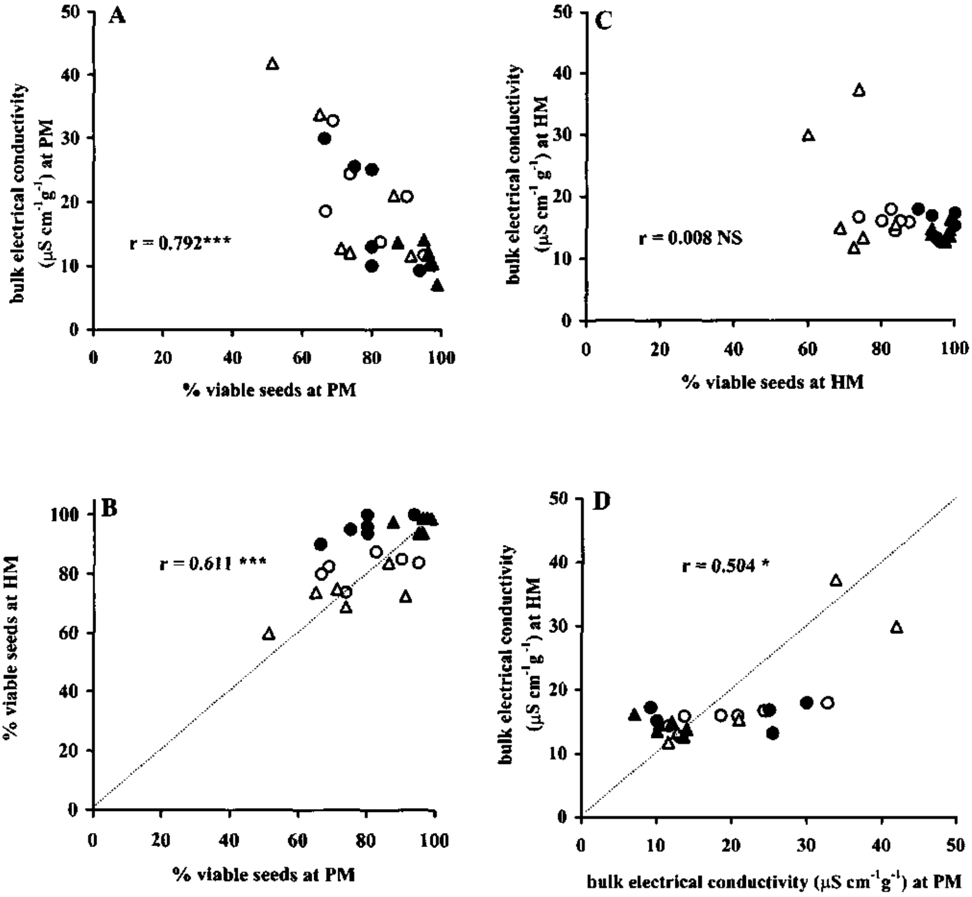

Fig. 2. Associations between percentage viable seeds, determined by the tetrazolium test, and electrical conductivity in seeds from 24 crops at physiological maturity (A) and harvest maturity (C), and associations between percentage viable seeds at physiological maturity and harvest maturity (B) and electrical conductivity at physiological and harvest maturity (D). Dotted lines in C and D indicate equal quality at physiological and harvest maturity. Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. ***: $P<0.001$, *:$0.01 \leq P<0.05$, NS: $P \geq 0.05$. Correlation coefficients (r) were calculated over all seed lots except the two deviating seed lots.

Seed quality at physiological maturity and harvest maturity

At physiological maturity, the percentage viable seeds of the seed lots produced under different conditions ranged between 51 and 99%, and the bulk EC ranged between 7 and 42 µS cm^{-1} g^{-1} (Fig. 2A). A lower percentage viable seeds was closely associated with a higher EC value of the same seed lot (Fig. 2A). The percentage viable seeds was higher in Eldoret than in Kitui, which was entirely accounted for by the better performance of cv. Mwezi Moja in Eldoret (Table 2). EC values were not significantly different at the two sites (Table 2).

At harvest maturity, seed lots produced under different conditions still greatly varied in percentage viable seeds (Fig. 2C), but little variation occurred in EC, which in most seed lots was around 15 µS cm^{-1} g^{-1} (Fig. 2C). Only 2 out of 24 seed lots had high EC values. Consequently, lower percentage viable seeds was no longer associated with high EC values (Fig. 2C). The percentage viable seeds was higher in Eldoret than in Kitui in both cultivars (Table 2).

Over all seed crops, higher percentage viable seeds at physiological maturity were associated with a higher percentage viable seeds at harvest maturity (Fig. 2B). Higher EC values at physiological maturity were weakly associated with higher EC values at harvest maturity, even when the two deviating seed lots were excluded from the analysis (Fig. 2D), due to the small variation in bulk EC at HM.

Between physiological maturity and harvest maturity, seed quality in individual crops could still change (Figs 2B and D). On average, the percentage viable seeds increased, but this was almost exclusively caused by the increase in cv. Rosecoco in Eldoret (Table 2). EC on average did not change significantly between physiological and harvest maturity (Table 2), whereas its variation among seed lots reduced. A decrease in quality in individual seed lots could not be assessed as statistically significant.

Associations between temperature and seed quality

Temperatures during the different (but partly overlapping) growth phases (vegetative mass accumulation, flowering, pod set, seed filling and maturation drying) were correlated ($P<0.01$) indicating temperatures were warmer or colder throughout the growing periods. Differences in temperature between sites were much larger than the differences over time within the sites, with Kitui having higher temperatures than Eldoret.

At physiological maturity, higher temperatures in all preceding growth phases were associated with lower percentages of viable seeds, but this effect was only significant in cv. Mwezi Moja (Table 3). This is illustrated in Fig. 3A for temperature during the pod set period. Cv. Mwezi Moja seemed to benefit more from low temperatures and

Table 2. Percentage of viable seeds and electrical conductivity at physiological maturity and at harvest maturity for common bean cultivars Rosecoco and Mwezi Moja produced in six different periods in Eldoret and Kitui.

	Physiological maturity				Harvest maturity				Significance of time effect		
	Eldoret	Kitui	Average over sites	Significance of site effect	Eldoret	Kitui	Average over sites	Significance of site effect	Eldoret	Kitui	Average over sites
Percentage viable seeds											
Rosecoco	79.2	79.7	79.4	NS[1]	95.8	82.3	89.1	***	**	NS	**
Mwezi Moja	95.3	73.0	84.2	**	97.2	72.5	84.8	***	NS	NS	NS
Average over cultivars	87.3	76.3	81.8	*	96.5	77.4	87.0	***	**	NS	*
Significance of cultivar effect	**	NS	NS		NS	*	NS				
Electrical conductivity (μS cm^{-1} g^{-1})											
Rosecoco	18.8	20.3	19.6	NS	15.5	16.1	15.8	NS	NS	NS	NS
Mwezi Moja	11.2	22.2	16.7	NS	14.2	20.4	17.3	NS	NS	NS	NS
Average over cultivars	15.0	21.3	18.1	NS	14.8	18.3	16.6	NS	NS	NS	NS
Significance of cultivar effect	*	NS	NS		NS	NS	NS				

[1] NS, *, **, *** *** indicate statistically non-significant ($P \geq 0.05$), significant at $0.01 \leq P < 0.05$, $0.001 \leq P < 0.01$ and $P < 0.001$, respectively according to the t-test.

Table 3. Associations between the average daily temperature and rainfall in different growth phases and seed quality, and individual seed quality variation at physiological maturity and harvest maturity as established by linear regression analysis over all seed lots.

	Physiological maturity					Harvest maturity				
	Seed quality as measured by		Individual seed quality variation in electrical conductivity as measured by			Seed quality as measured by		Individual seed quality variation in electrical conductivity as measured by		
	Percentage viable seeds	Electrical conductivity	Standard deviation	Coefficient of variation (%)	Range 0-75%	Percentage viable seeds	Electrical conductivity	Standard deviation	Coefficient of variation (%)	Range 0-75%
Average temperature during										
Vegetative mass accumulation	-[1, 2]	+[3]	NS[6]	NS[7]	NS	-[4]	-	+	NS	NS
Flowering	NS[2]	+	NS	NS	NS	-[5]	-	+	+	NS
Pod set	NS[2]	+	NS	NS	NS	-[5]	-	+	NS	NS
Seed filling	NS[2]	+	NS	NS	NS	-[5]	-	+	NS	NS
Maturation drying						-[5]	-	+	NS	NS
Average rainfall during										
Vegetative mass accumulation	NS[3]	NS	NS	NS	NS	-[3]	NS	NS	NS	NS
Flowering	NS	NS	NS	NS	NS	+[3]	NS	NS	NS	NS
Pod set	NS	NS	NS	NS	NS	+[3]	NS	NS	NS	NS
Seed filling	+	NS	NS	NS	NS	+[3]	NS	NS	NS	NS
Maturation drying						+[3]	NS	NS	NS	NS

[1] -: Negative association ($P<0.05$), +: positive association ($P<0.05$), NS: non-significant ($P\geq0.05$).

[2] Adding cultivar to the regression model significantly increased R^2. Separate analysis for the two cultivars revealed a negative association in cv. Mwezi Moja but no significant association in cv. Rosecoco.

[3] Adding site to the regression model significantly increased R^2. Separate analysis for the two sites revealed no significant associations within a site.

[4] Adding cultivar and site to the regression model significantly increased R^2. Separate analysis for the different sites and cultivars only revealed a significant negative association for cv. Mwezi Moja in Kitui.

[5] Adding cultivar and site to the regression model significantly increased R^2. Separate analysis for the different sites and cultivar revealed no significant associations within a site and cultivar.

[6] Adding site to the regression model significantly increased R^2. Separate analysis for the two sites revealed no significant association in Eldoret but a positive association in Kitui.

[7] Adding cultivar and site to the regression model significantly increased R^2. Separate analysis for the different sites and cultivar revealed no significant association within cultivars and a positive association within sites.

suffer more from higher temperatures than cv. Rosecoco. EC increased with increasing temperature over all seed lots (Table 3, Fig. 3B).

Also at harvest maturity, higher temperatures were associated with lower percentages viable seeds (Table 3, Fig. 3C). This effect was confounded with the site effect: both cultivars had a higher percentage viable seeds at low temperature (Eldoret)

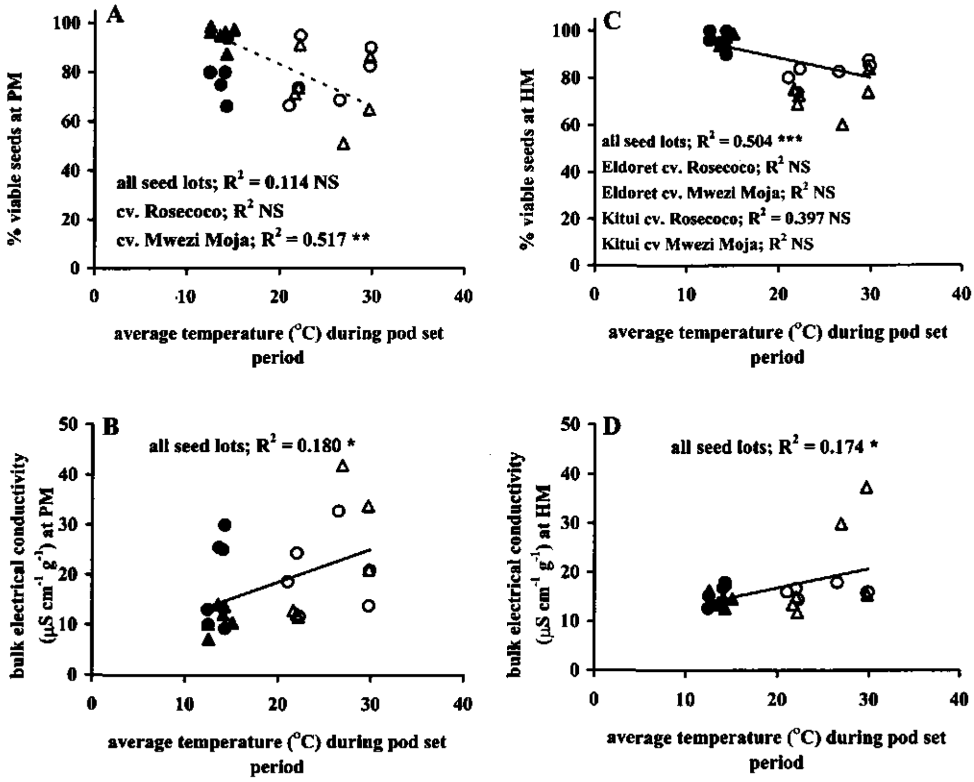

Fig. 3. Associations between the average daily temperature during the pod set period and percentage viable seeds (A, C) or electrical conductivity (B, D) of seeds from 24 crops at physiological maturity (A, B) and harvest maturity (C, D). Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. Straight lines show significant (P<0.05) regression lines over all seed lots, dashed lines show significant regression lines within specific site and/or cultivar combinations. The latter were only calculated when adding site and/or cultivar to the regression model significantly (P<0.05) improved the R^2 of the model. R^2 presented are for the regression model over all seed lots and for individual site and/or cultivar combinations when relevant. When no value is presented for the R^2, the residual variance exceeded the variance of the response variate, ***: P<0.001, **: 0.001≤P<0.01, *:0.01≤P<0.05, NS: P≥0.05.

and a lower at high temperature (Kitui). Within a cultivar and site, temperature effects on percentage viable seeds could not be established. EC was higher when temperatures were higher (Table 3), but this positive trend was only found because two seed lots of cv. Mwezi Moja had much higher EC values than most seed lots (Fig. 3D). Sometimes effects were confounded with site effects (Table 3).

Associations between temperature and individual seed variation

At physiological maturity, individual variation in EC and temperature in the various preceding growth phases were usually not associated (Table 3). Only in Kitui, individual seed variation, as characterized by SD or the range 0 - 75%, was higher when temperatures during vegetative mass accumulation were higher (Table 3). Individual seed variation as characterized by CV% was not associated with temperature at all.

At harvest maturity, individual seed variation as characterized by SD was higher when temperatures during any of the preceding periods was higher, but individual seed variation in the bulk of the seeds (the range 0 - 75%) was not related to temperature in any of the preceding periods (Table 3). Individual seed variation as characterized by CV% was only higher when temperatures during flowering were higher.

Associations between temperature and variation in development within crops

The duration of flowering was not associated with temperature during vegetative mass accumulation or flowering (Table 4), but the duration of the pod set period was shorter when temperature during pod set was higher (Table 4, Fig. 4A).

Plant-to-plant variation in number of flowers as estimated by the range 25 - 75% or SD was lower when temperatures during vegetative mass accumulation and flowering were higher (Table 4). Within sites, the latter was only significant in Eldoret. Plant-to-plant variation as estimated by CV% in flower number was not associated with temperature (Table 4).

Associations between rainfall and seed quality

At physiological maturity, only a significant association existed between average daily rainfall during seed filling and percentage viable seeds: higher rainfall was associated with higher percentage viable seeds (Table 3). No significant associations were present between daily rainfall in any of the other preceding periods and percentage viable seeds or EC (Table 3, Fig. 5A, B).

At harvest maturity, the percentage viable seeds was higher when average daily rainfall during flowering, pod set, seed filling and maturation drying was higher (Table 3). These effects were mainly due to site effects (Table 3, Fig. 5C). Within a site no significant associations were found. EC was not significantly associated with rainfall (Table 3, Fig. 5D).

Associations between rainfall and individual seed variation

None of the parameters, by which individual seed variation in EC was assessed, showed any significant relationship with rainfall, either at physiological maturity or at harvest maturity (Table 3).

Associations between rainfall and variation in development within crops

The duration of flowering was not associated with average daily rainfall (Table 4). Longer duration of pod set was associated with higher average daily rainfall during pod set (Table 4, Fig. 4B).

Table 4. Associations between temperature and rainfall during the different growth phases and flowering duration, podding and duration, plant-to-plant variation in number of flowers (standard deviation, coefficient of variation and range 25 - 75%) over all seed lots.

	Duration of		Plant-to-plant variation		
	Flowering	Pod set	Standard deviation	Coefficient of variation (%)	Range 25-75%
Average temperature during					
Vegetative mass accumulation	NS [1]	NS [2]	-	- [3]	-
Flowering	NS	NS [4]	- [5]	NS [3]	- [5]
Pod set		-	- [5]	NS [3]	- [6]
Average rainfall during					
Vegetative mass accumulation	NS	NS	NS	NS [3]	NS
Flowering	NS	NS	+	NS [3]	+
Pod set		+	+	NS [3]	+

[1] NS – non-significant ($P \geq 0.05$), – significant decrease ($P<0.05$), + significant increase ($P<0.05$).

[2] Adding site to the regression model significantly increased R^2. Separate analysis for the two sites revealed a no significant associations within the two sites.

[3] Adding cultivar to the regression model significantly increased R^2. Separate analysis for the two cultivars revealed no associations within the two cultivars.

[4] Adding site to the regression model significantly increased R^2. Separate analysis for the sites revealed a no significant association in Eldoret but a positive association in Kitui.

[5] Adding site to the regression model significantly increased R^2. Separate analysis for the sites revealed a negative association in Eldoret and a no significant association in Kitui.

[6] Adding site and cultivar to the regression model significantly increased R^2. Separate analysis for the sites and cultivars revealed a negative association in Eldoret site cv. Rosecoco and a positive association in Kitui cv. Mwezi Moja.

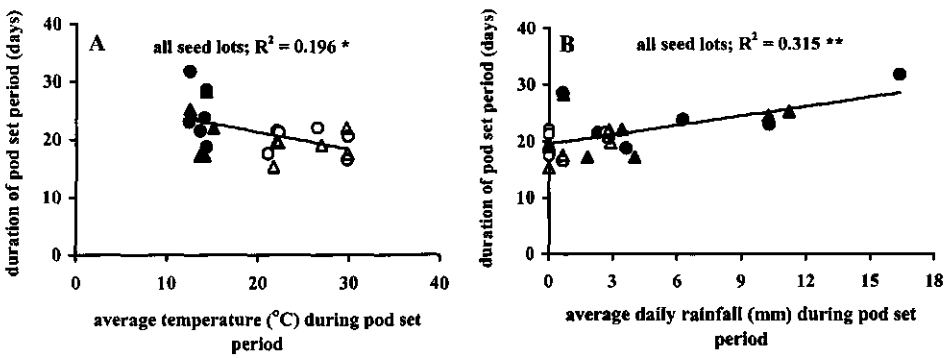

Fig. 4. Duration of the pod set period in relation to the average daily temperature (A) and rainfall (B) during the pod set period for 24 seed crops. Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. For significance, see Fig. 3.

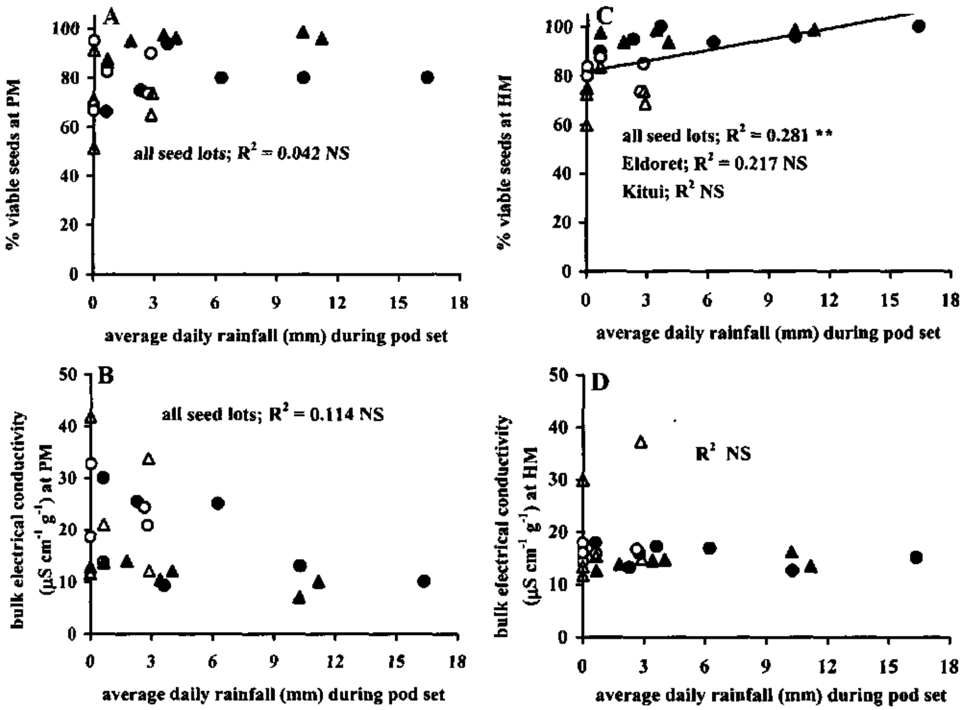

Fig 5. Associations between the average daily rainfall during the pod set period and percentage viable seeds (A, C) or electrical conductivity (B, D) of seeds from 24 crops at physiological maturity (A, B) and harvest maturity (C, D). Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. For explanation of R^2, line types and significance, see Fig. 3.

Higher plant-to-plant variation in number of flowers was related to higher average daily rainfall during flowering, when plant-to-plant variation was measured as SD or the range 25 - 75% (Table 4). No associations between rainfall and plant-to-plant variation as measured by CV% were significant.

Associations between variation in development within crops and seed quality

At physiological maturity, quality was not associated with the duration of the flowering and pod set period (Table 5, Figs 6A and B). However, quality was higher when plant-to-plant variation in number of flowers as characterized by the range 25 - 75% was higher, whereas higher plant-to-plant variation as characterized by SD was only associated with better quality as measured by EC (Table 5).

At harvest maturity, longer pod set period was associated with higher percentage viable seeds (Table 5, Fig. 6C), but this effect was caused by the different sites and/or cultivars. Within a site × cultivar combination, associations between duration and quality were not significant. There were no associations between the durations of the relevant periods and quality as measured by EC (Table 5, Fig. 6D). In case significant associations were found between plant-to-plant variation and quality, they showed a better quality in situations where plant-to-plant variation in number of flowers was high (Table 5): higher SD and CV% in flower number was associated with higher percentage of viable seeds while higher range 25 - 75% in flower number was related to lower EC values in Kitui.

Associations between variation in development within crops and individual seed variation

At physiological maturity, individual seed variation as characterized by SD was associated with the length of the pod set period: longer pod set periods were associated with a larger variation (Table 5, Fig. 7B). Also higher individual seed variation as characterized by CV% was associated with longer pod set periods but only in Kitui (Table 5). Individual seed variation in the bulk of the seeds (the range 0 - 75%) was not associated with the length of the flowering or pod set periods (Table 5, Fig. 7A). However, individual seed variation at physiological maturity was not associated with plant-to-plant variation in number of flowers (Table 5, Figs 8A and B).

At harvest maturity, individual seed variation was not significantly related to the duration of the flowering or pod set period (Table 5, Figs 7C and D). Individual seed variation was lower, when the plant-to-plant variation in number of flowers as characterized by the range 25 - 75% was higher, but only in Kitui (Table 5, Fig. 8C). Individual seed variation was not associated with plant-to-plant variation in number of flowers as characterized by SD or CV% (Table 5, Fig. 8D).

Table 5. Associations between within-crop variation and seed quality, and individual seed quality variation, at physiological maturity and harvest maturity, over all seed lots.

Parameters describing within-crop variation	Physiological maturity					Harvest maturity				
	Seed quality		Individual seed quality variation			Seed quality		Individual seed quality variation		
	Percentage viable seeds	Electrical conductivity	Standard deviation	Coefficient of variation (%)	Range 0-75%	Tetrazolium test	Electrical conductivity	Standard deviation	Coefficient of variation (%)	Range 0-75%
Duration of										
Flowering	NS[1]	NS	NS	NS	NS	NS[2]	NS	NS	NS[2]	NS
Pod set	NS	NS	NS[3]	+	NS	+[2]	NS	NS[2]	NS	NS
Plant-to-plant variation in number of flowers										
Standard deviation	NS	+	NS	NS	NS	+[2]	NS	NS	NS	NS[4]
Coefficient of variation (%)	NS	NS	NS[5]	NS[5]	NS	NS[3]	NS	NS	NS	NS
Range 25 – 75%	+	+	NS	NS	NS	NS[2]	NS[3]	NS	NS	NS[4]

[1] -: negative association ($P<0.05$), +: positive association ($P<0.05$), NS: non-significant ($P\geq0.05$,

[2] Adding site to the regression model significantly increased R^2. Separate analysis for the two sites revealed no significant associations within the sites.

[3] Adding site to the regression model significantly increased R^2. Separate analysis for the two sites revealed no significant associations in Eldoret site and a positive association in Kitui site.

[4] Adding site to the regression model significantly increased R^2. Separate analysis for the two sites revealed no significant associations in Eldoret site and a negative association in Kitui site.

[5] Adding cultivar to the regression model significantly increased R^2. Separate analysis for the two cultivars revealed no significant associations.

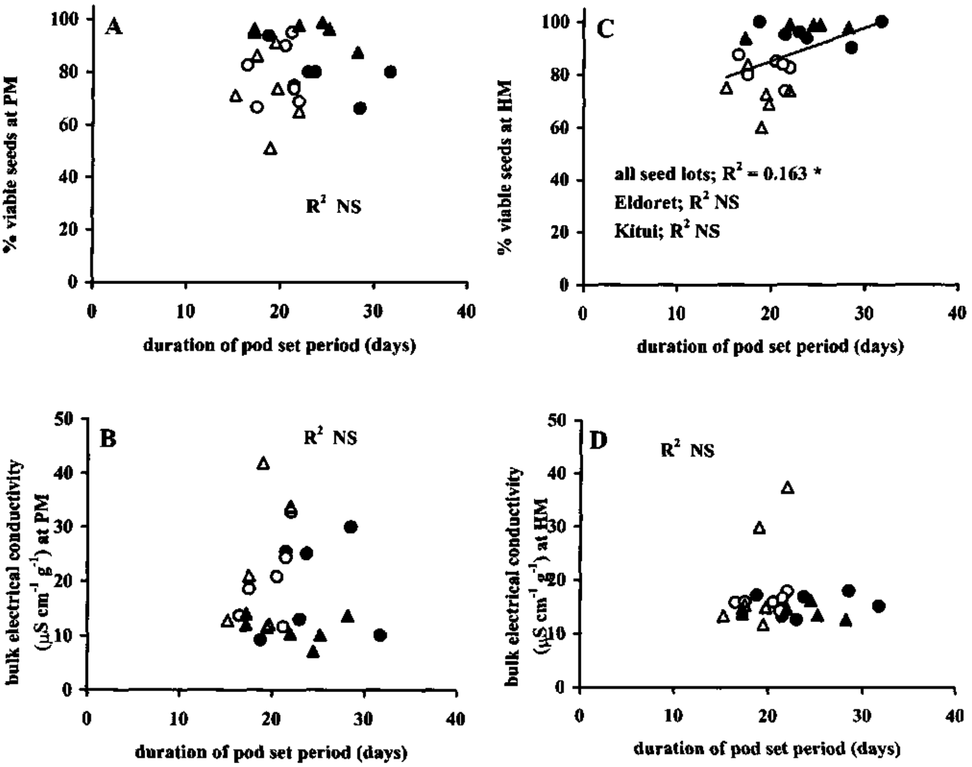

Fig. 6. Associations between the duration of the pod set period and percentage viable seeds (A, C) or electrical conductivity (B, D) of seeds from 24 crops at physiological maturity (A, B) and harvest maturity (C, D). Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. For significance, see Fig. 3.

Associations between seed quality and seed yield or weight

High quality at physiological maturity, indicated by high percentage viable seeds and low EC, was associated with high yield of seeds per m² (Figs 9A and B) and high individual seed weights (Figs 10A and B). The same was found for percentage viable seeds at harvest maturity (Figs 9C and 10C), but for yield effects were confounded with site and cultivar effects. Only for Rosecoco in Eldoret also a positive association was found for yield and quality within a cultivar. No associations existed between EC and seed yield (Fig. 9D) or weight per seed (Fig. 10D).

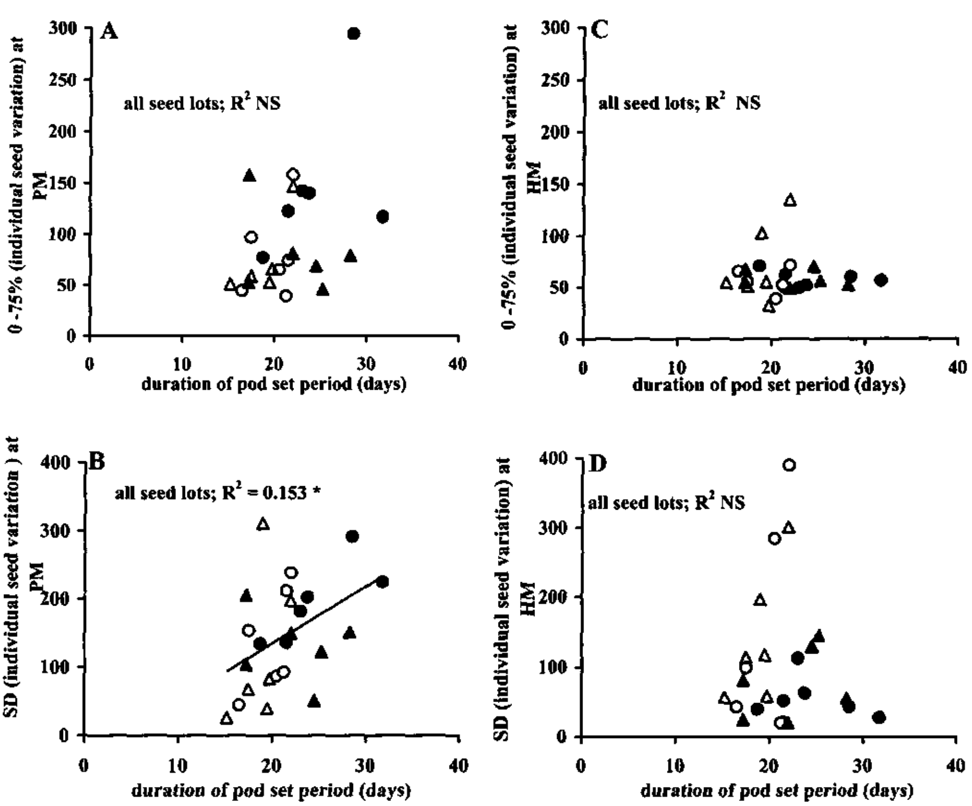

Fig. 7. Associations between parameters characterising variation in development within 24 seed crops and parameters characterising individual seed variation in EC in seeds from these crops. Associations between the duration of the pod set period and individual seed variation in EC characterized by the range 0 - 75% (A, C) or SD (B, D) at physiological maturity (A, B) and harvest maturity (C, D). Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. For explanation of R^2, line types and significance, see Fig. 3.

Discussion

Effects of temperature and rainfall on seed quality

The lower seed quality found at higher growing temperatures (Table 3, Fig. 3) and less rainfall (Table 3, Fig. 5) is in accordance with the well described effects of high temperature (e.g. Spears et al., 1997) and drought (e.g. Dornbos Jr and Mullen, 1991) on seed quality. The purpose of this work was to explain whether these weather

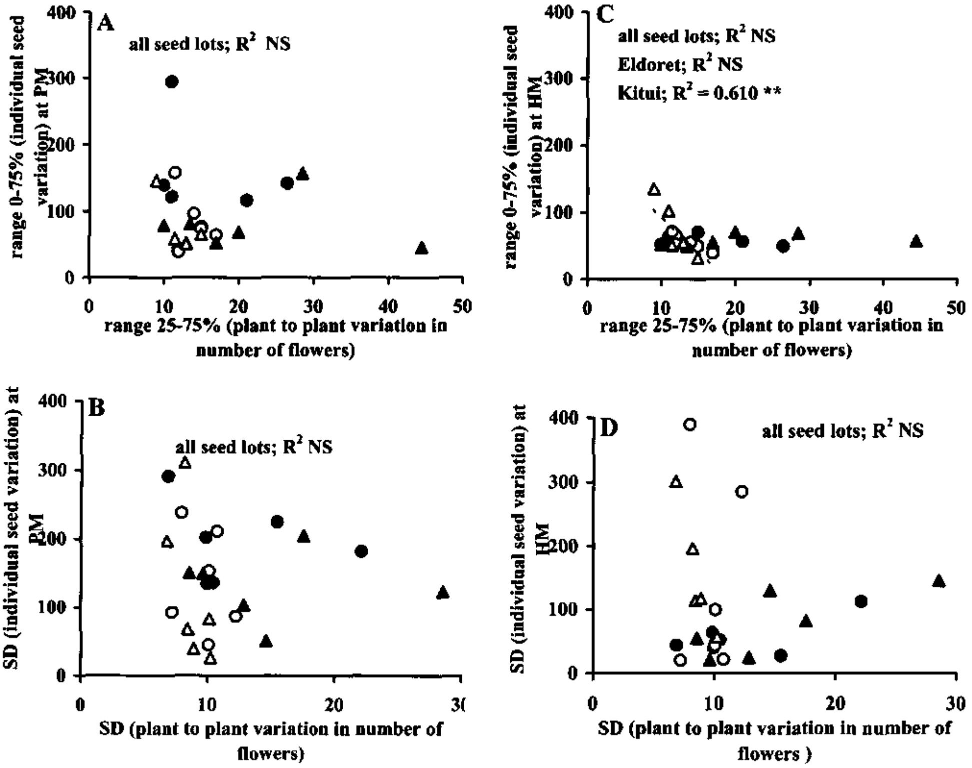

Fig. 8. Associations between plant-to-plant variation in number of flowers and individual seed variation in EC as characterized by ranges (A, C) or SD (B, D) at physiological maturity (A, B) and harvest maturity (C, D). Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. For explanation of R^2, line types and significance, see Fig. 3.

conditions exerted their effects mainly through influencing the maximum attainable quality at physiological maturity, through deterioration of quality between physiological and harvest maturity and/or through increasing variation within a crop. The latter might create large differences between individual seeds in the harvested seed lot, which reduces its quality (Muasya et al., 2001d).

On the basis of the results, we regard the maximum attainable quality a more important explanatory factor for quality differences at harvest than quality deterioration. However, the concept of quality being highest at physiological maturity (Harrington, 1972; Egli, 1998) proved not to be always tenable in the situations included in this study. In some cases, the percentage viable seeds still increased between physiological

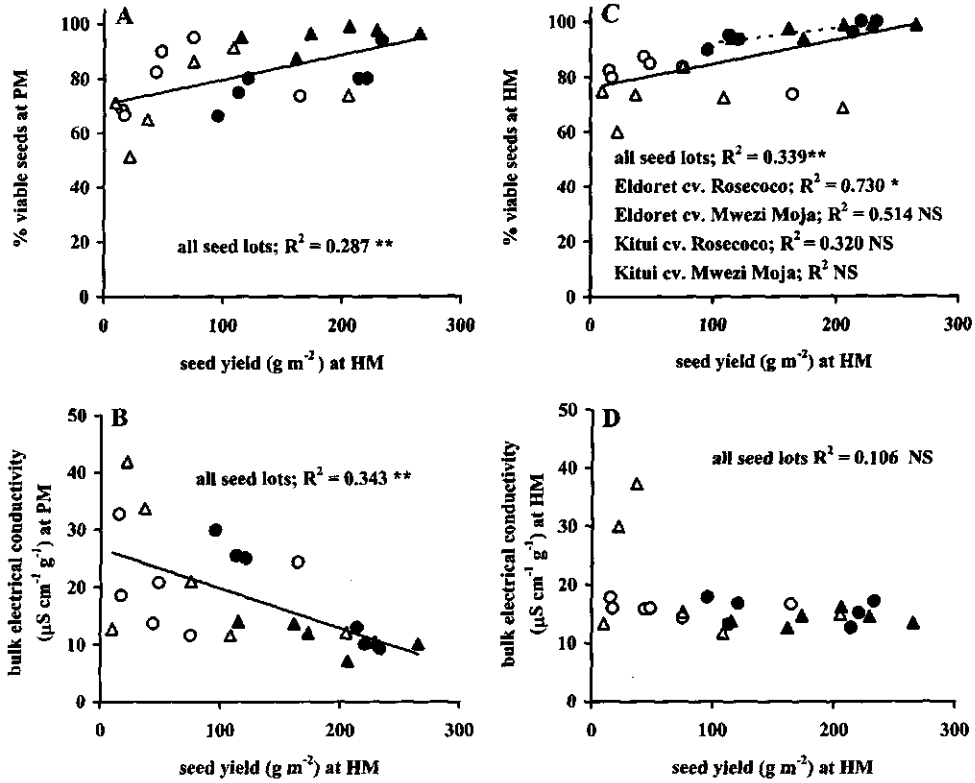

Fig. 9. Associations between seed yield (dry weight basis) at harvest maturity and percentage viable seeds (A, C) or electrical conductivity of seeds (B, D) in 24 common bean crops at physiological maturity (A, B) and harvest maturity (C, D). Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. For explanation of R^2, line types and significance, see Fig. 3.

maturity and harvest maturity. This occurred especially under the more favourable production conditions in Eldoret. At this site, the percentage viable seeds in cv. Rosecoco, whose growth habit is not uniform, increased from 79.2 to 95.8% during this period (Table 2). The more uniform cultivar Mwezi Moja already had achieved a high percentage viable seeds at physiological maturity. Increase in the percentage viable seeds of common bean after PM already was found by Muasya et al. (2001b). Also Zanakis et al. (1994) showed that maximum viability might occur after reaching physiological maturity. Nevertheless, over all crops, high quality at physiological maturity was positively associated with high quality at harvest maturity (Figs 2B and D). Quality sometimes seemed to decrease between physiological and harvest

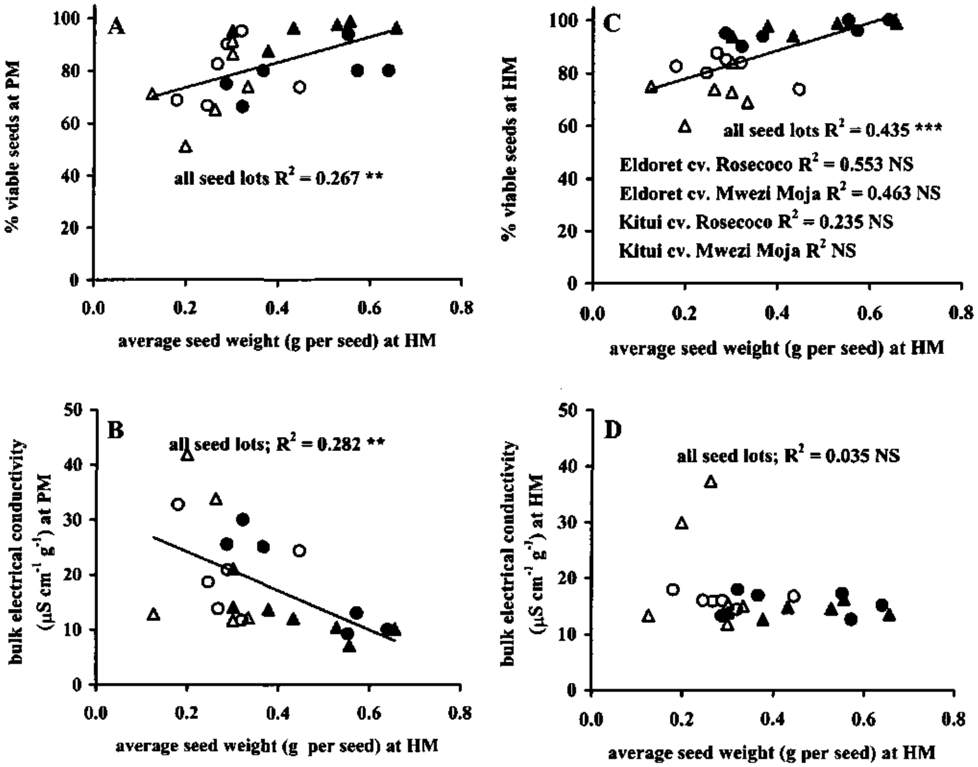

Fig. 10. Associations between average seed weight (dry weight basis) at harvest maturity and percentage viable seeds (A, C) or electrical conductivity of seeds (B, D) in 24 common bean crops at physiological maturity (A, B) and harvest maturity (C, D). Closed symbols: Eldoret, open symbols: Kitui. Circles: cv. Rosecoco, triangles: cv. Mwezi Moja. For explanation of R^2, line types and significance, see Fig. 3.

maturity, but this could not be proven statistically for individual seed lots.

There was no proof that external conditions reduced the quality of the seed lots produced through increasing variation within the crop, as measured by the length of the flowering period, the length of the pod set period or plant-to-plant variation in flower numbers. Often there were no significant associations between seed quality and these parameters (Table 5). When associations were significant they showed the opposite: longer pod set periods were associated with higher percentages of viable seeds at harvest maturity (Fig. 6C), and higher plant-to-plant variation in number of flowers was associated with better seed quality in some cases (Table 5). Longer flowering or pod set periods also were usually not related to higher variation in quality between individual seeds (Table 5, Fig. 7A, F, E). Only longer duration of the pod set period was associated with a higher individual seed variation in EC at physiological maturity, when this was measured as SD (Fig. 7B), or as CV% in Kitui (Table 5). Larger variations between plants in number of flowers measured as range 25 - 75% in Kitui were even associated with lower variation in quality between individual seeds at harvest maturity (Table 5, Fig. 7 B).

In fact, the results support the conclusion that conditions leading to good seed quality, could also lead to larger variation within a crop, i.e. longer flowering or pod set periods and larger variation in flower number between plants. Variation in flower number over plants was high when plants flowered abundantly (results not shown).

Relative importance of temperature and rainfall in different periods

Because crops were exposed to natural conditions, temperature and rainfall effects cannot be separated from each other. Because phases also were partly overlapping and temperatures during different phases were strongly correlated it is difficult to identify the most critical phases. In addition, temperature effects were confounded with site effects and factors other than only temperature may have exerted their influence as well. Nevertheless, the negative effect of a higher temperature was obvious and was found both at PM and at HM for both percentage of viable seeds and EC. The positive effect of sufficient rainfall, however, was mainly expressed at harvest maturity and only for the parameter percentage of viable seeds (Table 3, Fig. 5). We therefore surmise that in the range of conditions studied, temperature effects are more determinant for seed quality than rainfall effects.

Irrigation as a means of improving seed quality when temperatures are high and rainfall limited therefore does not seem to be prospectful. This is supported by the fact that when conditions in the warm area of Kitui still allowed reasonable yield to be achieved (Fig. 9C) seed quality was still relatively poor. Due to equipment failure, no data on relative humidity are available.

Electrical conductivity versus Tetrazolium

The two parameters used to assess seed quality, percentage viable seed as estimated by the tetrazolium test and electrolyte leakage as estimated by the EC test, were very different in their evaluation of seed quality at harvest maturity (e.g. Fig. 2C). EC seemed a less appropriate parameter to characterize seed quality differences between seed lots at that moment, because it was not very discriminating. EC values of 10 - 20 $\mu S\ cm^{-1}\ g^{-1}$ are generally regarded to indicate good seed quality (ISTA, 1995). All except two seed lots were in this low range, whereas for the same seed lots the percentage viable seeds as estimated by the tetrazolium test showed large differences, with values ranging between 69 and 100% (Fig. 2C). No other quality tests were carried out. However, because production conditions were chosen to cover a range from good to bad, tetrazolium test results better reflected the expected future performance of the seed lots produced. The reason for the poor discriminating value of the EC test at harvest maturity remains unknown, but probably could be related to characteristics of the seed coat. Panobianco et al. (1999) showed differences in electrolyte leakage between soybean cultivars differing in lignin content in the seed coat. Because of hand harvesting in our experiments, leakage could not have been caused by mechanical seed coat damage. Also no hard-seededness occurred in the samples tested.

Phenological assessment of physiological maturity and harvest maturity by colour markers

Our results show that phenological assessment of physiological and harvest maturity by use of colour changes in pods is not accurate. Especially at harvest maturity, seeds could still be far from attaining the desired moisture content of 20% when they were harvested at the moment pods had changed colour from green yellow to straw yellow colour. Nevertheless, pod colour change is a generally accepted method on small scale farms to estimate maturity. It is also a very common procedure in assignment of development scales in many crops for example in soybean (Fehr, 1971) and common bean (Chamma et al., 1990). The results imply that processes determining seed development and maturation and those affecting pod colour changes are not synchronized, and that conditions can differentially affect the two processes (cf. Housley et al., 1982; Egli, 1998).

Relationship between seed quality and seed yield and size

A general notion in seed production is that conditions conducive to high yields are also conducive to high seed quality. However, Adam et al. (1989) showed that, for soybean crops grown in different periods, the highest quality was not always attained when the highest yield was achieved. Our results confirm the general trend, but show that in production conditions where yield was low or seed sizes were small, quality was also not good (Figs 9 and 10). On the other hand, fairly good yields and fairly large seed sizes were not a guarantee for good quality. The seed crops deviating from the trend (those with fairly high yield but poor quality (Fig. 9C) were not those produced under the most extreme conditions because under those conditions also yield was reduced (results not shown).

Conclusions

- Weather conditions during seed lots production did not lead to lower quality seed lots through increasing the variation within the crop, as measured by duration of flowering or pod set periods or plant-to-plant variation in number of flowers.
- Averaged over a large number of seed crops, seed quality as measured by the tetrazolium test still increased between physiological maturity and harvest maturity. However, seed quality as measured by EC did not change significantly during the same period.
- The pattern of seed quality development between physiological maturity and harvest maturity was affected by weather conditions and cultivar. Low temperatures may allow prolonged increases in the percentage viable seeds.
- Over the range of conditions studied, high temperatures seemed more detrimental to seed quality development than limited rainfall. The two cultivars used differed in susceptibility to high temperature.
- For the common bean cultivars used, which lacked mechanical damage of the seed coat, EC was unsuitable as a method for detecting quality differences at harvest maturity, because in almost all seed lots quality was indiscriminately classified as "good".
- Processes determining the changes in pod colour and those determining changes in seed moisture content are differentially affected by external conditions.
- Phenological assessment of harvest maturity by changes in pod colour from green yellow to straw yellow or seed colour from green yellow to red purple is an unreliable method when seeds have to be harvested at a moisture content of 20% or lower.
- Production conditions conducive to low seed yield or low individual seed weights were also conducive to low percentage of viable seeds. However, conditions conducive to high seed yield, may not guarantee high percentage viable seeds.

Chapter 8

General discussion

8. General discussion

Introduction

In order to suggest improvements of common bean seed quality in Kenya, better scientific insight into growth and development of common bean seed, within-plant variation, and effects of weather conditions on plant-to-plant variation and within- and between-seed lot quality variation, was needed. To achieve these aims, the following research objectives were formulated:

- To increase insight into how common bean seeds within a crop develop with time and how viability, vigour and morphological markers are related to changes in the seed during seed development at the whole crop and in pods differing in earliness.
- To increase insight into how large the variation between seeds is and how the variation develops in common bean crops and in pods differing in earliness with time.
- To test whether large variation between individual seeds at harvest is associated with poor quality.
- To increase insight into how weather conditions at different sites affect seed quality, e.g. through the variation within a crop, through affecting maximum quality achievable during crop development and/or through quality deterioration between crop physiological and crop harvest maturity.

This general discussion concentrates on the major highlights emerging from the research and focuses on the differences in development of seeds from the entire crop and from pods differing in age, the development of seed quality over time, different methods of testing seed quality, and ways in which weather conditions affect quality in seed lots. Subsequently, suggestions for the appropriate moment and method of harvesting common bean seed crops in Kenya are made. Finally the practical implications of the research findings for the improvement of common bean seed quality in Kenya are outlined.

Seed quality development in common bean over time

The patterns of seed quality development in common bean as observed in this research are shown in Fig. 1. Seed dry weight initially increased slowly and more sharply thereafter as seed filling progressed attaining a maximum at a moisture content of 58%. This pattern of common bean seed development observed conforms to the general observations that seeds attain physiological maturity (PM) when moisture content is still high as reported in Chapter 1. However, the moisture content we observed was higher than the 52% reported in common bean by others (e.g. van de Venter et al., 1996). The difference could be attributed to differences in methodology of establishing the moment of PM or large variations in the environment and cultivar. Egli (1998) reported that inaccurate estimates of the moment at which dry weight is maximum, combined with rapid changes in seed water status around that time contribute to the large variation in estimates of seed moisture content at PM commonly reported in literature. However, the procedure used in this thesis did not estimate the moment of maximum dry weight and the moisture content at that moment, but directly estimated the moisture content at which maximum seed dry weight was achieved using non-linear regression analysis between moisture content and seed dry weight. Harvest maturity (HM) in this thesis was defined to occur at 20% moisture content in common bean unless stated otherwise. In mungbean, HM was reported at 19% (Dharmalingam and Basu, 1990) and in dry bean reports vary between 17% (van de Venter et al., 1996) and 20 - 25% (Kelly, 1988).

The observation that viability was still increasing beyond PM and reached a peak close to HM (Chapter 8, Fig. 1; Chapter 3) contradicts the general pattern that maximum viability is achieved before PM and that at PM it is maximum (Miles, 1985; Dornbos Jr., 1995; TeKrony and Egli, 1997; Egli, 1998; Chapter 1, Fig. 1). Our results suggest that in common bean seed development could still be going on at PM. It is not clear whether viability declined after achieving the peak. A decrease could be expected because of gradual seed deterioration as a result of ageing, but in our experiments this decrease was not significant (Chapter 3).

Seed vigour as measured by electrical conductivity (EC) increased linearly to a maximum at PM and remained constant thereafter (Fig. 1; Chapter 3). This observation is contrary to the general trend shown in Chapter 1 where vigour was maximum at PM and started to decline thereafter as reported by Miles (1985), Dornbos Jr (1995), TeKrony and Egli (1997) and Egli (1998) in soybean. The fact that EC remained constant after PM until HM suggests that conditions during our experiments (Chapter 3) did not favour deterioration of seed vigour between PM and HM.

Differences in seed quality as measured by tetrazolium test and electrical conductivity

Viability as measured by tetrazolium test (TZ) and vigour as measured by EC indicated different seed quality levels in common bean (Fig. 1). While viability achieved its maximum after PM and close to HM, vigour was already maximum at

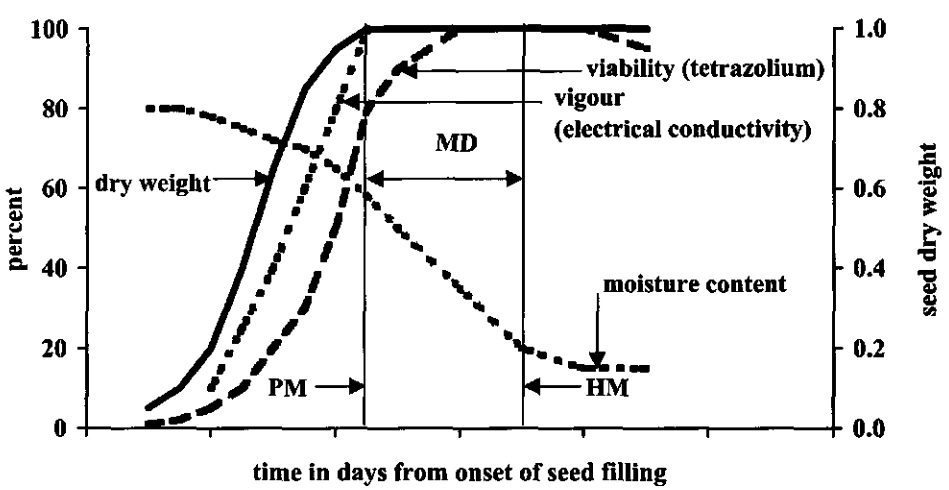

Fig. 1. Development of common bean seed dry weight (relative values), viability (relative values) as measured by tetrazolium test, vigour (relative values) as measured by the electrical conductivity test, and moisture content (percentage) over time from the onset of seed filling. Indicated are the moments of physiological maturity (PM), harvest maturity (HM) and the period maturation drying (MD).

PM. These observations would imply that at PM seed quality will be measured differently depending on the test used and at HM both viability and vigour are shown as maximum. Nevertheless, over seed lots produced under different conditions both tests were correlated (Chapter 7). However, TZ and EC indicated different levels of quality such that most seed lots could show low EC values indicating quality was good but the same seed lots showed low percentage viable seeds indicating poor quality (Chapter 7). TZ better reflected the expected quality of the seed lots. In addition, little variation in EC occurred between seed lots at harvest maturity.

It is unknown if this could be partly attributed to characteristics of the seed coat in the common bean cultivars used, restricting electrolyte leakage. Panobianco et al. (1999) reported a significant negative relationship between EC and seed coat lignin in soybean genotypes and concluded that the higher the amount of lignin in the seed coat, the lower the levels of seed exudates to the soaking solution and consequently the lower the EC, indicating higher vigour. No hard seeds were found in the seed lots produced in our experiments. In addition, no mechanical damage occurred due to hand harvesting. EC therefore could be a less appropriate parameter to characterize seed quality differences between seed lots at harvest if mechanical damage is absent.

Differences in seed physical attributes and quality within a crop

Seed dry weight

Differences were observed between seeds from different pod classes. Seeds from early pods showed a higher seed filling rate, achieved maximum dry weight earlier and achieved a higher maximum dry weight than seeds from medium and late pods (Fig. 2A; Chapter 2). Seeds from whole crop pods were comparable to the seeds from medium and late pods (Fig. 2A; Chapter 2). Consequently, PM was in fact achieved earlier in seeds from early pods than in seeds from late, medium and crop pods. Seed weight remained fairly constant after the maximum was achieved. These observations are attributed to differences in timing of development of pods of different pod classes. At the time the early pods are formed the crop is at the maximum vegetative mass with a high amount of available assimilates such that there is almost no competition between vegetative growth and reproductive growth. Early pods are fewer and are formed before seeds from the majority of the pods are formed and therefore they experience less competition in accumulating seed reserves (Cocks, 1990). Seeds from early pods also benefit from a high influx of assimilates during the phase the plant is switching from the vegetative to the reproductive phase (Bewley and Black, 1994). One of the reasons for the lack of differences between seeds from medium, late and whole crop pods could be the fact that the majority of the pods were classified as late and therefore showed the same pattern of dry weight increase as seeds from late pods.

Moisture content

In general seed moisture content in different pod classes declined gradually, followed by a sharper decline and finally a gentle decline (Fig. 2B; Chapter 2). Eventually a more or less stable moisture content was achieved. PM (58% moisture content) and HM (20% moisture content) were achieved earlier in seeds from early pods than in seeds from medium, late and crop pods but in all pod classes moisture content at PM was the same (Fig. 2B). By definition moisture content at HM was also the same in all pod classes. Maturation drying period was longer for seeds from early pods than in seeds from the other pod classes in cv. Mwezi Moja (Chapter 2). These results imply that seeds from early pods may take longer at high moisture content to dry from PM to HM than seeds from all the other pod classes. Due to their slower decline in moisture content, seeds from early pods may undergo higher in-plant deterioration when

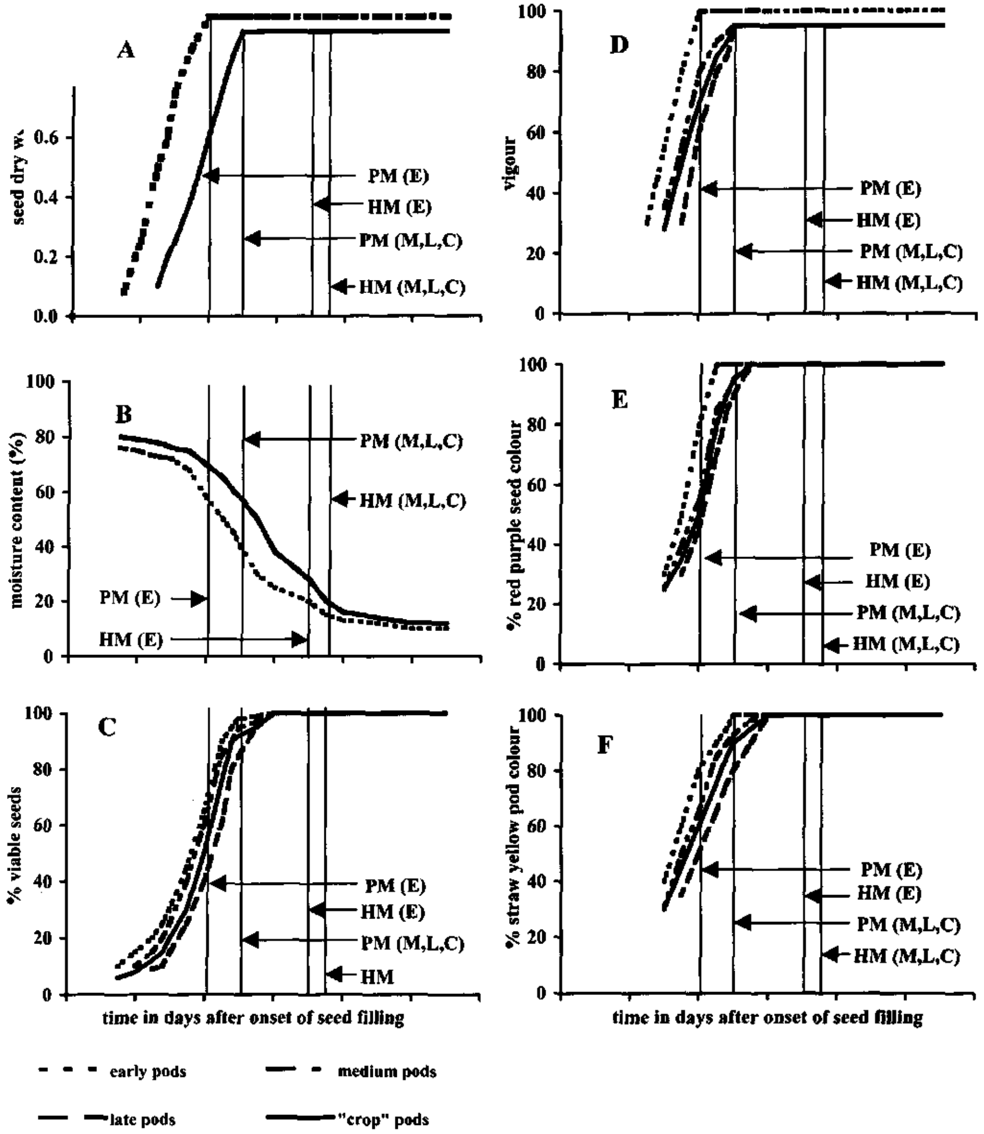

Fig. 2. Development of common bean seed dry weight (relative values), moisture content, viability as measured by tetrazolium test, vigour (relative values) as measured by electrical conductivity, seed colour and pod colour over time in days from the onset of seed filling in seeds from different pod classes within a crop. Vertical lines represent the moment of physiological maturity (PM) and harvest maturity (HM) for early pods (E), and medium, late and "crop" pods (M, L, C, respectively).

adverse weather conditions are experienced during maturation drying than seeds from later pods.

Viability

Viability showed logistic increase but we could not establish significant differences in the increase between seeds from different pod classes. No consistent differences were detected in the moment or moisture content at which maximum viability was achieved and the maximum achieved was the same in all pod classes (Fig. 2C; Chapter 3). Maximum viability was achieved well beyond PM in all pod classes which supports the hypothesis that seed development continues during maturation drying. At HM, there were no differences in viability between pod classes.

Vigour

Vigour as measured by EC increased sharply over time in all pod classes with seeds from early pods achieving maximum level earlier than seeds from medium and late pods (Fig. 2C; Chapter 3). Seeds from crop pods achieved maximum vigour level at a moment comparable to seeds from medium and late pods. This observation was associated with differences in timing of seed development with seeds from earlier pods achieving maximum quality level earlier than seeds from later pods. Maximum vigour in all pod classes was achieved around PM and was higher in seeds from early pods than in seeds from medium and late pods. Seeds from crop pods were comparable to seeds from medium and late pods.

Variation over time

The differences in development with time within the crop (Chapters 2 and 3) imply that there is variation in seed attributes and in development of seed quality attributes within the crop over time.

Seed variation in dry weight increased during seed filling and tended to level off after PM (Chapter 4). The increase in seed dry weight variation was earlier in seeds from earlier pods than in seeds from later pods which is attributed to seed filling starting earlier in the earlier pods. At PM maximum dry weight is achieved and variation is bound to stabilize because the weight does not change, and consequently variation cannot change anymore.

Seed moisture content standard deviation (SD) also increased earlier in seeds from early pods than in seeds from later pods (Chapter 4). The increase must have been partly caused by differences in the course of seed moisture decline as dry matter accumulation progressed. In seeds from all pod classes moisture content SD was lower at PM than during seed filling or after PM (Chapter 4). It is likely that this would be caused by a decrease in SD because of a gradual reduction in the absolute moisture content during seed filling, whereas differences in the timing of the onset of rapid moisture decline after PM would cause SD in moisture to increase temporarily. It is unknown why the low SD would coincide with PM since moisture content was declining gradually around the moment of PM was achieved. The very high variation in seed moisture content SD after PM as a result of rapid decrease in moisture content during maturation drying (Chapter 4) implies that there are differences in the timing of maturation drying in individual seeds.

Seed quality differences between seeds from the whole crop and from individual pods

It was hypothesized that the quality of seed from a uniform selection of pods of similar age might be higher than from all pods combined. However, in this research proper comparisons were hampered by the fact that the majority of the pods at the whole crop level were late (Chapters 2 and 3). Differences in timing between medium and late pods were small.

We observed that seeds from the whole crop were usually comparable in timing to seeds from later pods or had an intermediate behaviour. The moment maximum viability and vigour was achieved did not differ between seeds from the whole crop and those from pods differing in earliness in both seasons of our experiment (Chapter 3 and Fig. 2C, D). Maximum viability achieved did not differ in seeds from all pod classes probably because viability was approaching the maximum achievable in all pod classes and was strongly maintained (Dornbos Jr, 1995). Minimum EC was lower in seeds from early pods in both seasons, especially when calculated in relation to the change in moisture content and when compared to seeds from medium, late and whole crop pods (Chapter 3). Variation in individual seed EC over time was found to be higher in seeds from the whole crop than in seeds from pods differing in earliness (Chapter 4). These observations show that a crop is a mixture of seeds in different stages of development and may show lower vigour than selected pods at their optimum moment of harvesting.

Variation in seed quality in seed lots produced under different conditions

Conditions during production may create differences between individual seeds in a crop resulting in variation in quality within the seed lot, and between seed lots. This was found to be true in this research for seed lots harvested at PM and HM. At PM higher temperature during vegetative mass accumulation was associated with higher individual seed quality variation as quantified by SD and the range 0 - 75% in Kitui. Higher temperature before HM was associated with higher individual seed quality variation as quantified by SD at HM while higher temperature during flowering also was associated with higher individual seed variation as quantified by CV%. Rainfall was not associated with individual seed variation in EC.

Higher values of parameters quantifying individual seed variation namely, range 0 - 100%, mean - median, variance and SD, were found within seed lots with one or more seeds with high values, but the ranges 0 - 75% and 25 - 75% characterized individual seed quality variation in the bulk of seeds (Chapter 5). Coefficient of variation (%) did not characterize individual seed quality variation well nor was associated with bulk quality (Chapters 5 and 6). Higher variation in individual seed quality was found to be associated with a poor final bulk quality of the seed lots (Chapter 6). These results show that the final bulk quality of seed lots produced under different sets of conditions is related to variation in individual seed quality.

Mechanisms by which weather affects seed quality

Weather conditions could affect seed quality either directly by exerting their effect on the maximum obtainable quality at PM or through quality deterioration, or indirectly by creating large within-crop variation which would result in large differences between individual seeds and consequently reduce quality.

At PM, EC increased with increasing temperature over all seed lots produced at different sets of conditions and at HM higher temperatures were associated with lower percentage viable seeds (Chapter 7). At PM and HM higher rainfall was associated with a higher percentage viable seeds but no association was observed between rainfall and bulk EC. The higher seed quality found at lower growing temperatures and sufficient rainfall is in accordance with the well described effects of high temperature and drought on seed quality (Meckel et al., 1984; Gibson and Mullen, 1996). Cv. Mwezi Moja seemed to benefit more from low temperatures and suffer more from higher temperatures than cv. Rosecoco (Chapter 7).

Quality decrease between PM and HM was noticed but could not be assessed as significant for individual seed lots and therefore maximum attainable quality was considered a more important explanation for quality differences at harvest than quality deterioration.

Associations between bulk quality and within-crop variation

It was hypothesized that large variation in seed quality could result from large variation within the crop. Our results prove that weather conditions did not lead to lower quality seed lots through increasing the variation within the crop, as measured by long flowering or pod set periods or a high plant-to-plant variation in number of flowers. Instead, larger variations between plants in number of flowers were even associated with lower variation in quality between individual seeds at HM (Chapter 7).

We observed that longer duration of pod set period was associated with a higher individual seed quality variation when the variation was measured as SD or CV% in Kitui (Chapter 7). This was as expected but we also observed that larger variation between plants in number of flowers in Kitui were even associated with lower variation in quality between individual seeds at harvest maturity. Also, conditions supportive for high yields or high weight per seed were generally found to support high quality (Chapter 7).

Associations observed between seed quality and within crop variation led to the conclusion that conditions leading to a good quality seed could also lead to a larger variation within a crop (Chapter 7). For example, higher rainfall could lead to longer flowering period, podding period and may lead to higher within crop variation (Meckel et al., 1984; Chapter 7), but could also give rise to better seed development leading to better quality (Chapter 7; Egli, 1998).

Estimating when to harvest

The questions of how to predict the moment of PM and HM accurately in the field and how to avoid loss of seed quality through ageing are often faced by common bean seed growers. Based on seed moisture content PM in common bean has been reported (e.g. Kelly, 1988; van de Venter et al., 1996). We identified PM at around 58% seed moisture content and defined HM at around 20% moisture content (Fig. 1; Chapter 2). Morphological markers for PM and HM based on plant, pod or seed colour have been identified in other crops. Morphological markers of PM have been identified, e.g. kernel black layer in maize (Daynard and Duncan, 1969), yellow or black pod colour in soybean (Crookston and Hill, 1978; Egli, 1998) and brown seed colour in common bean (Chamma et al., 1990). In soybean 95% brown pod colour has been identified as a morphological marker for HM. Results presented in Chapter 2 showed that change of the colour of pods from green to green yellow was less well related to the moment of PM than the moment 100% of the seeds achieved their red purple colour pattern (Fig. 2F; Chapter 2). Regardless of cultivar, season and pod class, the moment that 100% of the seeds achieved the red purple colour pattern was at PM or just after PM (Fig. 2E; Chapter 2). However, in practice phenological assessment of the moment of PM by pod and seed colour led to seeds harvested around the targeted moisture content of 58%, but obvious exceptions occurred where seeds were harvested at PM when moisture content was around 35% (Chapter 7). This makes the moment all seeds within a crop or crop fraction achieve the final red purple colour pattern a very good, but not completely accurate indicator of PM. These results imply that through the use of pod or seed colour PM could not be accurately identified in all seed lots. Because in our experiments maximum quality was achieved well after PM, identification of the moment of PM may not have a practical value.

The 100% change of the colour of pods from green yellow to straw yellow proved not a reliable indicator of HM (i.e. a moisture content of 20% or less) for pods from the whole crop and pods differing in earliness. Early and medium pods had already achieved their final straw yellow colour at moisture contents that were much higher than 20%, whereas late pods achieved their final straw yellow colour at moisture contents higher or lower than 20% (Chapter 2). For pods in the whole crop, 100% of the pods achieved their final straw yellow colour at moisture content above or below 20% (Chapter 2). Phenological assessment of HM by pod colour and seed colour proved to be even more difficult than phenological assessment of PM for seed lots produced under different set of conditions. Almost half of the seed lots indeed had much higher moisture contents than the desired 20% with a maximum of 58%, whereas the remaining seed lots had moisture contents of 14 - 18% which could be expected when seeds were rapidly drying around HM (Chapter 7).

Although leaf colour has been used to indicate different stages of crop growth, it is usually more variable, showing greater environmental and cultivar effects than seed characteristics such as weight or moisture content and therefore cannot be reliably used to indicate a specific moment of seed development (Housley et al., 1982).

We therefore conclude that seed moisture content was a more reliable method of estimating when to harvest than using pod, seed or leaf colour markers in common bean.

Method of harvesting

It was hypothesized that harvesting of common bean crops simultaneously could lead to seed lots consisting of seeds differing in age and may show high individual seed variation and subsequently poor bulk quality. Indeed our results prove that higher individual seed quality variation was associated with final bulk quality of the seed lot (Chapter 6). Within a crop vigour of seeds from earlier pods at their optimum moment of harvesting was higher than the vigour of seeds from all pods combined (Chapter 3). Also, individual seed variation in EC over time was found to be higher in seeds from whole crop pods compared to seeds from early pods (Chapter 4). These results imply that selective harvesting of pods of the same age could improve the uniformity of the seeds within the seed lot produced and subsequently the final bulk quality. This was actually shown for the seeds from early pods.

Implications for improving common bean quality in Kenya

- Picking pods of the same age selectively, based on 20% seed moisture content, should improve the uniformity among the seeds harvested and consequently the final quality.
- Pod or seed colour should not be used as an indication of when to harvest common bean crops because colour changes are not consistent over the whole crop and are influenced by external conditions in a way different from the effects on seed quality.
- Within a cultivar, seed lots with small seeds are more likely to possess lower quality than seed lots with larger seeds. However, large seed is not a guarantee for good seed quality in common bean.
- When quantifying the quality of common bean seed lots, electrical conductivity (EC) should not be used alone because it may not be a reliable parameter to characterize seed quality in common bean in the absence of mechanical damage.
- Conditions in Kitui are not suitable for common bean seed production because seed lots produced there always showed lower quality than those produced in Eldoret.
- Cv. Mwezi Moja is more sensitive to high temperature than cv. Rosecoco with regard to the quality of the seed produced.
- In production conditions where yield was lower, seed quality was also lower.

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Summary

Introduction

In Kenya, common bean is the most important pulse crop second only to maize as a food crop. The total area under bean cultivation per annum is estimated to be 500,000 ha with actual bean yields reported as 250 kg ha^{-1} in mixed cropping and 700 kg ha^{-1} in pure stands. This yield is low compared to potential yield of up to 5000 kg ha^{-1}.

Use of poor quality seed, low soil fertility, adverse weather conditions and incidence of pests and diseases have been identified as some of the major constraints to bean production in Kenya. While substantial research work has been done on breeding for improved cultivars, response to soil fertility, and pest and disease control, this thesis focused on production of good quality seeds.

In Kenya, common bean is produced by small-scale farmers using farm-saved seed produced and stored as a grain crop without adhering to standard seed quality regulations e.g. isolation and roguing of offtype plants and without focusing on high viability or seed vigour, weeds, pests and diseases. The use of this type of seed can lead to production of a seed of reduced vigour because both poor genetic make-up and physiological quality of seed sown can be transmitted to the seed produced.

It was hypothesized that increased insight into development of common bean seed quality during crop production and into how conditions during production affect the seed quality could ultimately lead to production of better quality seed, and consequently increase common bean yields in Kenya.

The research objectives were:

- To increase insight into how common bean seeds within a crop develop with time and how viability, vigour and morphological markers are related to changes in the seed during seed development at the whole crop and in pods differing in earliness.
- To increase insight into how high the variation between seeds is and how the variation develops in common bean crops and in pods differing in earliness with time.
- To test whether large variation between individual seeds at harvest is associated with poor quality.
- To increase insight into how weather conditions at different sites affect seed quality, e.g. through the variation within a crop, through affecting maximum quality achievable during crop development and/or through quality deterioration between crop physiological and crop harvest maturity.

Seed quality development in common bean over time

In Chapter 2, seed development over time was studied. Physiological maturity (PM) was defined as the moment maximum seed dry weight was achieved. The pods of different earliness were made up of early, medium, and late pods as compared to the whole crop. Consistently over two seasons, two cultivars and in all pod classes, PM was achieved when moisture content was 58%. The moment of PM tended to be earlier in seeds from earlier pods. The moment all seeds within a crop or pod fraction achieved the final red purple colour pattern indicated PM well, though not completely accurate. Harvest maturity (HM) was defined to occur at 20% moisture content. The period of seed drying from 58 - 20% moisture content was longer in seeds from earlier pods in cv. Mwezi Moja, but not in cv. Rosecoco. Changes in pod colour could not be used to predict the moment of harvest maturity consistently over pods from different classes.

In Chapter 3, maximum seed viability as measured by the percentage of viable seeds (tetrazolium test) was achieved well beyond PM and closer to HM. This was contrary to the expectation that maximum viability would be achieved before PM. No differences were found in the moment at which maximum viability was achieved in different pod classes in Season 1. However, in Season 2, maximum viability was achieved earlier in seeds from early (cv. Rosecoco) or medium (cv. Mwezi Moja) pods than in seeds from late pods. Seeds from whole crop pods did not differ from seeds from pod earliness classes in the maximum viability achieved during the growing seasons in these experiments. Vigour was measured by electrical conductivity divided by the seed weight (EC). Maximum seed vigour (minimum EC) was achieved around the moment of PM in all pod classes. Minimum EC tended to be achieved earlier in seeds from earlier pods than in seeds from later pods. At the moment maximum vigour was achieved, earlier seeds also showed a better vigour than the later seeds, especially in Season 2. The vigour of seeds from the individual pod classes at their optimum moment of harvesting was higher than the vigour of seeds from all pods combined.

In Chapter 4, results of individual seed variation in seed and quality attributes over time showed that the highest seed variation in moisture content was observed after PM but before HM. Minimum electrical conductivity variation was achieved around PM in seeds from all pod classes in both cultivars and seasons. Maximum seed variation in weight and moisture content over time was earlier and lower in seeds from early pods than in seeds from medium, late or "crop" pods. Electrical conductivity variation was lower in seeds from early pods than in seeds from medium and late pods, at least in one season. Seed variation in weight, moisture content and electrical conductivity was higher in seeds from whole crop than in the seeds from pods of the different classes of earliness.

Effects of weather conditions on seed quality

Variation in seed quality in seed lots produced under different conditions

In Chapter 5, it was observed that conditions during seed lot production could create large differences between individual seeds. This resulted in large variation in quality within a seed lot but also between seed lots. Several variation parameters that quantify variation in EC per seed were assessed. Electrical conductivity of individual seeds in seed lots usually was not normally distributed, but showed a skewness towards high values indicating there were seeds within the seed lots deviating from the majority. The variation observed was quantified differently by different variation parameters. Higher values of variation parameters range 0 - 100%, mean - median, variance and SD were found in seed lots with one or more seeds with very high values (outliers). The ranges of which the samples contained 0 - 75% and 25 - 75% characterized individual seed quality variation in the bulk of seeds. CV% was not a good parameter for quantifying individual seed variation in EC within seed lots because, it was lower in seed lots with many outliers than in seed lots with only one outlier. Ln transformation of the EC data before calculation of the variation parameters reduced skewness of the distribution pattern, but did not give rise to more suitable parameters describing variation in EC.

The purpose of Chapter 6 was to test whether a larger variation between individual seeds at harvest in seed lots from different production conditions was also associated with a poorer quality of the entire seed lot. Variation between individual seed electrical conductivity (EC) was quantified using the parameters mean - median, SD, CV% and the range 0 - 75%. Over all 24 seed lots, low variation in individual seed EC as quantified by the parameters mean - median, SD and the range 0 - 75%, was found to be associated with good quality as measured by low bulk EC and high percentage viable seeds at PM. At HM most seed lots showed bulk EC values comparable to each other. Also differences between most seed lots in parameters quantifying individual seed variation were smaller. Consequently associations found were clearer at PM than at HM. No relationship was found between variation in individual seed EC measured by CV% and bulk quality over seed lots. The associations between bulk quality and individual seed variation were different for seed lots produced at different sites, indicating that both the degree of variation and the level of individual seed quality determined bulk quality.

Association between bulk quality and within-crop variation

In Chapter 7, the negative influence of high temperature and limited rainfall on seed quality was confirmed. Different weather conditions did not lead to lower quality seed lots through increasing the variation within the crop, as measured by long flowering or pod set periods or a high plant-to-plant variation in number of flowers. Instead, larger variations between plants in number of flowers were even associated with lower variation in quality between individual seeds at HM. The most important effects of weather conditions seemed to be on the maximum quality obtained during crop growth. The pattern of seed quality development between PM and HM was affected by weather conditions and cultivar. Low temperatures may allow further increases in the percentage viable seeds between PM and HM. Over the range of conditions studied, high temperatures seemed more detrimental for seed quality than limited rainfall. Cv. Mwezi Moja was more susceptible to temperature than cv. Rosecoco.

Processes determining the changes in pod colour and those determining changes in seed moisture content apparently were differently affected by external conditions. Therefore phenological assessment of HM by changes in pod colour from green yellow to straw yellow was an unreliable method when seeds have to be harvested at moisture contents of 20% or lower.

Differences in seed quality as measured by tetrazolium test and electrical conductivity

In the general discussion (Chapter 8), differences in seed quality as measured by tetrazolium test (TZ) and electrical conductivity (EC) are further discussed. While maximum viability as measured by tetrazolium test (TZ) was achieved beyond PM and close to HM, vigour as measured by electrical conductivity (EC) was already maximum at PM implying seed viability development was still continuing after PM. At HM both viability and vigour were still at their maximum level. EC and TZ, however, classified common bean quality at harvest differently.

In almost all seed lots produced under different sets of conditions seed quality measured by EC was classified as "good" whereas at the same time seed quality as measured by TZ varied between PM and HM. TZ reflected more the expected quality.

Future perspectives

The objectives of this research have been met and the practical implications of the research findings to the improvement of common bean seed quality in Kenya have been outlined. However, there is need to verify the findings of this research at farm level and assess the level of seed quality improvement due to the adoption of scientific insights.

Samenvatting

Inleiding

De landbouwstamboon (*Phaseolus vulgaris* L.) is de belangrijkste peulvrucht in Kenia. Na maïs is het zelfs het tweede voedselgewas. Het totale areaal boon bedraagt jaarlijks ongeveer 500.000 ha. De actuele boonopbrengsten zijn 250 kg ha^{-1} in mengteelt en 700 kg ha^{-1} in monocultuur. Dit zijn lage opbrengsten vergeleken met potentiële opbrengsten die wel 5000 kg ha^{-1} kunnen bedragen.

Tot de belangrijkste opbrengstbeperkende factoren in Kenia behoren een slechte zaaizaadkwaliteit, lage bodemvruchtbaarheid, ongunstige weersomstandigheden en ziekten en plagen. Waar het produceren van betere rassen, de effecten van bodemvruchtbaarheid, en de beheersing van ziekten en plagen al veel aandacht hebben gekregen in het onderzoek, richt dit proefschrift zich op de productie van goed zaaizaad.

In Kenia wordt de boon geteeld op kleinschalige bedrijven die tevens hun eigen boerenzaaizaad produceren. Dit zaaizaad wordt geproduceerd en bewaard alsof het bonen voor consumptie betreft. Tijdens de teelt wordt geen aandacht besteed aan de speciale maatregelen die in de zaaizaadproductie normaal zijn, zoals isolatie, ziekzoeken en verwijderen van afwijkende planten. Evenmin wordt er veel aandacht besteed aan maatregelen ter bevordering van de vitaliteit of het groeivermogen van het zaaizaad of aan de beheersing van onkruiden, ziekten en plagen. Het gebruik van dergelijk zaaizaad kan dan ook leiden tot een volgende generatie zaaizaad van matige kwaliteit. Immers, verminderde genetische en fysiologische kwaliteit kan van de ene generatie op de andere generatie zaaizaad worden overgedragen.

Het uitgangspunt van het onderzoeksprogramma was de veronderstelling dat meer inzicht in de ontwikkeling van de zaaizaadkwaliteit van de boon gedurende de zaaizaadproductie en in de effecten van omstandigheden daarop uiteindelijk zou kunnen leiden tot een betere zaaizaadkwaliteit en derhalve tot hogere boonproducties in Kenia.

De doelstellingen van het onderzoek waren:

- Het vergroten van het inzicht in het verloop van de ontwikkeling van de zaden binnen een gewas in de tijd en inzicht in hoe de vitaliteit, groeikracht en morfologische merkers gekoppeld zijn aan veranderingen in het zaad gedurende zijn ontwikkeling, zowel op het niveau van het gehele gewas als op het niveau van klassen van peulen die verschillen in vroegheid;
- Het vergroten van het inzicht in de variatie tussen zaden en hoe deze variatie in de tijd verloopt in het gehele gewas en in klassen van peulen van verschillende vroegheid;

- Het nagaan of een grote variatie tussen individuele zaden bij de oogst gepaard gaat met een slechte zaaizaadkwaliteit;
- Het vergroten van het inzicht in hoe de weersomstandigheden op verschillende locaties de zaaizaadkwaliteit beïnvloeden. Dergelijke invloeden kunnen werken via effecten op de variatie binnen een gewas, het verlagen van de maximaal haalbare kwaliteit gedurende de gewasontwikkeling en/of de afname van de kwaliteit gedurende de periode van fysiologische rijpheid tot oogstrijpheid op het niveau van het gehele gewas.

Ontwikkeling van de zaaizaadkwaliteit van de boon in de tijd

In Hoofdstuk 2 werd de ontwikkeling van het zaad in de tijd onderzocht. Fysiologische rijpheid (FR) werd gedefinieerd als het moment waarop het maximale drooggewicht van het zaad werd bereikt. De peulen werden onderverdeeld in drie klassen, te weten vroege peulen, middelvroege peulen en late peulen en deze werden vergeleken met de peulen van het gehele gewas. Fysiologische rijpheid werd in beide seizoenen, voor beide rassen en in alle peulklassen steeds bereikt bij een vochtgehalte van 58%. Het tijdstip van fysiologische rijpheid leek vroeger te vallen voor de vroege peulen. Het moment waarop alle zaden in een gewas of in een peulklasse het uiteindelijke rood-paarse kleurpatroon bereikten, was een goede indicator voor de fysiologische rijpheid, zij het dat het moment niet geheel accuraat werd voorspeld. Oogstrijpheid (OR) werd per definitie bereikt bij een vochtgehalte van het zaad van 20%. De periode van het drogen van het zaad van 58% vocht naar 20% vocht duurde bij het ras Mwezi Moja langer bij zaden van vroege peulen, maar dit gold niet voor het ras Rosecoco. Veranderingen in peulkleur konden niet worden benut om het moment van oogstrijpheid consistent over peulen van verschillende vroegheidsklassen te voorspellen.

In Hoofdstuk 3 werd de maximale zaadvitaliteit (vastgesteld op basis van de tetrazoliumtest) ruim na FR bereikt en dichter bij het moment waarop de zaden oogstrijp werden. Dit was in strijd met de verwachting dat de maximale vitaliteit al vóór FR bereikt zou zijn. In het eerste groeiseizoen werden geen verschillen tussen de verschillende peulklassen waargenomen ten aanzien van het moment waarop de maximale zaadvitaliteit werd bereikt. Echter, in het tweede groeiseizoen werd de maximale vitaliteit eerder bereikt in zaden van vroege peulen (bij Rosecoco) of middelvroege peulen (bij Mwezi Moja) dan in zaden van late peulen. Zaden uit peulen van het gehele gewas verschilden niet van zaden uit de verschillende peulklassen in de maximale vitaliteit die bereikt werd tijdens de groeiseizoenen in deze experimenten. De groeikracht van de zaden werd vastgesteld met behulp van de electrische geleidbaarheid per eenheid zaadgewicht. Maximale groeikracht (minimale geleidbaarheid) werd in alle peulklassen bereikt rond het moment van FR. De minimale geleidbaarheid leek vroeger te worden bereikt in zaden van vroege peulen dan in zaden van late peulen. Op het moment dat de maximale groeikracht werd bereikt, bleken de zaden van vroege peulen een betere groeikracht te hebben dan de zaden van de latere peulen. Dit gold vooral voor het tweede groeiseizoen. De groeikracht van zaden uit de afzonderlijke peulklassen was op het optimale tijdstip groter dan de groeikracht van zaden van alle peulklassen samen.

Onderzoek beschreven in Hoofdstuk 4 toonde aan dat de variatie in fysische zaadeigenschappen en in zaadkwaliteit tussen individuele zaden in de tijd veranderde en wel zodanig dat de hoogste variatie in vochtgehalte werd waargenomen na het bereiken van de FR, maar vóór het bereiken van de OR. De variatie in electrische geleidbaarheid was het kleinst rond FR. Dit gold voor de zaden van alle peulklassen, in beide rassen en beide groeiseizoenen. De maximale variatie in zaadgewicht en in vochtgehalte werd vroeger bereikt en was kleiner voor zaden van vroege peulen dan voor zaden van middelvroege of late peulen, en ook dan voor zaden uit het gehele gewas. De variatie in electrische geleidbaarheid was lager in zaden van vroege peulen dan in zaden van middelvroege of late peulen, althans in één groeiseizoen. De variatie in zaadgewicht, vochtgehalte en electrische geleidbaarheid was hoger in zaden van het gehele gewas dan in zaden van peulen van de verschillende vroegheidsklassen.

Effecten van weersomstandigheden op zaaizaadkwaliteit

Variatie in zaaizaadkwaliteit in partijen zaaizaad die onder verschillende omstandigheden werden geproduceerd

In Hoofdstuk 5 werd gevonden dat de omstandigheden tijdens de productie van het zaaizaad grote verschillen konden veroorzaken tussen individuele zaden. Dit resulteerde in een grote variatie in zaaizaadkwaliteit binnen een partij, maar ook in een grote variatie tussen partijen. Verschillende parameters werden berekend die de variatie in electrische geleidbaarheid (EC) tussen individuele zaden kwantificeren. De EC van individuele zaden binnen partijen vertoonde meestal geen normale verdeling. Er was sprake van scheefheid naar hogere waarden hetgeen duidt op zaden binnen een partij die sterk afweken van het gros van de zaden. De verschillende variatieparameters kwantificeerden de waargenomen variatie verschillend. Hogere waarden van de variatieparameters "bereik 0-100%", "gemiddelde minus de mediaan", "variantie" en "standaardafwijking" werden gevonden in partijen zaaizaad waarvan de monsters één of meer zaden met zeer hoge waarden bevatten. Het "bereik 0-75%" en het "bereik 25-75%" gaven een waardevolle karakteristiek van de variatie tussen individuele zaden voor de bulk van de zaden. De "variatiecoëfficiënt" bleek geen nuttige parameter te zijn om de variatie tussen individuele zaden te kwantificeren, omdat lagere waarden werden gevonden in partijen met veel extreme waarden dan in partijen met slechts één extreme waarde. Indien de waarden voor de electrische geleidbaarheid ln getransformeerd werden alvorens de variatieparameters werden berekend, dan bleek de scheefheid van de verdeling af te nemen. Transformatie leidde echter niet tot parameters die de variatie in electrische geleidbaarheid beter kwantificeerden.

Het doel van Hoofdstuk 6 was na te gaan of bij de oogst een grotere variatie tussen individuele zaden van partijen zaaizaad die onder verschillende omstandigheden waren geproduceerd, gekoppeld was aan een slechtere kwaliteit van de totale partij. Variatie in electrische geleidbaarheid tussen individuele zaden werd gekwantificeerd met behulp van de parameters "gemiddelde minus de mediaan", de "standaardafwijking", de "variatiecoëfficiënt" en het "bereik 0-75%". Voor alle 24 partijen zaaizaad bleek een lage variatie in de electrische geleidbaarheid van individuele zaden zoals vastgelegd in de parameters "gemiddelde minus mediaan", de "standaardafwijking" en het "bereik 0-75%" te zijn gekoppeld aan een goede zaaizaadkwaliteit van de gehele partij, gemeten als een lage electrische geleidbaarheid en een hoog percentage vitale zaden bij FR. Op het moment van OR bezaten de meeste zaaizaadpartijen vergelijkbare waardes voor de electrische geleidbaarheid. Bovendien waren de verschillen in de parameters die variatie tussen individuele zaden kwantificeren, tussen de meeste partijen kleiner. Daarom waren de bovengenoemde koppelingen duidelijker bij FR dan bij OR. Er werd geen verband gevonden tussen de variatie in electrische geleidbaarheid van individuele zaden zoals bepaald door de "variatiecoëfficiënt" en de bulkkwaliteit over de verschillende partijen. De relaties tussen bulkkwaliteit en variatie tussen individuele zaden waren verschillend voor zaaizaadpartijen geproduceerd op verschillende locaties. Dit duidt er op dat zowel de mate van variatie als het niveau van kwaliteit van het individuele zaad de bulkkwaliteit kunnen bepalen.

Relaties tussen bulkkwaliteit en binnen-gewas variatie

In Hoofdstuk 7 werd de negatieve invloed van hoge temperaturen en beperkte regenval op de zaaizaadkwaliteit bevestigd. Verschillende weersomstandigheden bleken niet te leiden tot een lagere zaaizaadkwaliteit van partijen als gevolg van een toename in de variatie binnen een gewas, zoals die werd vastgesteld op basis van een lange periode van bloei of peulzetting of op grond van een hoge tussen-plant variatie in aantal bloemen. Integendeel, grotere variaties tussen planten in het aantal bloemen leken bij OR gekoppeld aan een lagere variatie in kwaliteit tussen individuele zaden. Weersomstandigheden leken voornamelijk de maximale kwaliteit te beïnvloeden die tijdens de gewasgroei bereikt kon worden. Het ontwikkelingspatroon van de zaadkwaliteit tussen FR en OR werd beïnvloed door weer en ras. Lage temperaturen maken het wellicht mogelijk dat het percentage vitale zaden tussen FR en OR verder toeneemt.

Voor het traject van omstandigheden dat onderzocht werd, gold dat hogere temperaturen schadelijker voor de zaaizaadkwaliteit leken te zijn dan een tekort aan neerslag. Het ras Mwezi Moja was gevoeliger voor temperatuur dan het ras Rosecoco.

De processen die de veranderingen in peulkleur bepalen en de processen die de veranderingen in het vochtgehalte van de zaden bepalen worden kennelijk op een verschillende wijze beïnvloed door de externe omstandigheden. Derhalve was het niet mogelijk om het moment van OR op een fenologische wijze vast te stellen door de verandering in peulkleur van groengeel naar strogeel te bepalen, als zaden moeten worden geoogst bij vochtgehaltes van 20% of lager.

Verschillen in zaaizaadkwaliteit op basis van een tetrazoliumtest of metingen van de electrische geleidbaarheid

In de Algemene Discussie (Hoofdstuk 8) werd nader ingegaan op de verschillen in zaaizaadkwaliteit zoals gemeten met de tetrazoliumtest (TZ-test) of met de methode van electrische geleidbaarheid (EC-test). De maximale vitaliteit zoals bepaald met de tetrazoliumtest werd pas na de FR en vlak voor de OR bereikt. De groeikracht zoals bepaald op basis van electrische geleidbaarheid was al maximaal bij FR. Dit suggereert dat de ontwikkeling van de zaadvitaliteit nog doorging na de FR. Bij de OR waren overigens zowel de vitaliteit als de groeikracht nog steeds maximaal.

De EC- en TZ-testen waardeerden de zaaizaadkwaliteit van boon echter verschillend. In vrijwel alle partijen zaaizaad die onder verschillende omstandigheden werden geproduceerd, werd de kwaliteit op grond van de EC-test als "goed" gekwalificeerd, terwijl volgens de TZ-test het percentage vitale zaden varieerde tussen 69% en 100%. De resultaten van de TZ-test gaven beter de verwachte resultaten weer.

Toekomstperspectief

De oorsponkelijke doelstellingen van het onderzoek beschreven in dit proefschrift zijn gerealiseerd. Daarnaast is in het proefschrift aangeduid wat de practische implicaties van de resultaten zijn voor het verbeteren van de zaaizaadkwaliteit van de boon in Kenia. Het is echter noodzakelijk om de resultaten in de praktijk nader te toetsen. Bovendien dient nog nader te worden vastgesteld in hoeverre het benutten van wetenschappelijke inzichten ook inderdaad leidt tot een betere zaaizaadkwaliteit in de praktijk.

Curriculum vitae

Reuben Masambia Muasya was born on 12th September 1960 in Kitui district, Kenya. After his primary and secondary education, he joined the Eldoret Institute of Agriculture where he was trained as an agricultural assistant and later worked with the Ministry of Agriculture before joining Moi Teachers College as an instructor in agriculture. In 1988, Reuben joined the University of Eastern Africa Baraton for his bachelor's degree in crop science. On completion in 1992, he joined Moi University as a graduate assistant in the department of Crop Production and Seed Technology, Faculty of Agriculture and immediately registered for a master of philosophy degree (M.Phil.) in agronomy, which he completed in 1996. On completion, Reuben was appointed as a tutorial fellow in the department and later was promoted to a lecturer. He took part in teaching of grain legume seed production and cereal seed production courses at postgraduate level. Other than a member of the Faculty of Agriculture Board in Moi University, he is also a member of the faculty board of examiners and the Faculty Graduate Studies Committee. He is also involved in research on alternative sources of nutrient replenishment using locally available agricultural residues. In September 1997, Reuben joined Wageningen University as part of a linkage programme between Moi University and Wageningen University to pursue a sandwich PhD study in seed quality development and variation in common bean. Reuben is married and a father of two boys and one girl.

Other publications by the author

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