NON-DESTRUCTIVE SEED EVALUATION WITH IMPACT MEASUREMENTS AND X-RAY ANALYSIS

W.J. van der Burg, H. Jalink, R.A. van Zwol, J.W. Aartse and R.J. Bino
DLO - Centre for Plant Breeding and Reproduction Research, P.O. Box 16,
6700 AA Wageningen, The Netherlands

Additional index words: acoustic impulse elasticity radiography image analysis imbibition germination usable transplants point of inflection

1. Summary

Non-destructive testing is important in the search for seed characteristics that relate to quality. It provides a means for consecutive testing on a seed by seed basis. If the tests are fast and can be automated they can form the basis for rapid new analysis methods or online sorting. X-ray analysis of tomato seeds enables us to predict seedling morphology. Acoustic analysis provides rapid physical assessment of seeds, which correlates with seed coat conditions. Both techniques can be automated and provide potential new sorting techniques. The X-ray technique provided us with some evidence for the origin of vigour differences in tomato seeds: morphological differences in seed embryos and the availability of endosperm proved to be crucial.

2. Introduction

Many techniques have been developed to estimate the quality of agricultural products including seeds (Mohsenin, 1986; Chen and Sun, 1991). Most of them however, result in the destruction of the sample which is investigated. Examples are moisture, germination, vigour, viability and health tests. If one wants to develop methods that can be used for sorting, the measurement techniques must be non-destructive. Other prerequisites are that the tests can be automated and that they are fast. Non-destructive methods also have the advantage of enabling more tests on one and the same seed. As a result correlations between test methods can be made on a seed-by-seed basis. Also, development processes can be followed in time. This can, for instance, be carried out with X-ray analysis (figure 1).

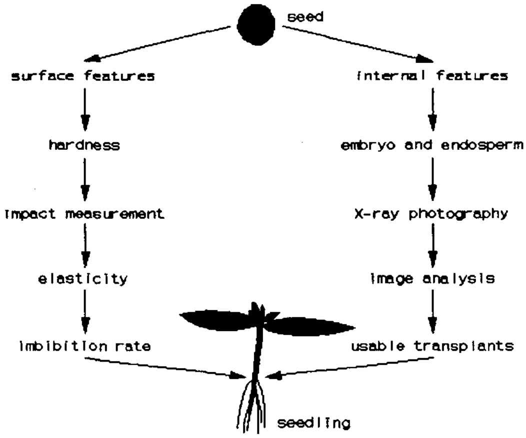


Figure 1. Different information obtained by two different non-destructive approaches.

A disadvantage of many physical techniques is that they provide physical information of which the correlation with physiological phenomena still has to be demonstrated. An example of this type of analysis is acoustic analysis.

3. Acoustic measurements

Acoustic measurements can be performed in different ways. Firstly one can drop an object onto a hard surface and register the sound of the impact: a true acoustic measurement. Secondly one can drop the seed onto a sensor and register the acceleration with an accelerometer or the force with a force transducer. Thirdly one can administer vibrations to one side of a seed and register the vibrations on the other side. After an outline of the different techniques, some results with the impact measurements will be presented.

True acoustic measurements

We have not experimented much with this technique because it has several drawbacks: firstly, one needs a 'silent room', a box which isolates the place of measurement from noises of the outside world. The frequencies produced by dropping seeds are in the range of up to around 30 kHz. So mostly in the area of frequencies which are all around us. Because real-time seed analysis has to be performed in relatively noisy areas, such as seed processing plants, this is a disadvantage. Someone banging a door or dropping a case would seriously affect the measurements. A second disadvantage is that one needs to transform the time signal into a frequency spectrum: a Fourier transform is needed. Although special Fast Fourier chip technology is available, it means a delay. And thirdly and most important of all, modelling and curve fitting in the frequency domain proved to be extremely difficult.

Impact measurements

The advantage of impact measurements using an accelerometer or a force transducer is that there is no signal when the seed is not in contact: the sensor produces a zero-signal with only some electrical 'noise', as in all electrical systems. We did some experimenting with accelerometers initially, but they proved to be impractical. The sensor must be able to move as a result of the impacting seed. The signal produced is the acceleration of the sensor rather than of the seed. The sensors tend to vibrate after an impact, which interferes with a following measurement. The sensor must be large enough to 'catch' the seed, which results in too much mass. If one would use a smaller sensor one should provide it with a contact disc in order to have enough surface. Such a disc again makes the mass too large and has the tendency to vibrate also, which interferes with the frequency spectrum. These were reasons to try force transducers.

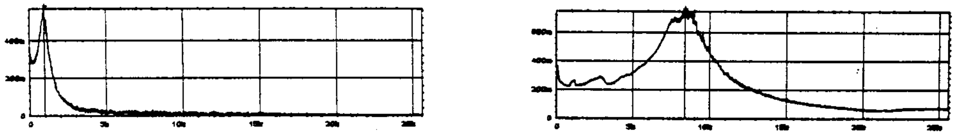
Force transducers have to be mounted on a solid support, eg a granite block. The signal is produced by strain in a piëzo-electric layer, there are no movements in the sensor. This means that the sensor can accept a new seed instantaneously.

Measurements in transmission

An other interesting option is the measurement of the physical nature of the seed interior by transmission measurement. To this end the seed is typically placed between two sensors, eg force transducers. One of the transducers is forced to vibrate with a sine wave or noise. The difference between the ingoing and outcoming signals is determined by dividing the latter by the first. In this way the Frequency Response Function (FRF) can be determined. Each material has its typical FRF, some are given in figure 2. Because each seed has to be placed between the sensors, the technique can be used for scientific investigations, rather than for real-time sorting. Some proposals have been made to use the principle for the testing of fruits (Yamamoto and Haginuma, 1982; Peleg et al., 1990)). We have concentrated on impact measurements so far.

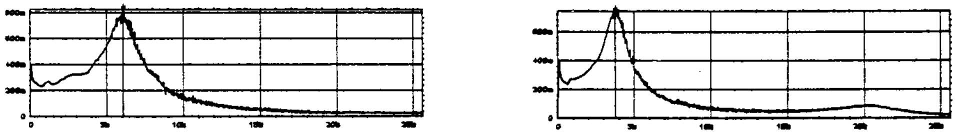
Impact analysis of pea seeds

The principle of the measurements is presented in figure 3. Seeds are dropped onto



rubber

pea



pvc

berry

Figure 2. Frequency response functions of some materials: rubber, pvc, pea seed, dry *Juniperus* berry.

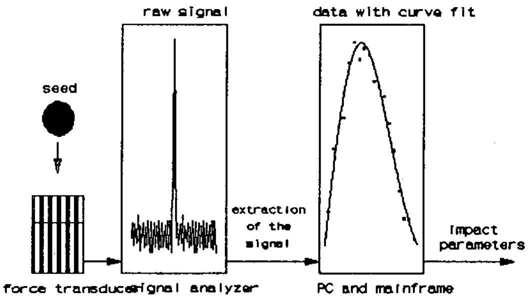


Figure 3. Principle of impact measurements.

a sensor placed on a rigid surface. The signal is multiplied and transferred to a signal analyzer (Brüel & Kjaer 2032) or a fast A/D converter. From there the signal is transferred to a PC, where the signal is isolated using our own programs written using a LabWindows™ software shell and transferred to a mainframe computer for storage. Signals are analyzed with a fit model developed by our group. This model produces values for several variables used in the model, eg the maximum force F_{max}, the pulse length [*p*], the elasticity [E], the coefficient of restitution [R], which is the speed at which the seed leaves the sensor divided by the speed at first contact and provides a measure for the loss of energy, and the asymmetry of the curve [Θ].

As soon as a seed makes contact (time = t_0) the signal starts to build up until a maximum when the seed has reached its maximum distortion. At that time F_{max} is reached. After that the seed starts to restore its shape until the moment t_p when the seed leaves the sensor. A rubber ball, a radish tuber, a fresh pea and other soft and elastic bodies produce regular symmetric bell-shaped curves (figure 4), the time-force signal. If damage is caused by the impact, eg when one drops the object from too far above the sensor, this is visible as irregularities in the first half of the curve. We hypothesize that the asymmetry is a measure for the 'perfectness' of the collision: if the curve is not symmetric, as can be seen in figure 5, right hand graph, where the second half of the curve is less steep, this would be an indication that the material is less elastic due to viscous losses or inhomogeneities.

Viscous losses were expected to be of less importance than losses to eg cracks or diseased spots, because seeds are rather dry and hard, more elastic than plastic. There-

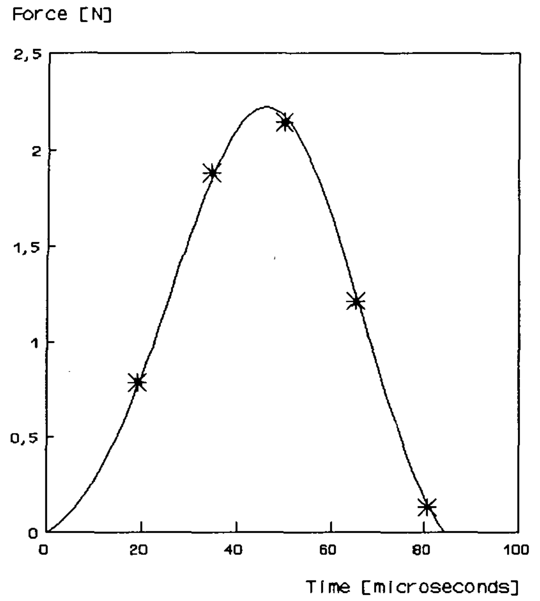


Figure 4. Typical bell-shaped force-time curve of the impact of an elastic body onto a rigid surface.

fore we investigated the influence of damage and disease on the time-force signal in order to compare this with physiological measurements. Because we have results per seed, we preferred an individual measure for seed quality. Since germination, viability and vigour are properties of populations, these could not be used. The individual measurement of seed leachate conductivity provides an accepted method, with proven correlations (AOSA, 1983; ISTA, 1987). However, the maximum leakage at a given point in time does not cater for the time-difference that can be observed between the start of leakage of different peas. We felt that the shape of the leakage curves (figure 6) would be more informative than the maximum per se. A mathematical model was developed (publication in preparation) to fit these curves. Using the first derivative the point of maximum leakage rate was determined, which coincides with the moment where the curve changes from concave to convex. We call this the point of inflection (PI) of the curve (figure 6). PI was compared with the elasticity measured for a test set of peas (figure 7). The elasticity correlates with the log of PI. Although the correlation is not very strong, it can be observed

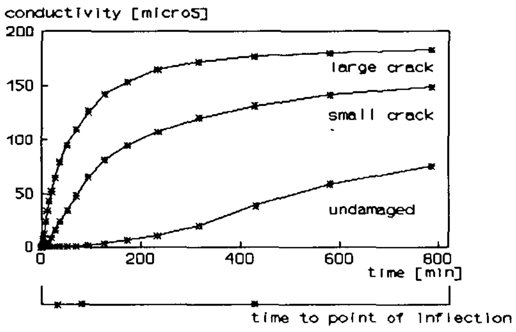


Figure 6. Conductivity of three peas showing a different time to point of inflection (PI).

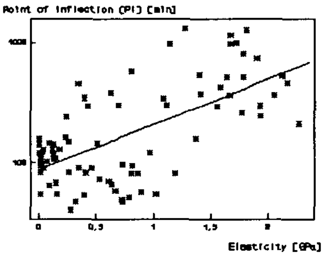


Figure 7. Correlation between acoustic data (elasticity) and conductivity (point of inflection) of peas.

that the softer seeds (these are the seeds with cracks and other forms of damage) form a cluster with very low PI values. This indicates that the seeds imbibe very rapidly, hardly forming any physical barrier to the penetrating water. Thus peas which have seed coat damage could be detected with impulse measurements.

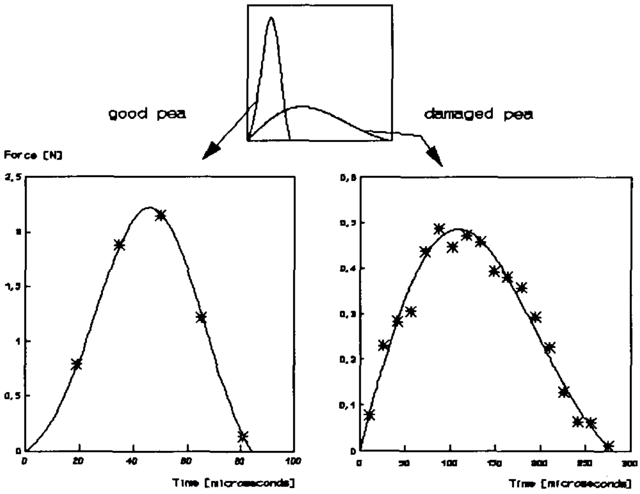


Figure 5. Force-time curves of a good (left) and a damaged (right) pea seed.

The next step will be to establish correlations with other physiologically important phenomena: the imbibition itself, germination and vigour. From the practical point of view it will be interesting to speed up the process and to develop a rapid seed sorting machine based on these principles. At the moment we are building new sensors to be able to measure small seeds also. Seeds like those of *Brassica* tend to produce resonances in the present sensors, which we would like to eliminate for a better evaluation.

4. X-ray analysis

X-ray provides a means of looking into seeds (Simak, 1991). The tomato seed is particularly suited for morphological study, since it contains a clearly defined embryo and separate endosperm (perisperm). The process of seed production of tomatoes is complicated and often results in poor quality seed. This is due to several factors, amongst which the growing conditions and processing steps are the most important. Regular evaluation of seed quality during the production stages is necessary. Our studies are aimed at understanding the mechanisms of seed formation and how the observed abnormalities arise. This will assist in establishing the most important parameters to look for.

Usable transplants

In the modern tomato growing industry where tomatoes are grown in greenhouses for the fresh market, the quality of seedling transplants has become a factor of major importance. The transplants are grown by specialised producers and sold to the crop growers. Thus, the aspect of the seedling has become very important in contrast with the past when the producers did their own sowing and transplanting. At present the quality of the seedling is often poor and subject to much debate, and the percentage usable transplants (UT) has become the major physical quality factor. The standard ISTA (ISTA, 1985) or AOSA (AOSA, 1989) germination tests do not predict the percentage UT. Therefore, grow out tests in the greenhouse are performed, but these are difficult to standardize. An X-ray evaluation procedure would not only assist the producers, but could also provide a rapid test as an alternative to the greenhouse procedure.

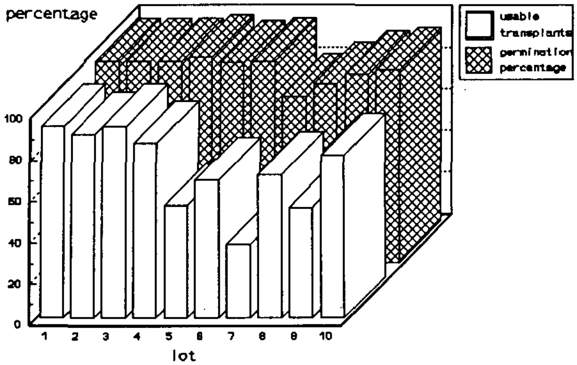


Figure 8. Standard germination test results and UT performance in the greenhouse.

Embryo morphology

It appears that the embryo lies in two basic configurations: either 'coiled' or 'spectacles' (Van der Burg et al., 1993). Although the coiled type seems to be the wildtype situation, *Capsicum* seeds only have coiled embryos, for instance, the spectacles type is not necessarily unfavourable. In both types, but more in the spectacles type, one can distinguish numerous forms of abnormality. Very unfavourable are those which show twisting or wrinkling and other forms of crippling of the cotyledons. In most cases the resulting seedling will have difficulty with coming out of the seed coat. These abnormalities often result in cotyledons which remain stuck in the seed coat for too long. Depending the treatments the seeds have undergone, the root may also show thinner root tips (eg with primed seeds), blunt root tips (with pregerminated seed) and partially emerged root tips (with sprouted seeds).

In our studies we have classified the embryos as 'normal' or 'abnormal'.

Endosperm and free space

We have the impression that the amount of endosperm should not be less than a certain critical limit. It was observed that seeds can have reduced amounts of endosperm and still germinate well under favourable conditions. If conditions are unfavourable, such seeds may produce abnormal and/or retarded seedling, or even not germinate at all: this is a clear example of a factual cause for lack of vigour. In many seeds, especially the primed ones, one can observe empty spaces between embryo and endosperm, which is referred to as free space.

In our studies we have classified the free space as 'none' or 'little', which were both considered normal, and 'extensive', which was evaluated as insufficient.

Seedling morphology

All seeds were then grown in the greenhouse in peat soil. The seedling were scored using very strict criteria employed by certain transplant growers and seed houses. Only small abnormalities like small indents or a small part missing from the tip, were regarded as usable transplants (UT). All others were not.

Prediction of seedling morphology

The two embryo classes combined with the three free space classes combined into six 'categories'. The empty seeds comprised a seventh, and seeds which we could not evaluate clearly formed an eighth.

The prediction was done using two different methods: method 1 regarded seeds with normal embryos and none or little free space as potentially UT; Method 2 used all categories but used a sum of the frequency of these categories multiplied with their probability factor p_i as predictor, as follows:

$$\mathit{percentage\ usable\ transplants} = \frac{\Sigma(f_i * p_i)}{\Sigma f_i} \times 100$$

where p_i is the probability of UT and f_i is the number of seeds for category i (i = 1...8).

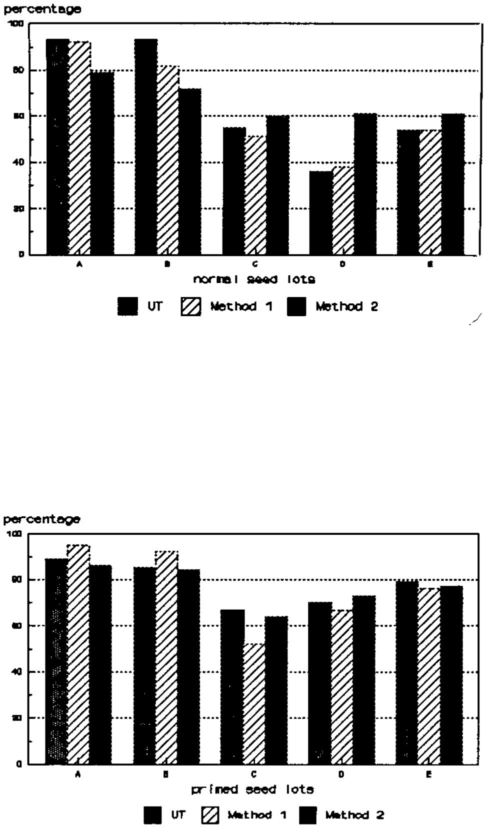


Figure 9. Prediction of UT by two methods of X-ray evaluation compared with UT obtained in the greenhouse. Above 5 lots of normal seeds, below 5 lots of primed seeds.

Method 1 predicts better with normal (unprimed) seeds (mean of paired unsigned differences (MPD) 3.6, figure 9), whereas Method 2 performs best with primed seeds (MPD 2.4). The latter is dependent on exact classification of the X-ray images, hence its better performance with primed seeds. These seeds can be evaluated more easily, because they usually have some free space, which facilitates evaluation. In future studies the factor *p* must be further developed for each category, and its dependence on factors such as cultivar, production conditions, and period of sowing will have to be determined. Notwithstanding that, both X-ray evaluation methods give a much better prediction of the percentage UT than the standard germination test (figure 8). Therefore, it may be concluded that a test based on X-ray images has considerable potential as a standard method to predict UT.

Fresh priming and the origin of free space

The origin of free space was discussed by Argerich and Bradford (1989). It has been known that free space occurs more often in primed seed, and the authors hypothesized that free space might, in part, be attributed to the greater seed volume. We have measured the area of the whole seed, the embryo, the free space and the endosperm with an interactive image analysis system (TCL-Image™, TNO Institute of Applied Physics, Delft, The Netherlands, 1990 version 4.6) (Liu et al., 1993).

Free space was not found when seeds were osmoprimed in the wet state, directly after harvest from the fruit. We called this 'fresh priming'. Free space however, could be induced in these seeds by a second hydration and dehydration step. This demonstrates that the first desiccation process is a requirement for the occurrence of free space. Apparently the endosperm loses its flexibility upon dehydration. This loss of flexibility may also explain the rapid decline in endosperm area in primed seeds: the hydration results in a proportionally larger swelling of the embryo, and a compression or displacement of the endosperm in 'vertical' direction (increased thickness).

Hydroprimed seeds (imbibed in fresh water for 40 hours, and brought in equilibrium with 32% RH thereafter) were compared with osmoprimed seeds (imbibed with a -0.1 MPa PEG-6000 solution for 7 days (Michel and Kaufmann, 1973)). In both cases dead (autoclave-killed) and living seeds were used.

The measurements showed that free space was larger in osmoprimed seed (+10.8% on average), than in hydroprimed seed (+8.1%). This was respectively largely and entirely due to a decrease in endosperm area. Some induction of free space was observed in some dead seeds. It may be concluded that the embryo swells during imbibition, but regains its original size after dehydration.

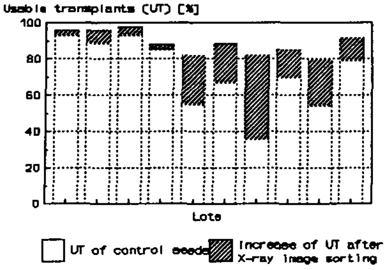


Figure 11. Improvement of seed quality, expressed in UT, with hypothetical sorting based on visual evaluation of X-ray images.

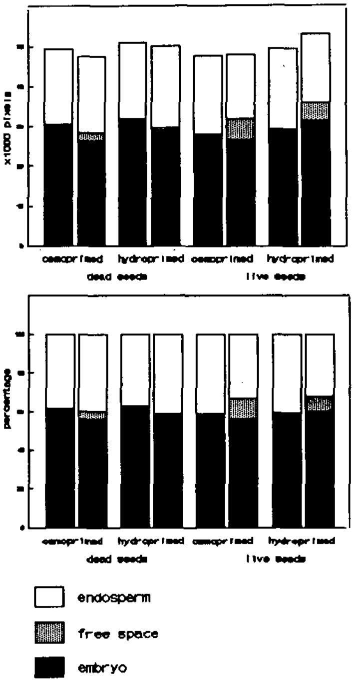


Figure 10. Development of free space in dead and live seeds during osmopriming or hydropriming. Left columns are before and right columns are after the treatment. Above: actual values, below: percentages.

Options for selection and sorting

The evaluation of X-ray photographs is traditionally done with the human eye. An illustration of what one could achieve if a machine could judge seeds as a person can, is presented in figure 11. Here a hand sorting based on visual interpretation of radiographs was made. The increases in UT are substantial: all lots were brought above 80% UT. Next to this technique, we also read the radiographs in the computer with a normal CCD video camera, after which we employed image analysis (see above). But it is also possible to capture the images directly into a computer with the aid of an X-ray camera. Although the resolution of these images is still less than of a radiograph, it provides the possibility for quick automatic analysis, or even sorting. The principle of an analysis system which we have built is given in figure 12. The personal computer controls the sample disk which contains eg 100 seeds. The system photographs all seeds in a batch process automatically, and stores the images on the mainframe computer. When analysis is desired the operator can start special image analysis programs which will retrieve the images, and calculate the parameters in a

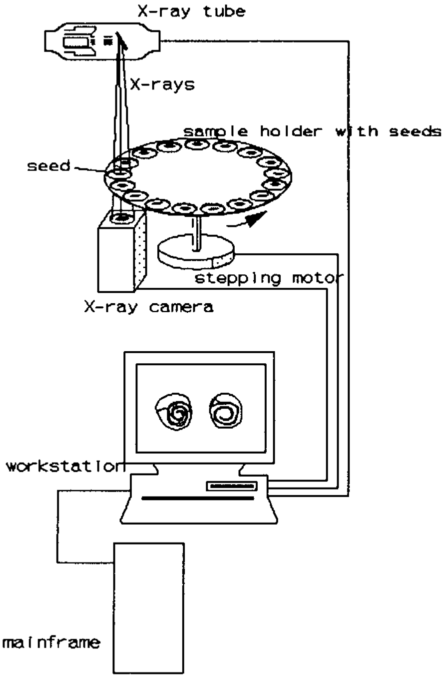


Figure 12. Automated analysis system for online evaluation by X-ray.

batch process again, and store the results in a data file. In this way much of the labour intensive photographing is avoided and due to the unbiased image analysis programs, the analyses have become objective and exact.

5. References

AOSA (1983). Seed vigor testing handbook. AOSA, USA.

AOSA (1989). Rules for Testing Seeds. Journal of Seed Technology 12(3), 1988; Revised 1989.

Argerich, C.A. and Bradford, K.J. (1989). The effects of priming and ageing on seed vigour in tomato. Journal of Experimental Botany 40:599-607.

Chen, P. and Sun, Z. (1991) A review of non-destructive methods for quality evaluation and sorting of agricultural products. Journal of Agricultural Engineering Research 49: 85-98.

ISTA (1985). International Rules for Seed Testing. Seed Science and Technology 13(2).

ISTA (1987). Handbook of vigour test methods. ISTA, Zürich.

Liu, Y., Van der Burg, W.J., Aartse, J.W., Van Zwol, R.A., Jalink, H. and Bino, R.J. (1993). X-ray studies on changes in embryo and endosperm morphology during priming and imbibing of tomato seeds. Seed Science Research (September 1993).

Michel, B.E. and Kaufmann, M.R. (1973) The osmotic potential of polyethylene glycol 6000. Plant Physiology 51: 914-916.

Mohsenin, N.N. (1986). Physical properties of plant and animal materials. Gordon and Breach, New York.

Peleg, K., Ben-Hanin, U. and Hinga, S. (1990). Classification of avocado by firmness and maturity. Journal of Texture Studies 21: 123-139.

Simak, M. (1991). Testing of forest tree and shrub seeds by X-radiography, p. 14-1 to 14-28. In Gordon, A.G., P. Gosling, B.S.P. Wang (eds.). Tree and Shrub Seed Handbook. International Seed Testing Association, Zürich.

Van der Burg, W.J., Aartse, J.W., Van Zwol, R.A., Jalink, H., Bino, R.J. (). Prediction of tomato (*Lycopersicon esculentum* Mill.) seedling morphology by X-ray analysis of seeds. J. Amer. Soc. Hort. Sci. (in press)

Yamamoto, H. and Haginuma, S. (1982). Vibrating reed method and nondestructive acoustic impulse response method for measuring textural quality of apple flesh. J. Japan. Soc. Hort. Sci. 51: 210-218.