e-ISSN:2320-0189
p-ISSN:2347-2308

Research & Reviews: Journal of Botanical Sciences

Seed Quality Markers: A Review

Jeevan Kumar SP, Rajendra Prasad S*, Madan Kumar#, Chandu Singh#, Sinha AK# and Avinash Pathak
ICAR-Indian Institute of Seed Science, Kushmaur, Mau-275103, Uttar Pradesh, India

Review Article

Received date: 06/03/2016
Accepted date: 17/04/2016
Published date: 19/04/2016

***For Correspondence**

Rajendra Prasad S, ICAR-Indian Institute of Seed Science, Kushmaur, Mau-275103, Uttar Pradesh, India, Tel: 0547-2530326

E-mail: srprasad1989@yahoo.co.in

Keywords: Antioxidants, Cell cycle markers, Embryo, Endosperm, Ethylene, Seed Quality.

ABSTRACT

Seed is a basic input for agriculture productivity and germplasm conservation. Seed quality predominantly comprises of germination and vigour which are quintessential for successful stand establishment. On the other hand, seed industries are thriving to improve the crop yield through control of seed vigour. Possibilities of seed vigour could be improved by seed priming and post-harvest processing. So far, during seed production at a commercial scale, maturity used to monitor visually which necessitates the expertise of growers. It is the need of the hour to develop markers that aid in identifying optimum harvest time. Markers could be developed by clearly understanding the physiological, biochemical, molecular mechanisms involved in seed quality (germination and vigour). From the recent studies, several promising markers such as cell cycle markers, electrolyte leakage, ethylene production, sugar metabolism, proteins, antioxidants and telomeres have been suggested as reliable markers to monitor the seed quality process. Such markers not only help to identify the seedling stages but also facilitate a better understanding of seed priming treatments.

INTRODUCTION

Seed provides food, feed, fibre and most importantly conserve the species in the form of a tiny size to adapt to the environment. Seed is the harbinger for agricultural productivity; however, burgeoning population, reduction in land holding size due to urbanization and tremendous demand for quality food is an upcoming challenges to be confronted. Under such odds, it is inevitable to have quality seeds that enable successful stand establishment and crop improvement. Seed quality comprises of seed germination, vigour, genetic purity and health estate of seed [1]. Quality seeds germinate completely at faster rate with vigorous seedlings of little sensitivity to climatic changes that enable them to adapt broad range of environmental conditions [2]. Today seed companies are mushrooming to cater the demand by improving the crop yield through control of seed vigour. Nevertheless, it is need of the hour to develop reliable markers to evaluate the seed performance of quality seed in field conditions. Normally, in seed production chain at commercial scale, seed maturity is evaluated visually, which exclusively relies over the expertise of the growers. On the other hand, several physiological tests applied suffer lack of preciseness and leads to variability between the seed lots. Therefore, it is intriguing that development of markers to understand the seed performance can be achieved by understanding the mechanisms involved in the various physiological processes. Biochemical, molecular and proteomics studies revealed the potential markers at respective stages which not only facilitate to improve the crop stand through easy pick of high quality seeds but also enable to control the seed vigour through seed priming treatments.

Recent studies have demonstrated some evidence as potential markers at biochemical, molecular levels. Antioxidants, integrity of membrane determination by electrolyte leakage, ethylene production during imbitions phase, changes in raffinose family oligosachharides and 11 S globulin β- subunit, late embyrogenesis abundant protein and heat shock protein changes are reliable indicators of seed germination, performance, maturity and seed priming treatments [3]. Some more markers are anticipated due to embrace of new 'OMICS' studies of seeds in various stages to understand clearly the basic mechanism in the respective stages.

Antioxidants

Seed quality maintenance during seed production is inevitable as reactive oxygen species (ROS) synthesis takes place during metabolic process. ROS comprises of superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen which has adverse

effects on lipids, DNA, RNA, and proteins that ultimately leads to cell death. The condition could be more deteriorative if the seed biochemical composition is enriched with oil/lipid content. For example, ROS damages the integrity of cell membranes, alters the permeability and fluidity through peroxidation of lipids [4]. This exacerbate, particularly, in soybean seeds whose chemical composition is endowed with oil content [5]. Recent studies show that antioxidants play an important role in controlling the ROS [6]. An intriguing fact has been hypothesized that reduction of ROS concentrations could act as a second messenger in seed germination, seed dormancy (maturation and drying), endosperm weakening and more profoundly linked to seed longevity [7,8].

Antioxidants such as tocopherols, phenols, carotenoids, ascorbic acid and thiols are non-enzymatic in nature whereas, catalase (CAT), ascorbate peroxidase, superoxide dismutase (SOD), glutathione reductase are the major enzymatic moieties. The enzymatic antioxidants mainly execute its function through detoxification mechanisms. For example, superoxide (free radical) has been dismutated by SOD to hydrogen peroxide and oxygen. Further, catalase detoxifies the hydrogen peroxide into water and oxygen. These enzyme activities are directly correlated with ROS maintenance at non-toxic levels which may serve as markers. Bailley et al. [9] has reported that scavenging potential of ROS due to enzymatic activity had been increased during seed development in bean [9]. Another study demonstrates that osmo-priming restored the scavenging potential of enzymatic activities which is directly correlated to germination rate and antioxidant potential in sunflower seeds [10]. These findings clearly show that the antioxidants absorbs/neutralizes the free radicals, singlet and triplet oxygen are quenched and the peroxides are being detoxified/decomposed [11].

Membrane Integrity

Unlike recalcitrant seeds, orthodox seeds undergo desiccation tolerance after maturation. Membrane integrity is one of the important features to confront the desiccation tolerance to remain seed viability [12]. On set of ageing leads to loss of membrane integrity, and the degree of leakage indicates the quality of membranes to solute diffusion and seed vigour. The leakage of solutes is indirectly proportional to the seed quality; in other words, more the leakage lesser will be the quality of seeds [3]. However, in case of sweet corn, where high sugar contents are present it may not serve as a good indicator. Similarly, the crops with high phenolics in seed coat may also not suitable for the membrane integrity test [13]. Nevertheless, international seed testing association (ISTA) has recommended the test for large seeded legumes [14]. This indicator is quite helpful to determine the quality seed during imbibitions.

Ethylene as Indicator

Ethylene synthesis in higher plants takes place through 1-aminocyclopropane 1-carboxylic acid (ACC) oxidase activity [15]. Since the ACC activity depends on membrane integrity, ethylene production is a good indicator of membrane conditions. Less synthesis of ethylene due to low conversion of ACC is a sign of membrane damage observed in *Araucaria angustifolia* embryos [16,17]. Similar results were found in other species where seed vigour is correlated with ACC dependent ethylene production [18,19]. Therefore, ethylene formation is one of the good sign of membrane conditions and can be assessed during imbibitions.

Raffinose Family Oligosaccharides (RFO)

In addition to the above markers, some molecules such as RFO are of particular interest. RFO accumulates during seed maturation and helps to maintain the intracellular contents in glassy state through increase in cytoplasmic viscosity and glass to liquid transition temperatures. These modifications help in to restrain ageing effect [20,21]. In addition, soluble sugars accumulation is correlated with seed dehydration rates which might be a good indicator for seed drying conditions [22]. For instance, in pea positive correlation exists between RFO/sucrose and electrolyte leakage. Furthermore, several research studies suggest that the sugar content, precisely oligosaccharides to sucrose ratio, could be a good indicator for seed storability [23-26]. Apart from RFO family sugars, heat shock proteins (HSPs), late embryogenesis abundant protein [(LEA), are also important markers for seed germination. However, much more research is emancipated in these aspects [27,28].

Cell Cycle Markers

In higher plants, the embryo development needs endosperm nourishment. In angiosperms, the embryo is formed by the fusion of one nucleus from male gametophyte and the other from female gametophyte. Thus, the embryo is in 2 n stage representing a dploid genome (2n). In seeds, at different developmental stages mitotic/endoreduplication takes place, as a result, physiological state of a seed could be easily understood. In embryo, a typical proliferative cell undergoes mitosis where 2 n is duplicated to 4 n condition after 'S' phase; thereafter, in mitosis where the DNA content is divided into two daughter nuclei (4 n to 2 n) as depicted in **Figures 1 and 2.** In case of non-proliferative cells it undergoes quiescent G_0 stage from G_1 phase. On other hand, endosperm forms by fusion of one nucleus from male gametophyte with two nuclei from the female gametophyte whose ploidy condition is 3 n. After 'S' phase of endosperm cell, the ploidy condition would turn to 6 n **(Figure 3).**

Unlike embryonic cells, some endosperm cells undergo endoreduplication where repeated rounds of DNA replication occur in nuclei without the mitotic stage which leads to formation of endopolyploid cells. This striking feature could be studied through flow cytometry to estimate the DNA content with fluorochrome [29]. Moreover, studying the replication of DNA could be advantageous in knowing the seed vigour; as hypothesized, that low quality seeds would require longer time for DNA repair and also suffers with integrity during imbibitions [30]. Since, active cells could complete its cell cycle within short span; 4n/2n ratio might be a good marker for seed germination and quality [31]. In early developmental stages of embryo, cell cycle activity occurs at a faster rate.

For instance, in sugarbeet, pepper and soybean the ploidy of 4 n condition is attained at 45%, 30% and 15-20% respectively [32-34]. After maturation, cessation of DNA replication occurs with a ploidy state of 2 n and enters probably G_0 state. Similarly in endosperm, high mitotic activity is observed at higher rate initially followed by increased endoreduplication with concomitant zero mitotic index [35].

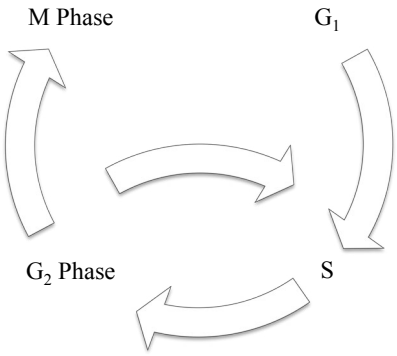

Figure 1. General overview of cell cycle in Angiosperms.
M phase: Mitotic phase, G_1 phase: Gap-1 phase; S Phase: Synthesis phase; G_2 phase: Gap-2 phase.

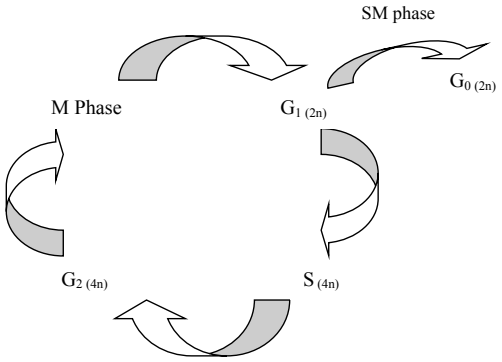

Figure 2. Typical proliferative cell cycle stages in embryo.
In embryo, a typical proliferative cell undergoes mitosis where 2n is duplicated to 4n condition after 'S' phase; thereafter, in mitosis where the DNA content is divided into two daughter nuclei (4n to 2n). In case of non-proliferative cells (seed maturation stage) it undergoes quiescent G_0 stage from G_1 phase.

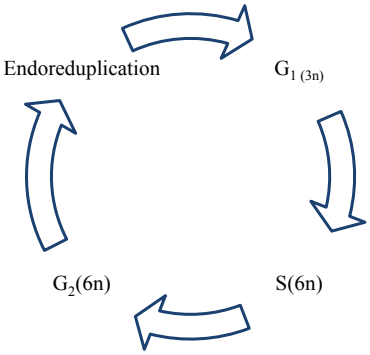

Figure 3. Probable route in some endosperm cells for polyploidy condition.
Some endosperm cells undergo endoreduplication where repeated rounds of DNA replication occur in nuclei without the mitotic stage. The continuous endoreduplication leads to the formation of endopolyploid cells.
M phase: Mitotic phase, G_1 phase: Gap 1 phase; S Phase: Synthesis phase; G_2 phase: Gap 2 phase; Endoreduplication occurs with repeated cycles with no M Phase.

From the above discussion, it is imperative to deduce a yardstick of seed maturity with the ratio of endosperm to embryo nuclei which will provide the insights. Several reports suggested the utility of endosperm to embryo ratio; for example, in sugarbeet, it is about 3:2 and 9:1 at 21 day old developing and 36 (mature) day old seeds respectively [33]. In the same way, cucumber seeds showed 45% at 21 days after pollination followed by 13% and 8% at harvest and processing stages respectively [36]. These results imply that the endosperm and embryo cells at early stages actively divide and maintained the ploidy condition (3:2) in sugarbeet. On the other hand, when cell attains maturity, the embryo and endosperm nuclei undergo G_0 and endoreduplication process respectively leading to reduction in ploidy condition of embryo nuclei in comparison to endosperm nuclei (polyploidy). Hence, it is easier to monitor the seed maturity based on the endosperm to embryo nuclei. However, in some cases the decrease in endoreduplication activity may not be attained to seed maturity. For instance, in pepper and Arabidopsis during seed development no endoreduplication could takes place [34,37].

Therefore, it is intriguing that based on the above discussion, in orthodox seeds, the seed maturity could be monitored effectively with the ratio of embryo/endosperm cell number along with DNA contents of corresponding nuclei [25].

ACKNOWLEDGEMENTS

The author is grateful to Mr. Avinash Kumar Pathak and Mr. Abhishek Kumar Rai for assistance in preparation of the manuscript.

REFERENCES

1. McDonald MB . Seed quality assessment. Seed Science Research. 1998;8:265-275.
2. Corbineau F, et al.A technique for improving seed quality. Seed Testing International. 2006;132:38-40.
3. Corbineau F. Markers of seed quality: from present to future. Seed Science Research. 2012;22:61-S68.
4. Avila CM, et al. Development of a new diagnostic marker for growth habit selection in faba bean (Vicia faba L.) breeding. Theor Appl Genet. 2007;115:1075-1082.
5. Ishibashi Y, et al. Regulation of soybean seed germination through ethylene production in response to reactive oxygen species. Ann Bot. 2013;111:95-102.
6. Donà M, et al. DNA profiling, telomere analysis and antioxidant properties as tools for monitoring ex situ seed longevity. Ann Bot. 2013;111:987-998.
7. Jeevan Kumar SP, et al. Seed birth to death: dual functions of reactive oxygen species in seed physiology. Ann Bot. 2015;116:663-668.
8. Sano N, et al. Staying Alive: Molecular Aspects of Seed Longevity. Plant Cell Physiol. 2015 .
9. Bailly C, et al. Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. Journal of Experimental Botany. 2001;52:701-708.
10. Bailly C, et al. Catalase activity and expression in developing sunflower seeds as related to drying. J Exp Bot. 2004;55:475-483.
11. Golovina EA, et al. The competence to acquire cellular desiccation tolerance is independent of seed morphological development. Journal of Experimental Botany. 2001;52:1015-1027.
12. McDonald MB. Seed deterioration: physiology, repair and assessment. Seed Science and Technology. 1999;27:177-237.
13. Hampton JG and TeKrony DM. Handbook of vigour test methods. Zurich, International Seed Testing Association. 1995.
14. Yang SF and Hoffmann NE. Ethylene biosynthesis and its regulation in higher plants. Annual Review of Plant Physiology.1984;34:155-189.
15. Espindola S, et al. Cellular and metabolic damage induced by desiccation in recalcitrant Araucaria angustifolia embryos. Seed Science Research. 1994;4:193-201.
16. Porter AJR, et al. Activity of the ethylene-forming enzyme in relation to plant cell structure and organization. Journal of Plant Physiology. 1986;125:207-216.
17. Chojnowski M, et al. Physiological and biochemical changes induced in sunflower seeds by osmopriming and subsequent drying, storage and aging. Seed Science Research. 1997;7:323-331.
18. Khan AA. ACC-derived ethylene production, a sensitive test for seed vigour. Journal of the American Society for Horticultural Science. 1994;119:1083-1090.
19. Bernal-Lugo I and Leopold AC. Seed stability during storage: raffinose content and seed glassy state. Seed Science Research. 1995;5:75-80.
20. Sun WQ. Glassy state and seed storage stability: the WLF kinetics of seed viability loss at T-Tg and the plasticization effect of water on storage stability. Annals of Botany. 1997;79:291-297.
21. Black M, et al. Water content, raffinose, and dehydrins in the induction of desiccation tolerance in immature wheat embryos Plant Physiol. 1999;120:463-472.
22. Corbineau F, et al. Effects of dehydration conditions on desiccation tolerance of developing pea seeds as related to oligosaccharide content and cell membrane properties. Seed Science Research.2000;10:329-339.
23. Sinniah UR, et al. Irrigation and seed quality development in seed rapid-cycling brassica: soluble carbohydrates and heat-stable proteins. Annals of Botany. 1998;82:647-655.
24. Sun WQ and Leopold AC. Glassy state and seed storage stability: a viability equation analysis. Annals of Botany. 1994;74:291-297.

25. Horbowicz M and Obendorf RL. Seed desiccation tolerance and storability: dependence on flatulence producing oligosaccharides and cyclitols - review and survey. Seed Science Research. 1994;4:385-405.

26. Dolezel J and Bartos J. Plant DNA flow cytometry and estimation of nuclear genome size. Ann Bot. 2005;95:99-110.

27. Leprince O and Buitink J. Dessication tolerance: From genomics to the field. Plant Science. 2010;179, 554-564.

28. Tunnacliffe A and Wise MJ. The continuing conundrum of the LEA proteins. Naturwissenschaften. 2007;94:791-812.

29. Osborne DJ. The physiology and biochemistry of seed dormancy and germination. Amsterdam, North Holland Biomedical Press. Nucleic acids and seed germination. In: Khan AA (Ed.). 1977;319-333

30. Sliwinska E. Nuclear DNA replication and seed quality. Seed Science Research. 2009;19:15-25.

31. Chamberlin MA, et al. Nuclear size and DNA content of the embryo and endosperm during their initial stages of development in Glycine max (Fabaceae). American Journal of Botany. 1993;80:1209-1215.

32. Sacande M, et al. Cell cycle events in developing neem (Azadirachta indica) seeds: are they related to intermediate storage behavior? Seed Science Research. 1997;7:161-168.

33. Sliwinska, E. Cell cycle activity during development of sugar beet seed. In: Maluszyn´ ska, J. (Ed.) Plant cytogenetics. Katowice, Silesian University Publishers (Prace Naukowe Uniwersytetu S ´ la?skiego 1696). 1998;pp. 51-59.

34. Portis E, et al. Molecular and physiological markers during seed development of peppers (Capsicum annuum L.). DNA replication and b-tubulin synthesis. Seed Science Research. 1999;9:85-90.

35. Dilkes BP, et al. Genetic analyses of endoreduplication in Zea mays endosperm: evidence of sporophytic and zygotic maternal control. Genetics. 2002;160:1163-1177.

36. Sadowski J, et al. Germination and cell cycle activity changes during development and storage of cucumber (Cucumis sativus L.) seeds. Polish Journal of Natural Sciences Suppl. 2008;5:139.

37. Raz V, et al. Sequential steps for developmental arrest in Arabidopsis seeds. Development. 2001;128:243-252.

This article was published under the special issue, **Plant Biotechnology and its Applications** handled by Editor. Deqiang Zhang, Beijing Forestry University, China