Rapid Determination of Seed Quality during Development and Maturation of Fodder Sorghum (*Sorghum bicolor* (L.) Moench) Seeds by using Digital Image Processing

M. Shanmugavalli and P.R. Renganayaki

Department of Seed Science and Technology
Tamil Nadu Agricultural University
Coimbatore - 3, India

ABSTRACT. *Studies were conducted to fix the physiological maturity of fodder sorghum (Sorghum bicolor (L.) Moench) cv. COFS 29 with standard seed quality analysis in comparison with the advanced Image Analysis System. The results revealed that the observation of routine seed quality parameters to fix physiological maturity coincides with the Image Analyzer. The machine vision (Image Analysis System) could be effectively used to determine the seed quality in seed development and maturation studies of crops.*

INTRODUCTION

Fodder sorghum (*Sorghum bicolor* (L.) Moench) cv. COFS 29 is a high yielding, highly palatable and nutritious variety released by the Tamil Nadu Agricultural University, India. The variety is mainly propagated through seeds. Availability of well-matured and quality seed is a limiting factor for extensive cultivation of this variety. In addition, the seed maturity is not uniform due to tillering, non-synchronized flowering among tillers and maturity habit. The stage of maturity is an important factor for variation in vigour and viability of seeds. Hence, the knowledge on physiological maturity and optimum time of harvest is important to reduce the quantitative and qualitative losses of seeds. The physiological maturity of seeds can be fixed by physical or physiological and biochemical indices.

The physiological changes relating to seed deterioration would set in the seeds on the mother plant itself after physiological maturity as revealed by Agrawal (1995). Physiological maturity of the seeds is widely defined as the stage at which the seed attains its maximum dry weight and related physical characters (length, breadth and size) (Shaw and Loomis, 1950), which was correlated by the maximization of viability and vigor (Knitile and Burries, 1976). This can be visualized by changes in color of either pod or seed or both (Khattra and Singh, 1995). Abdul-Baki and Baker (1973) used the fresh weight of seed as an index for differentiating the development and maturation of seeds. Rajasekaran (1997) in rice bean reported that seeds attained physiological maturity coinciding with the highest seed volume, length, breadth, dry weight, germination, vigor index and dry matter production, but the moisture content of the seed alone was in decreasing order. Hyde *et al.* (1959) recorded high moisture content for the first 10 days after pollination, which gradually decreased and reduced to about 40% and equilibrated with atmospheric humidity at maturity in rye grass. The decreasing trend of moisture content of maturing seed was noticed by Krishnarajan (1996) in guinea grass and Rajasekaran (1997) in rice bean.

Knowledge on the pattern of seed development and maturation and duration of maturation are highly warranted in seed production of any cultivated crop irrespective of agricultural, horticultural, fodder or silvicultural species. Delouche (1973) defined seed maturation as the morphological, physiological and functional changes that occur from the time of fertilization until the matured seeds are ready for harvest. However, Abdul-Baki and Baker (1973) differentiated seed development as the period between fertilization and maximum fresh weight accumulation, and seed maturation as the end process of seed development that continues up to harvest. Indeterminate growth habit, uneven maturity and seed shattering are some of the limiting factors causing poor seed yield of fodder crops. Identification of harvest date is one of the basic approach for getting maximum seed recovery. Similar to other valuable crops, the seed production techniques encompassing the optimum date of harvest (including physical, physiological and chemical indices), seed processing, seed treatment and storage are important for forage crops. Standardization of all these parameters for any newly released variety is foremost requirement which ensures the supply of good quality seed at appropriate time.

Apart from physical and physiological maturity indices various crops have also been assessed on the biochemical make up of seed during germination. Khattra *et al.* (1997) reported that water saturation deficit increased with progressive maturity and was positively correlated with soluble sugars and free amino acids. Total phenolic compounds were maximum at critical stage of development and minimum at final stage of maturation of rye grain (Weidner *et al*., 2000). Phenol content increased during hydrical step and then decreased rapidly during seed hydration in sorghum (Regnier and Masheix, 1996); whereas protein, carbohydrates, oil content was the maximum at the physiological maturity (Gambhir and Anand, 1981).

The physiological and biochemical indices are generally estimated under laboratory conditions and the physical indices by visualization, which are rather time consuming and expensive. By the time of estimation seeds, may have attained physiological maturity and start to shatter. The physical indices will be more useful if done through a machine (machine vision) and would be useful for fixing the physiological maturity. With this objective, a study was carried out to fix the physiological maturity by documenting the physical characters through an Image Analyzer and to study the seed characters contributing for fixing physiological maturity in comparison with other characters.

MATERIALS AND METHODS

Seed quality studies on fodder sorghum [*Sorghum bicolor* (L) Moench] cv. COFS 29 was carried out at the Tamil Nadu Agricultural University, Coimbatore, India during 2004-2005. The laboratory experiments were conducted at the Department of Seed Science and Technology and the field experiment was conducted in field number 37-A at the eastern block of the University. The crop was raised in the field June 2004 where all the agronomic practices were carried out as per recommendation. During anthesis, randomly selected panicles were tagged to study the physiological maturity. Panicles were harvested at seven-day intervals up to 35 days after flowering. The following observations were recorded at each stage of harvest.

Seed moisture content

The fresh seeds in a moisture bottle (weight of the moisture bottles, and the moisture bottles with fresh seeds were recorded separately) were placed in a hot air oven maintained at $103^0C\pm2^0C$ for 16 h. Thereafter, the bottles were then cooled in a desiccator for 20 min., and the weight of the moisture bottles with the dried seeds was recorded. The moisture content was calculated using the following formula:

$$\text{Moisture percentage} = \frac{(M_2 - M_3) \times 100}{M_2 - M_1}$$

Where;

M_1 = Weight of moisture bottle before drying
M_2 = Weight of moisture bottle + seed sample before drying
M_3 = Weight of moisture bottle + seed sample after drying

100 seed weight

Eight replicates, each carrying hundred seeds were counted and weighed. The mean value was expressed in milligram.

Electrical conductivity

The electrical conductivity of four replicates, each consisting of fifty seeds, was estimated as described by Presley (1958).

Phenol content

The phenol content of 500 mg seed sample was estimated following the method described by Malick and Singh (1980).

Starch content

The starch content of 500 mg seed sample was estimated by the anthrone method as described by Hedge *et al*. (1962), and the glucose content was calculated from the standard curve.

Alpha amylase activity

Alpha amylase activity was measured using the method described by Simpson and Naylor (1962).

Image analysis system

The developing and maturing seeds fodder sorghum cv. COFS 29 were subjected to image analysis system for observing the variation in the physical characteristics. This method has a potential usefulness as a rapid indicator of seed lot quality. The image analysis was carried out using the Delta - T® (Delta Instrument Device, Cambridge, UK) Image Analysis System by running custom written software "win DIAS" (Nick Webb and Dick Jekins, 2000). For every replication, 25 caryopses were placed on the lighting hood ensuring that the caryopses are facing the Image Analysis System and that the longitudinal axis of the capsule is running parallel to the surface of the camera lens. Seeds were viewed with a video camera (DSP Surveillance colour CCD camera CVS 200/3300) using transmitted light, and the binary image of the silhouette of the seed was recorded by the win DIAS. The image of the support was removed by software after image grabbing in the computer, thus, leaving an image of the objects consisting of five rows and five columns for geometric data measurements.

Before actual measurement, a calibration was done by placing a transparent plastic ruler on the lighting illuminated from below. Ruler was aligned diagonally across the field of view and the focus was adjusted to sharpen the image. The aperture adjustment was done until optimum color and contrast was achieved. Input length was given in centimeter. The object meter was selected to measure descriptors such as area, perimeter length and width. After the setting, the image was grabbed using the image grabber and color threshold was done until the entire area was highlighted. The overall data were extracted by clicking the entire objects. Data were viewed from the review and mean data for the each parameter were summed up for average value in the win DIAS itself. The entire images and their data were saved in the document file and interpreted. The parameter studied was as follows;

Area: the multiplication of the length and breadth of individual seeds
Perimeter: the circumference of the individual seed
Length: distance between two points marked on the screen using the mouse or the diameter of the smallest circumscribed circle that will fit around an object.
Width: Length measured in X-axis
Elongation: the ratio of the length and width. (E = width/length)
Circularity: the square root of the ratio of the actual area of the object (A) to the area of a circle (Ap) with the same circumscribed diameter or length of the object, with the same circumscribed diameter: $C = \sqrt{\frac{A}{Ap}}$
Shape factor: the ratio of the actual perimeter to that of a circle with the same area. S = P/PC, where P is the perimeter of the object and PC is the perimeter of a circle with the same area as the object. PC is calculated as follows.

$$PC = 2\ (II^*\ A)^{0.5}$$

Where; A is the actual area of the object.

RESULTS AND DISCUSSION

Physiological and biochemical aspects of seed development and maturation

Quality of a seed basically depends on seed filling, metabolic and synthetic efficiency of the fertilized ovule during seed development and maturation (Harrington, 1972). The developing seeds started to loss the moisture content continuously as seed matured. The highest moisture content of 54.7%, which was registered at 7 days after anthesis (DAA), declined gradually and reached the minimum of 27.8% at 35 DAA. The maximum reduction was noticed during first two stages of development. A marginal loss of moisture was observed between 7-21 DAA, coinciding with the faster and more accumulation of dry weight (Table1).

The hundred seed weight is the best parameter to study the seed maturity. Increased weight, during the course of maturation is due to accumulation of dry weight and storage reserves. The seed weight increased from 226 mg at 7 DAA to 773 mg at 28 DAA, with the maximum accumulation of 270-667 mg during 14-21 DAA (Table 1). The seed weight increased between 14-21 days, coinciding with the faster accumulation of dry weight and marginal decrease in moisture content indicates the entry of seed into the maturation stage. The study was inconformity with results of Reshma (2001) in Desmanthus.

Table 1. Physiological and biochemical changes during seed development and maturation in fodder sorghum cv. COFS 29.

Days after anthesis	Moisture content (%)	100 seed weight (mg)	Electrical conductivity (dS/m)	Phenol (mg/100g seed)	Starch content (%)	α-amylase (cm)*
7 DAA	54.72	226	0.093	2.25	32.4	0.4
14 DAA	45.20	270	0.058	2.90	34.5	0.5
21 DAA	39.51	667	0.022	4.10	81.9	0.8
28 DAA	32.74	773	0.020	4.21	83.3	1.2
35 DAA	27.82	756	0.020	4.15	83.0	1.2
Mean	39.99	538	0.043	3.50	63.0	0.8
SEd	0.3651	0.7303	0.004	0.0354	0.7746	0.0612
CD ($P \leq 0.05$)	0.7956	1.5912	0.008	0.0771	1.6877	0.1134

Note: DAA - Days After Anthesis; SEd - Standard Deviation; CD - Critical Difference.
*The distance covered by alpha amylase was measured in cm (Simpson and Naylor, 1962).

The initial electrical conductivity of the seed leachate (0.093 dS/m observed at 7 DAA) was reduced to 0.020 at 28 DAA and maintained at 35 DAA (Table 1). The results revealed that the electrical conductivity decreased as the maturation advanced, which could be attributed to an alteration in membrane structure. The loss of water from the seed at later stages of maturity made the seed coat hard, which did not permit the leaching of internal solutes. The electrical conductivity of the seeds at 28 and 35 DAA showed static maintenance indicating the attainment of integrity of seed outer membrane. Similar results were reported by Renganayaki (2001) in sunflower.

Phenols are the aromatic compounds with hydroxyl groups, and are of widely spread in the plant kingdom. At lower concentrations, these phenolic compounds induce defense mechanisms in plants, whereas at higher concentration, they inhibit germination of the seeds (Noggle and Fritz, 1991). In the present study, the minimum phenol content of 2.25 mg observed at 7 DAA, increased to 4.21 mg at 28 DAA. Thereafter, a slight decline was noticed at 35 DAA (4.15 mg), which was in par with 21 DAA (4.10 mg). Even though a reducing trend was noticed during later stages, the phenol content was two-fold as that of the initial stage. The results revealed that the total phenol content was the maximum at the critical stages of development and minimum at the final stage of maturation. These results are in conformity with those reported by Weidner *et al.* (2000) in rye grain and Renganayaki (2001) in sunflower.

In the present study, the starch and α-amylase content increased up to the stage of 28 DAA, confirming the completion of metabolic activity. The results on the seed development and maturation infer that fodder sorghum seeds have attained physiological maturity at 28 DAA with the crucial stage of development between (14-21 DAA). At this stage sudden increase in dry weight, 100 seed weight, phenol, starch and α-amylase activity (Table 1) were noted. A significant decrease in electrical conductivity at the same phase indicated that the seed is reaching maturity. The results revealed that an intrinsic mechanism built in the seed prevents the loss of synthesized food reserves thus, preparing the seed to germinate.

Image analysis system

An appropriate integration between automated machine vision techniques and standard seed education tests could provide more detailed information on the status of seed lot (Heijden *et al*., 1999). All the seed quality parameters, *i.e.* size and shape based on perimeter, area, elongation, circularity, length, width and shape factor, measured by the Image Analysis were found to be useful in fixing the pattern of maturity and to assess the physiological maturity of seed. The data obtained were also used to find out the differences in the distinguishable seed morphological characteristics.

According to Drezweiecki *et al*. (2000), the size and shape of seeds are unique for a species and variety. Many seeds are similar in shape, but differ widely in size. In the present study, the area of seeds showed significant differences during seed development and maturation. The initial and peak areas recorded were 0.022 and 0.048 cm^2 at 7 and 28 DAA, respectively (Table 2), and was static thereafter.

Table 2. Measurement of morphological seed descriptors using image analysis system – in fodder sorghum cv. COFS 29.

Days after anthesis	Area (cm^2)	Perimeter (cm)	Length (cm)	Width (cm)	Circularity	Elongation (cm)	Shape factor (cm)
7 DAA	0.022	0.70	0.25	0.09	0.75	0.32	0.9
14 DAA	0.035	0.82	0.27	0.10	1.00	0.45	1.2
21 DAA	0.043	0.85	0.27	0.11	1.10	0.46	1.8
28 DAA	0.048	0.85	0.28	0.12	1.20	0.51	1.8
35 DAA	0.048	0.85.	0.28	0.12	1.20	0.51	1.7
Mean	0.039	0.81	0.27	0.11	1.05	0.45	1.5
SEd	0.0021	0.0507	0.0068	0.0045	0.0714	0.0130	0.1000
CD ($P \leq 0.05$)	0.0045	0.1104	0.0149	0.0097	0.1555	0.0284	0.2179

Note: DAA - Days After Anthesis; SEd - Standard Deviation; CD - Critical Difference.

The perimeter of the seeds showed significant differences ($P<0.05$) during the seed development and maturation. Seed had the maximum perimeter of 0.85 cm on 21, 28 and 35 DAA. The minimum perimeter of 0.70 cm was recorded at 7 DAA (Table 2). Significant differences ($P<0.05$) in seed length and width were observed during seed development and maturation. The seed attained maximum length at 28 DAA (0.28 cm) from initial length of 0.25cm at 7 DAA, and was static thereafter (Table 2). The maximum width was noticed at 28 DAA and 35 DAA (0.12 cm) and the minimum of 0.09 cm was at 7 DAA (Table 2).

Circularity showed an increasing trend. The differences in values are significant (P<0.05) for all the stages. The maximum circularity of 1.20 cm was recorded at 28 DAA and 35 DAA (Table 2) whereas it was the minimum at 7 DAA (0.75cm). The maximum elongation of 0.51 cm was recorded at 28 DAA and 35 DAA. A significant elongation was observed between 7 DAA to 14 DAA (0.32 to 0.45). The maximum shape factor was recorded at 21 DAA and 28 DAA (1.8 cm), which was in par with 35 DAA (1.7 cm). The study of seed characteristic using the image analysis system showed that area, perimeter, length and width of the seed growth reached the highest value between 21-28 DAA, which coincided with the results of physiological and biochemical studies.

CONCLUSIONS

Geometric measurements obtained in fodder sorghum cv. COFS 29, confirmed that the maturity stages observed have more value for seed descriptors. A positive relationship was observed for growth stages with seed descriptor values. Seed descriptors using an Image Analysis System could be done quickly and large volume of samples could be subjected to observation within a shorter period of time for seed developmental studies. In the case of fodder sorghum, all the physical characters studied through Image Analyzer contributed to fix the physiological maturity and coincided with the results of other parameters.

REFERENCES

Abdul-Baki, A.A. and Baker. J.E. (1973). Are changes in cellular organelles or membranes related to vigor loss in seeds. Seed Sci. and Technol. 1**:** 89-125.

Agrawal, R.L. (1995). Seed Technology, Oxford and IBH Publishing Co. (Pvt.) Ltd., New Delhi.

Delouche, J.C. (1973). Seed maturation. In**:** Seed production manual. NSC and Rockfeller foundation, pp. 162-165.

Drezweiecki, J., Fornal, L., Filipowicz, A. and Konopka, S. (2000). Comparison of computers, image analysis and urea soluble proteins electrophoresis for the identification of malting barley varieties. Plant Varieties and Seeds. 13: 159-171.

Gambhir, P.N. and Anand, I.J. (1981). Oil and protein synthesis during grain development in sunflower. Ind. J. Plant Physiol. 24: 69-73.

Harrington, J.F. (1972). Seed Storage and Longevity. pp. 145-245. *In*: Kozlowski, T.T. (Ed). Seed Biology III, Academic Press, New York.

Hedge, J.E. and Hofreiter, B.T. (1962). Determination of Reducing Sugars and Carbohydrates. I. Analysis and Preparation of Sugars. *In*: Whistler, R.L. and Beniller, J.N. (Eds). Methods in Carbohydrate Chemistry, Academic Press, New York.

Heijden,V.D., Polder, G.W.A.M., Van Eck, J.W. and Vander Schoor, R. (1999). World seed conference, 6-8 September, 1999, U.K. Program and Abstracts, Pp. 14.

Hyde, E.O.C., Mcleavey, M.A. and Harris, G.S. (1959). Seed development in rye grass and in red and white clover. New Zealand J. Agric. Res. 2: 947-952.

Khattra, S. and Singh. G. (1995). Indices of physiological maturity of seeds - a review. Seed Res. 23(1**):** 13-21.

Khattra, S., Kaur, K. and Singh, O.S. (1997). Water loss from maturing *Crotalaria juncea* seeds. Indian J. Plant Physiol. 2(2): 145-147.

Knitile, K.H. and Burries. J.S. (1976). Effect of kernel maturation on subsequent seedling vigour in maize. Crop Sci. 16: 851-855.

Krishnarajan, M.V. (1996). Seed production, processing and storage technology in guinea grass cv. CO1. M.Sc. (Ag) Thesis Tamil Nadu Agric. University, Coimbatore.

Malick, C.P. and Singh, M.B. (1980). Plant Enzymology and Histo Enzymology, Kalyani Publishers, New Delhi . Pp. 286.

Nick-Webb and Dick Jekins. (2000). Win DIAS user Manual, Delta T. Devices Ltd., Cambridge, UK.

Noggle, G.R. and Fritz, G. (1991). Introductory Plant Physiology. Prentice Hall of India (Pvt.) Ltd., New Delhi. pp. 125-512.

Presly, J.T. (1958). Relation of protoplast permeability to cotton seed viability and predisposition to seedling disease. Plant Dis. Report, 42. Pp. 852.

Rajasekaran, S. (1997). Seed technological studies in rice bean. (*Vigna umbellate* Thunb). M.Sc. (Ag) Thesis, Tamil Nadu Agric. University, Coimbatore.

Regnier, T. and Macheix, J.J. (1996). Changes in wall bound phenolic acids, phenylalanine and tyrosin ammonia lyases and peroxidases in developing durum wheat grains. J. Agric. Food Chem. 44(7): 1727-1730.

Renganayaki, P. R. (2001). Seed production and senescence in sunflower (*Helianthus annuus* L.) hybrid KBSH-1. Ph.D. Thesis, Tamil Nadu Agric. University, Coimbatore.

Reshma, C. (2001). Studies on seed maturation, production, processing and storage of hedge lucerne cv TNDV 1. M.Sc. (Ag) Thesis, Tamil Nadu Agric. University, Coimbatore.

Shaw, R.H. and Loomis. W.E. (1950). Basis for the prediction of corn yield. Plant Physiol. 25: 225-247.

Simpson and Naylor. (1962). Dormancy studies of *Avena fastuca* - 3. A relationship between maltose, amylose and gibberellin. Can. J. Bot. 40: 1599-1673.

Weidner, S., Amarowiez, R., Learamac, M. and Fraczek, E. (2000). Changes in indigenous phenolic acids during development of cereals caryopsis and after dehydration treatment of vine ripe rye grains. Plant Physiol. Biochem. 38 (7/8): 595-602.