

Seed Tech News



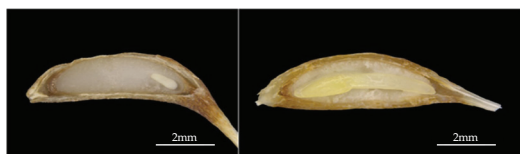
ISST:

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Morphological dormancy

Morphologically dormant (MD) seed embryos are not fully developed at maturity (underdeveloped and small relative to the size of the endosperm) and must grow/mature prior to germination. Embryos can be either undifferentiated (no clear structure) or underdeveloped but differentiated with some rudimentary structures visible (i.e. radicle and cotyledons). In seeds with MD, germination can be particularly slow even given the optimum germination conditions due to the required period of embryo development/growth prior to radicle emergence.



Internal morphology of a morphologically dormant (MD) seed of *Clematis linearifolia* (Ranunculaceae), a species producing seeds with a small, underdeveloped linear embryo (5 mm before radicle emergence occurs (image on the right). The embryo requires sufficient time to grow prior to germination and as a result, the sowing window must account for the period required for the embryo to reach maturity, which can only occur under specific soil moisture and temperature conditions.

Adopted from Baskin and Baskin, 2004b (Hort. Technology); Baskin and Baskin, 2004c (Seed Science Research); Erickson et al, 2016 (Pibara Seed atlas & Field guide, Australia)

Secretary : Sandeep Kumar Lal
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From President's Desk...

Dear Members,

Greetings from the Secretariat!

Science, technology and innovation (STI) are the key drivers of a country's development. Realising the importance of science, India adopted its first Science Policy Resolution (SPR) in 1958, and established national institutions to pursue all branches of basic and applied sciences. Since then, it has kept evolving its science policies over the years to the present Science, Technology and Innovation Policy (STIP) of 2013 to meet the changing demands and challenges of its people, while harmonising with the national and global positions. As a result of this, India has emerged today as the third largest scientific and technical manpower in the world. By 2015, it had 162 universities, including the PG education offered by the research laboratories under the CSIR, awarding 4,000 doctorates and 35,000 postgraduate degrees annually. In view of the demands of a large population and its principally agrarian economy, India paid greater attention to strengthen the Research, Education and Extension in agriculture. Presently there are 63 SAUs, 4 DUs and 3 CAUs offering UG and PG education in agriculture, conducting agricultural research to solve regional and national challenges and supporting agriculture extension through the majority of 724 KVKs attached with them.

The Government of India declared the period of 2010-2020 as the Decade of Innovation. The decade saw an era of new ways of thinking, innovating and delivering. With one of the largest pools of trained S&T professionals and focus on innovation, India has made an impressive progress in the Global Innovation Index (GII), climbing to 46th position in 2021 from 81st in 2015 (www.wipo.int/edcos/pubdocs/en/wipo_pub_gii/). Being innovative in scientific research and technology development is the key in finding sustainable solutions in every field. From agriculture to wellness; from managing the climate change to green energy technology; from healthcare for masses to education and skilling; from building eco-friendly dwellings to efficient transportation; from communication to defence and banking, the world is looking for innovative solutions.

Several innovative seed technologies have emerged during the last 15 years or so. These include hybrid seed production using apomixes; genome editing for eco-friendly management of abiotic and biotic stresses; quality assessment using chemi-luminescence properties of the seed coat; enhancing seed quality through a wide range of chemical or biological pesticide and seed stimulants, using machine visions and drone technology for identification of off-types; and preserving the highest quality during storage using drying beads technology or pesticide treated seed sacs, to name a few. However, unlike the last millennium, which was dominated by the scientific contributions from the public research institutions, most of these innovations have been introduced by the private sector seed companies and manufacturers of equipment, pesticides and bioregulators. Closer collaborations need to be forged between the public and private sectors for coming out with more effective, economic and innovative solutions in seed technology.

Malavika Dadlani

HONOURS AND AWARDS



The Executive Committee of ISSS announced Dr. Vijay Dunna, Principal Scientist, ICAR-IARI, New Delhi as the National Representative for India, in the Annual General Meeting on 13.08.2021. As a representative of ISSS, he will promote the International Society of Seed Science

and help in recruiting its members, provides information on regional events to the newsletter, as well as exchanging information to and from the members and the ISSS. He will also provide feedback to the Executive Committee of actions that will encourage and facilitate the participation of local members in the Society.

SCIENTIFIC BREAKTHROUGHS (Collated from various sources)

A shift in abscisic acid/gibberellin balance underlies retention of dormancy induced by seed development temperature

Through a combination of physiological, pharmacological, molecular and targeted metabolomics approaches, researchers showed that retention of wheat (*Triticum aestivum* L.) seed dormancy levels induced by low and high seed development temperatures during post-desiccation phases is associated with modulation of gibberellin (GA) level and seed responsiveness to abscisic acid (ABA) and GA via expression of TaABI5 and TaGAMYB, respectively. Dormancy retention during imbibition, however, is associated with modulations of both ABA level and responsiveness via expression of specific ABA metabolism (TaNCED2 and TaCYP707A1) and signaling (TaPYL2, TaSnRK2, TaABI3, TaABI4 and TaABI5) genes, and alterations of GA levels and responsiveness through expression of specific GA biosynthesis (TaGA20ox1, TaGA20ox2 and TaGA3ox2) and signaling (TaGID1 and TaGID2) genes, respectively. Expression patterns of GA signaling genes, TaRHT1 and TaGAMYB, lacked positive correlation with that of GA regulated genes and dormancy level observed in seeds developed at the two temperatures, implying their regulation at post-transcriptional level. Results overall implicate that a shift in ABA/GA balance underlies retention

of dormancy levels induced by seed development temperature during post-desiccation and imbibition phases. Consistently, genes regulated by ABA and GA during imbibition overlapped with those differentially expressed between imbibed seeds developed at the two temperatures and mediate different biological functions. For more details of the research, refer to the published paper in the *Plant Cell & Environment* vol. 44 during 2021 at <https://doi.org/10.1111/pce.13963>

Transcriptome and translome changes in germinated pollen under heat stress uncover roles of transporter genes involved in pollen tube growth

Plant reproduction is one key biological process that is very sensitive to heat stress and, as a result, enhanced global warming becomes a serious threat to agriculture. In this work, researchers have studied the effects of heat on germinated pollen of *Arabidopsis thaliana* both at the transcriptional and translational level. They have used a high-resolution ribosome profiling technology to provide a comprehensive study of the transcriptome and the translome of germinated pollen at permissive and restrictive temperatures. They have found significant down-regulation of key membrane transporters required for pollen tube growth by heat, thus uncovering heat-sensitive targets. A subset of the heat-repressed

transporters showed coordinated up-regulation with canonical heat-shock genes at permissive conditions. Researchers also found specific regulations at the translational level and have uncovered the presence of ribosomes on sequences annotated as non-coding. Results of this study demonstrate that heat impacts mostly on membrane transporters thus explaining the deleterious effects of heat stress on pollen growth. The specific regulations at the translational level and the presence of ribosomes on non-coding RNAs highlights novel regulatory aspects on plant fertilization. For more details of the research, refer to the published paper in the *Plant Cell & Environment* vol. 44 during 2021 at <https://doi.org/10.1111/pce.13972>

Endoplasmic reticulum stress pathway mediates the early heat stress response of developing rice seeds

A transient heat stress occurring during early seed development in rice (*Oryza sativa*) reduces seed size by altering endosperm development. However, the relationship between the timing of the stress and specific developmental stage on heat sensitivity is not well-understood. To address this, researchers imposed a series of non-overlapping heat stress treatments and found that young seeds are most sensitive during the first two days after flowering. Temporal transcriptome analysis of developing, heat stressed (35°C) seeds during this window shows that Inositol-requiring enzyme 1 (IRE1)- mediated endoplasmic reticulum (ER) stress response and jasmonic acid (JA) pathways are the early (1–3 h) drivers of heat stress response. Researchers propose that increased JA levels under heat stress may precede ER stress response as JA application promotes the spliced form of OsbZIP50, an ER response marker gene linked to IRE1-specific pathway. This study presents temporal and mechanistic insights into the role of JA and ER stress signaling during early heat stress response of rice seeds that impact both grain size and quality. Modulating the heat sensitivity

of the early sensing pathways and downstream endosperm development genes can enhance rice resilience to transient heat stress events. For more details of the research, refer to the published paper in the *Plant Cell & Environment* vol. 44 during 2021 at <https://doi.org/10.1111/pce.14103>

Cotyledons facilitate the adaptation of early-maturing soybean varieties to high-latitude long-day environments

Soybean (*Glycine max*), a typical short-day plant (SDP) domesticated in temperate regions, has expanded to high latitudes where daylengths are long from soybean emergence to bloom, but rapidly decrease from seed filling to maturity. Cotyledons are well known as the major storage organs in seeds, but it is unclear whether developing cotyledons store flowering substances at filling stage in SD for upcoming seedlings, or instead respond to photoperiod for floral induction after emergence of matured seeds in long-day (LD). Here, researchers report that cotyledons accelerate flowering of early-maturing varieties not resulting from stored floral stimuli but by perceiving photoperiod after emergence. Researchers found that light signal is indispensable to activate cotyledons for floral induction, and flowering promoting gene GmFT2a is required for cotyledon-dependent floral induction via upregulation of floral identity gene GmAP1. Interestingly, cotyledons are competent to support the entire life cycle of a cotyledon-only plant to produce seeds, underlying a new photoperiod study system in soybean and other dicots. Taken together, these results demonstrate a substantial role for cotyledons in flowering process, whereby researchers propose a 'cotyledon-based self-reliance' model highlighting floral induction from emergence as a key ecological adaptation for rapid flowering of SDPs grown in LD environments at high latitudes. For more details of the research, refer to the published paper in the *Plant Cell & Environment* vol. 44 during 2021 at <https://doi.org/10.1111/pce.14120>.

RESEARCH FINDINGS

Zeolite beads an effective low-cost tool for enhancing the seed storability in onion

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Onion seeds have one of the lowest storability among cultivated crops. Seed ageing is associated with ROS induced degradation of cellular components including DNA, RNA and proteins. The peroxidation of lipids leads to membrane degradation and leakage of solute from the cells, thus resulting in reduced vigour and germination. According to Harrington, the storability of seeds can be enhanced by reducing the storage temperature and or by reducing the seed moisture.

The reduction in the storage temperature requires setting up of cold storage unit, which is not only capital intensive but also involves recurring cost. Since > 80 % of the onion seed are produced by farmers (Gupta and Sharma, 2014), there is a need for on-farm seed storage technique. Desiccants like silica gel, aluminum silicates, lithium chloride, and calcium chloride are being used for drying seeds. These desiccants when kept in a closed container, reduce the RH of the air and the seeds lose moisture until it comes to equilibrium with storage atmosphere. Drying beads ® or zeolite beads are made of aluminum silicate. They have high affinity towards the water and can absorb moisture up to 20-25% of their dry weight. Unlike silica gels, aluminum silicates have a high affinity to water and works well even in low RH. It reduces the moisture quickly, is non-toxic can be regenerated by heating at > 200 °C for 3-4 hours.

Freshly harvested seeds of three onion varieties - Bhima Dark Red (BRD), Bhima Shakti (BST) and Bhima Safed (BSD) having initial germination of >80% and initial seed moisture ranging from 5.3-8.5% were dried in zeolite beads in 2: 1 bead to

seed ratio at ambient temperature (25-35 °C) for 96 hours. The dried seeds were hermetically sealed in a moisture impervious pack and stored for 12 months in ambient temperature (10 °C- 45 °C). Drying in zeolite beads rapidly reduced the seed moisture to 2.91%- 3.48% (Fig. 1). Ultra-drying of onion seeds had a significant effect on the germination (%). With an average germination of 85.33% at initial stage, the germination reduced to 80.55% at the end of 12 months. Whereas in control (having seed moisture of 5.3- 8.5%), the germination reduced to 51.78%. The ultra-dried seeds had 83.1% higher vigour index-I, 98.6% higher vigour index-II and 98.6% lower electrical conductivity in comparison to control indicating higher vigour and quality of seeds. The metabolic activity is higher in seeds with high moisture, which leads to higher reactive oxygen species (ROS) generation and rapid deterioration of seeds. Several studies have shown that seeds dried in zeolite beads had higher antioxidant activity as well as lower malondialdehyde content.

The dry chain concept, which refers to drying seeds to safe moisture levels and storing the seeds by hermetically sealing in moisture impervious bags is recommended for the storage of seeds in community seed banks and on-farm seed storage units (Bradford *et al.*, 2018). From the study, it is evident that onion seeds can withstand ultra-drying up to ~3% without any seed damage. Onion seed drying in zeolite beads (2:1 bead to seed) for 96 hours before packing can enhance the storability and vigour of onion seeds under ambient conditions.

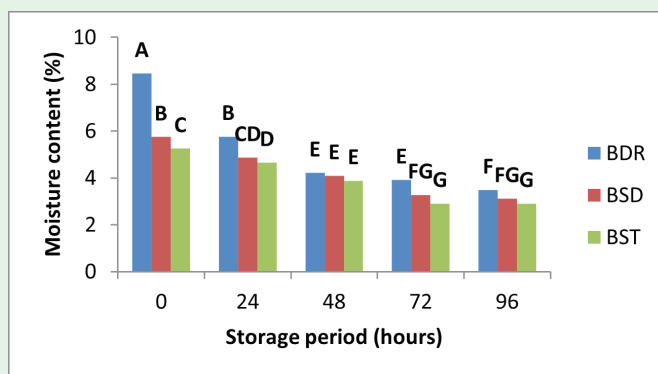


Fig. 1: Effect of zeolite beads on onion seed moisture.

INNOVATIVE SEED DISTRIBUTION SYSTEM

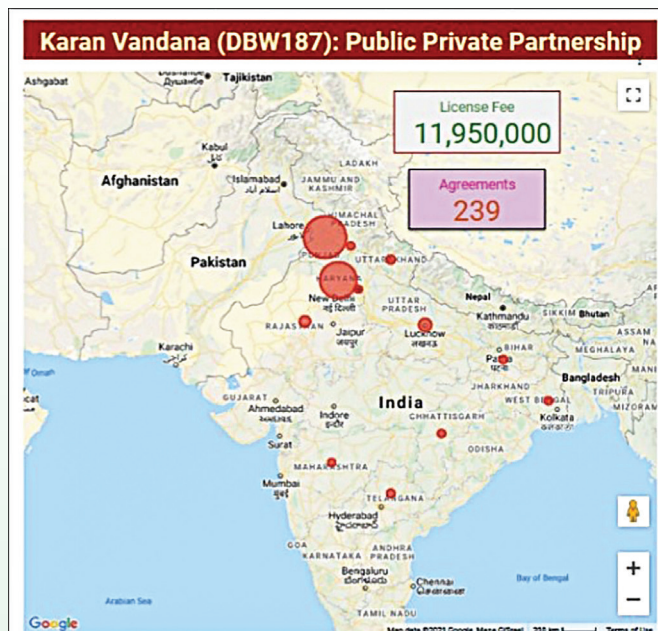
Karan Vandana (DBW-187) - Pivotal wheat variety for food and nutritional security

AK Sharma, CN Mishra, Umesh R Kamble, Satish Kumar and GP Singh

ICAR-Indian Institute of Wheat and Barley Research, Karnal

DBW 187 (Karan Vandana) is a high yielding wheat variety, developed by ICAR-Indian Institute of Wheat & Barley Research, Karnal. Firstly DBW-187 was released and notified by the 'Central Sub-Committee on Crop Standard Notification and Release of Varieties for Agricultural Crops' Govt. of India vide notification No S.O. 1498 (E) dated 01.4.2019 for commercial cultivation under timely sown, irrigated conditions in NEPZ which covers Eastern Uttar Pradesh, Bihar, Jharkhand, West Bengal (excluding hills), Odisha, Assam and plains of Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. However, variety has been released through area extension with gazette notification by 'Central Sub-committee on Crop Standards, Notification and Release of Variety' Ministry of Agriculture, Government of India vide No.S.O. 98(E) dated 06.01.2020 and S.O. 500(E) dated 29.01.2021 for cultivation in the North Western Plains Zone (NWPZ) of India which covers the states of 'Punjab, Haryana, Delhi, Rajasthan (except Kota and Udaipur divisions), Western Uttar Pradesh (except Jhansi division), Parts of Jammu and Kashmir (Jammu and Kathua Distt.), parts of Himachal Pradesh (Una Distt. and Paonta Valley) and Uttarakhand (Tarai region). DBW 187 was tested for two years in Special High Yield Potential Trial (SPL-HYPT 2018-2019 and 2019-20) under Early Sown High Fertility Conditions in NWPZ. It is a high input-responsive wheat genotype, high iron content (43.1 PPM), resistant to wheat blast and other diseases which shown yield superiority over other wheat varieties in both the zones.

ICAR-IIWBR, Karnal implemented following model of seed production forecasting breeder seed demand from various government and private



agencies and estimating demand from farmers of referred variety. ICAR-IIWBR, Karnal initially produced only 35.0 q of basic seed during 2018, however, same was accelerated up to 796.0q in 2019, 1220 q in 2020 and 3500 q in 2021 respectively. This quality seed production was achieved by inter-institutional programme in collaboration with varied ICAR institutes viz., ICAR-National Dairy Research Institute, Karnal, ICAR- Regional Station, Central Potato Research Institute, Modipuram (UP), ICAR-Indian Institute of Farming System Research, Modipuram (UP). Further, Foster Breeding Rights were given to the three centres viz., BAU, Sabour, BAU, Ranchi and BISA, Pusa Bihar in NEPZ by providing nucleus seed of DBW-187 and allowing faster multiplication of referred variety in the eastern region. In 2018, 2.5 Kg of minikit was distributed to farmers of eastern UP and Bihar which helped in rapid spread of variety in the region. In order to further expand seed availability in the eastern region, ICAR-IIWBR signed MOA (agreement) with RP-CAU, Pusa Bihar during 2021. Under public private partnership programme, 163

private seed companies signed agreement with ICAR-IIWBR during 2019 wherein 76 additional private seed companies signed contract with institute during 2020. In toto, 239 private seed companies are engaged in foundation and certified seed production of DBW-187 from the breeder seed supplied by ICAR-IIWBR, Karnal during last two years. ICAR-IIWBR generated revenue of RS. 119.50 lakh through licensing of variety and 122.5 lakh was earned through breeder & Truthfully labeled seed sale during 2019 and 2020. During last two years, TL seeds of DBW-187 were supplied to 12,000 farmers of Haryana, Punjab, Himachal Pradesh, Uttarakhand, Uttar Pradesh, Bihar, Jharkhand, West Bengal and Rajasthan. In addition, ICAR-IIWBR promoted new wheat varieties through vast network of Krishi Vigyan Kendra's (KVK's) and NGO's located in the eastern as well as western parts of the country for better farmers outreach.

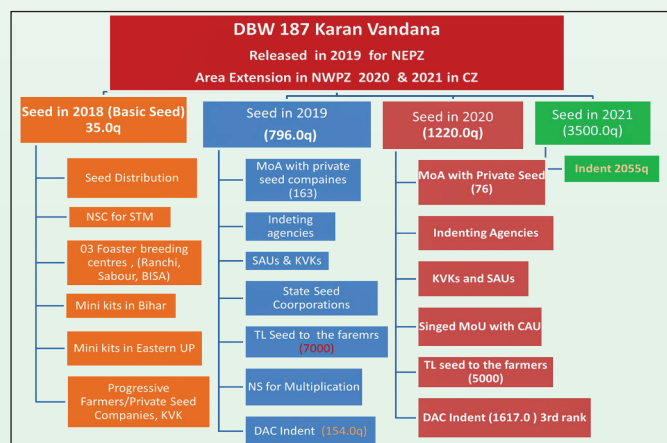


Fig.2. ICAR-IIWBR model of seed production for faster varietal diffusion

Assuring nutritional security by inclusion of biofortified wheat varieties in seed multiplication chain (Rabi BS Indents 2022-2023)

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Realizing the prominence of the nutritional quality of wheat and considering its nationwide consumption, research efforts were streamlined to develop and release a series of biofortified varieties in recent

times. The biofortified wheat varieties mentioned below are well equipped to provide required micro-nutrients for adequate growth and development in a cost-effective way. In toto, 16 varieties of wheat are included in breeder seed indents of Rabi 2022-23, having a total share of 33.25 % (4757.0q of 14306.8q) of total breeder seed indent. (Seednet India portal, 2022).

Details of Breeder Seed Indents of Biofortified Varieties in Wheat (2022-23)

S. No	Variety	Quality Trait	BS indent (q)
1.	DBW 187	Fe (41.3ppm) & Zn (43.7 ppm)	2055.10
2.	DBW 303	Protein (12.1%)	1326.30
3.	HI 8759	Fe (41.1 ppm) & Zn (42.8 ppm)	638.80
4.	PBW 1 ZN	Fe (40 ppm) & Zn (40.6 ppm)	175.60
5.	DDW 47 (d*)	Fe (40.1 ppm)	160.80
6.	WB-2	Zn (42 ppm) & Fe (40 ppm)	112.00
7.	DBW 173	Protein (12.5%) & Fe (40.7 ppm)	108.60
8.	HD 3298	Protein (12.1%) and Fe (43.1 ppm)	46.60
9.	PBW 752	Protein (12.4%)	33.20
10.	HD 3249	Protein (12.4%)	30.00
11.	HI-8777 (d*)	Fe (48.7 ppm) & Zn (43.6 ppm)	30.00
12.	PBW 771	Zn (41.4 ppm)	17.40
13.	PBW-757	Zn (42.3 ppm)	12.80
14.	HI 1633	Zn (41.1 ppm) and Fe (41.6 ppm)	7.00
15.	UAS-375	Protein (13.8%)	1.60
16.	HD-3171	Zn (47.1 ppm)	1.20
	*Durum Wheat	Total	4757.00
		Total BS Indent (2022-23)	14306.80

TECHNICAL ARTICLES

Recalcitrant Seeds and its Storage

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Generally, the seeds are classified into two categories *viz.*, “orthodox” and “recalcitrant” based on their desiccation and temperature sensitivity and storage. The orthodox seeds have the ability to tolerate reduction in moisture content and temperature, which increase the life span of seeds. These groups of seeds remain viable for longer periods even up to hundreds of years when they are dried and stored properly. They can be even stored at very low temperature of -196°C . However, recalcitrant seeds are killed if their moisture content is reduced below some relatively high critical value of 20 to 35%. It is estimated that 15% of the world’s flora possess recalcitrant seeds *i.e.*, approximately 37,500 species. In horticultural crops, except vegetables, few spices and fruits, most of the seeds have the recalcitrant behaviour.

Characters of recalcitrant seed

Recalcitrant seeds are well known for their sensitivity to desiccation and freezing temperatures. Generally recalcitrant seeds are large in size and weigh more as compared to orthodox seeds because of their high moisture content. Many recalcitrant seeds are covered with a fleshy layer, which is often edible, as in case of avocado, durian, jackfruit, jamun, litchi, mango, mangosteen, and rambutan. Similarly, some recalcitrant seeds are large and found in a single-seeded, simple fruit such as arecanut and coconut or as seeds in a composite fruit such as jackfruit. The shape and size of recalcitrant seeds of different species are differing greatly even within species or even within the same fruit. Some of the recalcitrant

seeds also possess the polyembryony nature. The other important property of recalcitrant seeds is their high moisture content, even after they have been shed from the mother plant. Unlike orthodox seeds, they do not undergo maturation drying. These recalcitrant seeds generally have high moisture content, ranging from 30 to 70%. The orthodox seeds can be stored for many years. However, the recalcitrant seeds cannot be stored for long period and its viability is few days to few months. Also, the recalcitrant seeds require special storage methods so as to prevent the moisture loss from the seed.

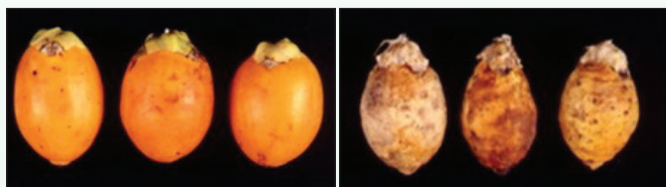
Critical moisture content

In recalcitrant seeds, decline in viability occurs abruptly below a certain moisture level, which is called “Critical Moisture Content (CMC) or “Lowest Safe Moisture Content (LSMC)”. Generally, the critical moisture content is 20-35% below which the seeds will be killed. For example, the critical moisture content of few recalcitrant seeds is given below:

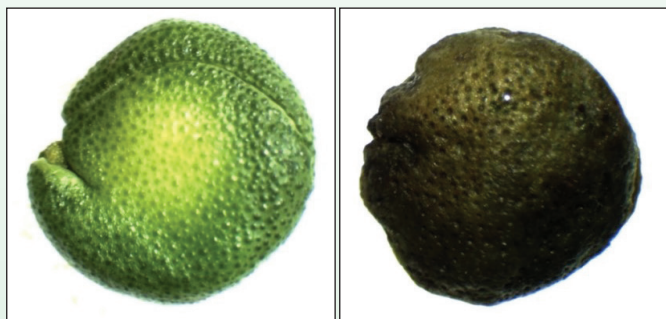
Species	Critical moisture Content (%)
1. Quercus robur	38
2. Nephelium lappaceum	20
3. Shorea talura	17
4. Hopea helferi	35
5. Hevea brasiliensis	15-20
6. Theobroma cacao	26
7. Litchi sinensis	40
8. Hancornia sp.	25
9. Myristica fragrans	45
10. Artocarpus heterophyllus	39
11. Euterpe edulis	39
12. Persia americana	49
13. Murraya koenigi	34
14. Areca catechu	33

Intra-varietal differences in critical moisture have also been found in number of species. For example, the critical moisture content for mango varieties is 25% in Alphonso, 32% in Totapuri (Doijode, 1990), 28% in Neelum and 34 % in Goa. The extent of

desiccation damage that occurs on the removal of water from desiccation sensitive seeds and thus the water content at which they lose viability, depends upon number of factors like the rate at which the seeds are dried and their metabolic activity when they are subjected to drying (which affect the 'metabolism-induced damage') as well as the extent to which any of the protective mechanisms are expressed.



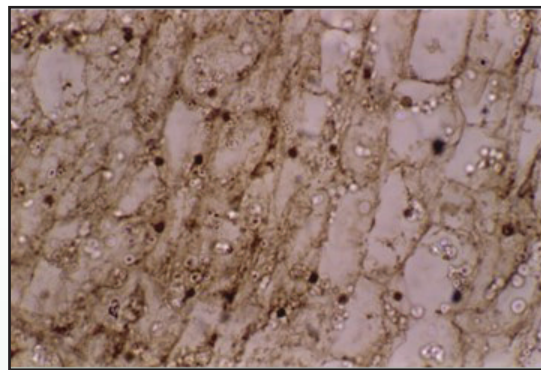
Viable and non-viable seeds in arecanut



Viable and non-viable seeds in curry leaf

Chilling Sensitivity

Generally, the orthodox seeds can be stored even up to the temperatures of -196°C without loss of viability. But the recalcitrant seeds cannot be stored at lower temperatures; even some species do not survive the temperatures of $10-15^{\circ}\text{C}$. It is because of chilling injury to the seeds, which varies according to species. Here there is a high moisture freezing limit which is the threshold and if it is exceeded, the viability of a seed sample will be reduced during storage. Thus, the chilling sensitivity in moist recalcitrant seeds is mainly due to the formation of ice crystals in between the cells when the moisture content is higher than 14 to 20%. For example, sharp reduction in storability was observed in arecanut seed at below 10°C .



Cellular damage at 5°C in arecanut seed

Recalcitrant seed species

Generally, recalcitrant seeds occur in humid forest environment. They may also occur in tropical, subtropical or temperate regions. The fruit crops *viz.*, mango, few species in citrus, avocado, jack, jamun, litchi, mangosteen, durian and rumbutan are coming under the recalcitrant seeds. The plantation crops like arecanut, coconut, cocoa, coffee, clove, nutmeg, rubber and tea and the spices like pepper, cardamom, curryleaf, cinnamon and cassia are also some of the examples of recalcitrant seeds. Forest tree species belonging to the Araucariaceae and Dipterocarpaceae have recalcitrant behaviour. Based on the tolerance to desiccation and freezing temperatures, some of the above species have been placed under 'intermediate' category of seeds.

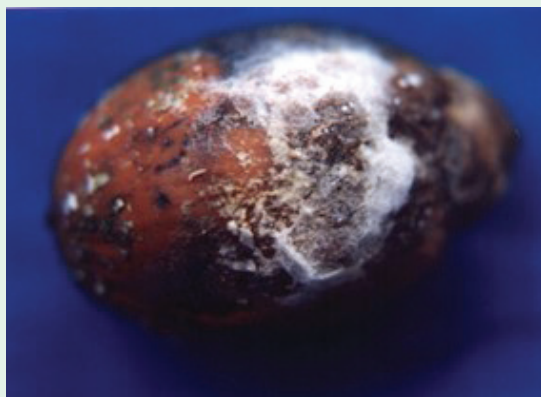
Storage of recalcitrant seeds

The longevity of recalcitrant seed is very short; it varies from a few days to a few months or a year under proper storage conditions. Therefore, recalcitrant seeds are to be sown immediately after collection. Then only they will give the maximum germination. In general, most of the above said horticultural crop seeds are sown immediately after collection. Because their longevity is very short, this varies from few days to few months. For example, the arecanut seed lose its viability within 24 days and curry leaf tolerates up to 14 days under ambient storage. Some seeds may be kept within the fruit itself for few days. The curry leaf seeds can be stored little more if it is stored along with fruit. However, due to the high moisture content

it may enhance the pathogen entry and create the germination loss. There can be no doubt in the mind of any investigator working on desiccation - sensitive seeds that microorganisms, more particularly fungi, play a significant role in post-harvest deterioration. Thus, the recalcitrant seeds can be stored through moist or imbibed storage, partial dehydration, controlled atmospheric storage and cryopreservation and *in vitro* conservation methods. Among them, moist or imbibed storage, partial dehydration and controlled atmospheric storage are short-term storage methods. Recently, the cryopreservation and *in vitro* conservation is gaining importance for long-term conservation of recalcitrant seeds.

a) Moist or imbibed storage

The recalcitrant seeds can be stored in moist media like damp charcoal, saw dust, moist sand and other moisture conserving materials and chemicals so as to avoid the moisture loss from the seed. This is a short-term storage method and the viability can be maintained up to three months or little bit more. For example, in arecanut, storage of fresh seeds with 5% moist sand or 0.2 M potassium dihydrogen phosphate premixed with sand at 5% level (seed: sand ratio 1:3) and packing loosely in 350 gauge polythene bags and stored in zero energy cool chamber recorded higher germination (85%) after four months of storage. In imbibed storage, the seeds are stored under water but only for a short period, for example the rubber seeds stored under water recorded only 60% germination, after one month of storage. But in this method the problem is the fungal growth during storage. Therefore, it is



necessary to give some chemical treatment to control the pathogens during storage.

Association of seed-borne microflora

b) Partial dehydration

In partial dehydration, the recalcitrant seeds are dried to certain critical moisture content by air at a temperature of 20°C and then stored. Rubber seeds can be stored for one year with 50% germination if they are cleaned, soaked in 0.3% benlate, surface air dried and stored in perforated polythene bags at an ambient temperature of 25°C. Similarly, the cocoa seeds can be stored up to 24 weeks with more than 50% viability.

c) Controlled atmospheric storage

The recalcitrant seeds can be stored in controlled atmosphere of carbon dioxide or sealed containers. For example, cocoa seeds can be stored up to 45 days in carbon dioxide atmosphere and durian seeds in sealed containers for about 32 days. Similarly, the wax-coated arecanut seeds stored in gunny bag under ambient condition were successfully extended the storage life up to 50 days with 60% viability. While, uncoated seeds stored in ambient condition loses its complete viability within 24 days. The litchi seed retained with fruits treated with benomyl (0.05%) and wax emulsion (6%) and sealed in polythene bags maintained 42% viability for 24 days. Coating of seed with wax also prevents the loss of moisture to certain extent in arecanut.

d) Cryopreservation and *in vitro* conservation

The recalcitrant embryos are tolerant to desiccation and low temperatures than whole seeds and together with their smaller size are amenable and practical for conservation. The strategy adopted for long-term genetic conservation of these recalcitrant seeds is to cryopreserve the embryos, which are more resistant to adverse conditions. For successful cryopreservation, at very low temperature, excised embryos must be dried to suitably low moisture content to avoid ice formation by ultra-low temperature.

Therefore, it is concluded that the short storage of recalcitrant seeds is a problem, as these seeds can't

tolerate the desiccation and freezing temperatures. It is also important to maintain the viability of these seeds for long-term for the genetic conservation to create genetic diversity. Hence, the optimum storage conditions for recalcitrant seeds should be determined for all the recalcitrant species.

Nanotechnology and its Application in Seed Science Research

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Nanotechnology encompasses engineering, production and application of physical, chemical and biological systems at the scale of atoms or molecules and infusing such concepts and principles in agricultural sciences to evolve processes and products that precisely deliver inputs in production systems that ensure food security and environmental safety is known as "Nano Agriculture". Richard Feynman, Nobel Laureate in Physics first envisioned the use of atoms and molecules for engineering the devices in 1959. Today nanoscale materials find use in areas covering all facets of human life and promising wide hope of breakthroughs in the area of agriculture. Nanoparticles (NP's) can be classified into three groups: (a) Natural nanoparticles, which includes particles originating from natural processes, e.g. clay particles (b) Incidental nanoparticles, includes particles generated as byproducts out of human activities like grinding and combustion (c) Engineered Nano Particles (ENP's) includes particles designed with specific properties and composition. Engineered nanoparticles include: fullerenes [molecule of pure carbon in varied shapes like hollow, tube, sphere etc e.g. carbon nanotubes (CNT's)], metal nanoparticles (pure metal NP's like Ag, Au, Fe and metal oxides like TiO₂, ZnO, CuO, SiO₂ etc), quantum dots (ENP's exhibiting size dependent optical or electronic properties) and organic polymers (polymers derived out of

nanoscale particles like dendrimers, polystyrene etc). The ENP's are being widely used for various applications in development of industrial goods along with potential application in the field of agriculture and allied sectors. ENP's exhibit dissimilar electrical, optical, magnetic, chemical and mechanical properties when compared to what they usually exhibit at macroscale. The increase in surface/volume ratio of NP's which results in increased activity of electrons in the vicinity are attributed to their differential behavior.

Seed is a vehicle for delivery of improved technologies and is a mirror for portrayal of inherent genetic potential of a variety/hybrid. Seed offers to integrate production, protection and quality enhancement technologies in a single entity in a cost-effective way. Scientific community is exploring the potential application of nanotechnology in the field of seed science research to tackle some of the most beleaguered areas. Nanotechnology can find its practicality in vital areas of seed science research as detailed below:

Nanoparticle Mediated Seed Invigoration: Metal nanoparticles can be grouped into two classes based on its chemical nature like pure metal NP's, which includes elemental silver (Ag), gold (Au), Iron (Fe) etc and metal oxide NP's, which include oxides (ZnO, TiO₂, SiO₂), nitrides (BN and TiN) and carbides (Fe₃C, BC) of elemental forms. These nanoparticles can be amalgamated to seed through process of dry dressing, priming (nanoprimering) or by novel technique known as electrospray. Mechanism of nanoparticles in seed hypothesised like they act as antioxidants through which they quench reactive oxygen species like super oxide anion radical (O₂⁻), hydrogen peroxides (H₂O₂) and consequently generate O₂ like catalase. Dry dressing and priming of seeds with NP's like Ag found to have profound influence in controlling surface microflora and insect pests. Metal NP's also shown the potential to overcome dormancy in the weed seeds, metal nano formulations were found to relieve dormancy in the bulbs of *Cyperus rotundus* and other weed

species which needs further investigation. Carbon Nano Tubes (CNT's) viz. Single Walled Nanotubes (SWNTs) and Multi Walled Nanotubes (MWNTs) can penetrate thick seed coat forming micropores and increases water uptake which is responsible for the significantly faster germination and higher biomass production in tomato. The Quantum Dots (QDs) can be used as a fluorescence marker coupled with immuno-magnetic separation proved useful to separate unviable and infected seeds.

Nano Seed Biotechnology: Nanotechnology offers a new set of tools to manipulate and delivery of genes using nanoparticles, nanofibres and nanocapsules. Properly functionalized nanomaterial serve as vehicles and could carry a large number genes as well as substances able to trigger gene expression or to control the release of genetic material throughout the plants. Such targeted delivery of genes to seeds could be used in transient gene expression at sight of interest for only one generation. Nanotechnologists are also hopeful of taking genetic engineering to new level down to atomic engineering. Atomic engineering could enable DNA of seed to be rearranged in order to obtain different plant phenotypes including colour, growth season and yield. In Thailand, Chaing Mai University has developed white grain rice variety from a traditional purple grained variety through nanotechnology-based mutation process, where a nano sized hole was drilled in embryos and it is bombarded with N^{2+} ions which stimulate rearrangement of genome and resulted in change in phenotype of rice variety. The GM seeds can be separated by the conventional seeds by the help of tagging the event involved in the GMO by radio nano-frequency tags which in turn detected by a specially modified processing unit by sensors which alerts about presence of GMO and allows its removal from seed lots.

Nano Smart Delivery System in Seeds (Smart Seeds): Existing commercial technologies allows coating of seeds with fertilizers, pesticides, herbicides, growth hormones etc. along with some inert material as a carrier with limitations of runoff,

leaching, narrow stability against degradation and uncontrolled release. In smart delivery system many nanoscale carriers like zeolite, magnesium sulphate, calcium carbonate and mesoporous silica core shells may be used to store, protect, deliver and control release of intended payloads in crop production processes. Nano lignocellulosic materials like organic polymers: dendrimers, polystyrene etc. in pure forms or in the biocomposite forms can be used for encapsulating the nanocarriers in controlled release formulation delivery systems. One of the advantages of nanoscale-controlled release formulation delivery vehicles in seed applications is improved stability of payloads, thereby increasing its effectiveness.

Nano Barcode Technology: Nowadays barcode is most widely used in marketing of goods both in national and international markets. Barcodes essentially carry digital signatures in the form of black and white horizontal bar upon which product details can be deciphered using electronic code reader. With advance in nanotechnology, nano-based bar codes are constructed by nanoscale electroplating of metal in desired pattern that can do the same functions of conventional barcodes for tracking and controlling the quality of seed moving in the market. In the era of proprietary rights seed sector can utilize nano barcoding system where novel genes are being incorporated into seeds and sold in the market. Nano barcodes can also be attached to seed packages for tracing of breeder, foundation and certified seeds with all relevant information like lot number, name of producer, varietal details, parentages, date of seed testing and its complete results etc.

Nano Biosensors: Nano biosensors are compact analytical devices incorporated with biologically derived sensitized element linked to the physico-chemical transducers. These sensors are developed by integration of immobilized bioreceptor probe that are highly sensitive to target analyte. Biologically sensitized elements (probe) composed of enzymes, antibodies, nucleic acids, molecular imprints or mere nanowire which receive signal from analyte

and in turn transfers the information to transducer before sending the information to microprocessors for amplification. The microprocessor can connect to user friendly devices like smartphone or computer to analyse the results. There are different classes of nano biosensors (mechanical, optical and nanoshell biosensors) which can find its potential application in the field of seed technology. Mechanical nano biosensors can be connected to user friendly devices through GPS can be effectively used for real time monitoring of contaminating pollen in seed production fields; such sensors can detect the contaminating pollen by its morphology and helps to take decision for corrective measures by seed grower. Optical or nano shell biosensors can be used for quick detection of seed quality in storage, such nano sensors known as electronic nose (E-nose) which uses pattern of response over array of gas sensors which detects early release of gases like volatile aldehydes during seed ageing processes and further helps in scheduling of mid storage correction treatments.

Nanotechnology in Seed Health Management:

Nano-coating using elemental forms of Zn, Mn, Pa, Pt, Au, Ag protect seed from pathogens. Seeds coated with nano particles encapsulated with specific bio-agents will reduce seed rate, ensure optimum field stand and improved crop performance by managing the seed and soil borne pathogens. Nano core shell biosensors with protein-ligand (antigen) and protein-nanoparticles can be employed for detection of pathogens associated with seeds. Nano biosensors can also be integrated into seed packages for detection of insect activity by sensing the metabolites released by insects at early stage. Nano cores loaded with insecticides can be treated with seeds for effective management of infestation. These nano cores can also be upgraded to programmed products known as “gut busters” which release insecticides only in alkaline environments like insect stomach.

Nanotechnology in Seed Packaging and Handling:

Ligno-cellulosic nanomaterials has opened up new area for novel and value-added nano biomaterials and products for example, cellulosic nano crystals can

be used as light weight reinforcement in polymeric matrix as nanocomposite, such application can be used for development of light weight, highly durable and cost-effective packaging material for seeds. In future bio and gas sensors could gain importance, when these sensors are integrated with packaging materials, they can monitor the changes in micro-environment.

Inference: Nanotechnology can play catalytic role in boosting the crop production. In the light of statement made by agricultural laureate M.S. Swaminathan (“Second green revolution through quality seed”) nanotechnology have the potential to revolutionize the 21st century agricultural technology and can answer the long-standing problems in the areas of seed science research. “The next big thing is really small” but with the capacity to move mountains in the arena of agricultural sciences.

ImageJ is a useful tool for measuring seedling length


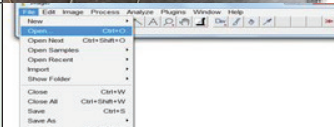
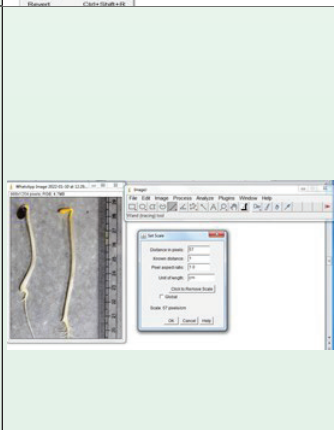
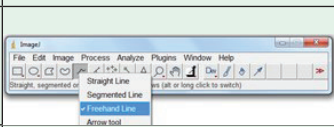
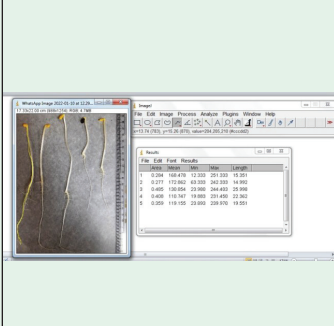
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Measuring seed vigour is an integral part of seed technology research. Seedling length and vigour index-I are important seed vigour parameters. Seedling length is measured manually by using a laboratory scale, which is laborious and time consuming. Often seed technologists have to measure 100s of seedlings in a day, since the seedlings cannot be stored, this exercise has to be completed on that given day. There are limited tools available for automation of seedling measurements; some of them require hardware like scanner and proprietary software which are often expensive. There are several open-source image processing software to name a few- ImageJ, FIJI, QUPATH, CELL PROFILER, CELL PROFILER ANALYST, ORBIT etc.

ImageJ is an open-source Java-based image processing program developed at the National Institute of Health and the Laboratory for Optical and Computational

Instrumentation (LOCI, University of Wisconsin, USA). It works on the principle of measuring image pixels, which is calibrated to known units of measurements. The user needs to define the pixel in a particular image in terms of centimeters or inches. It can process various formats of the image like- GIF, JPEG, TIFF (uncompressed), BMP, PNG, PGM, FITS and ASCII. Apart from measuring seedling length, it measures angle, area, mean, standard deviation, min and max of selection or the entire image.

Steps for measuring the seedling length.

<p>1) Take photographs of seedlings along with measuring scale</p>	
<p>2) Click open from the file menu to open the photograph</p>	
<p>3) Set scale- to define a pixel, on the measuring scale draw a line covering one cm. In the analyze menu select the set scale, in the known distance write 1 and unit as cm. In this example, 57 pixels is calibrated as 1 cm. This process has to be repeated for each image.</p>	
<p>4) Right-click on the line section menu and select the freehand line.</p>	
<p>5) With the help of the mouse trace/draw a line on the seedling and press control + m. The length of the seedling will be displayed. To measure another seedling trace the next seedling and press control + m.</p>	

ImageJ not only measures seedling length, it can be used to add a scale bar on an image, measure seed length, seed width, seed area etc. It is free, easy to use software. The measurements can be directly exported to MS-Excel for further analysis. Unlike the manual method of seedling measurement wherein seedling lengths are first recorded in a notebook and then typed in MS-Excel for tabulation, ImageJ saves time by eliminating the steps of manual data tabulation. Being an open-source software, source code is openly available which anyone can inspect, modify and enhance. ImageJ is a versatile tool that can be modified to suit the needs of seed technologists.

OIPNION

Need for Elaborating & Standardising the Seed Testing Procedure for Insect Assessment for Uniform Adoption by Seed Analysts and for Validating the Standards Prescribed In the IMSCS-2013

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Seed is prone to damage by insects both in the seed crop stage and during post-harvest stages, especially in subtropics / tropics which is enhanced by warm and humid environment common in many parts of India. The stored pests may begin with field level infestations and continue thereafter. Several insect species, mostly of Lepidoptera (Moths) and Coleoptera (Beetles & Weevils) families are known to damage seed; the type of damage being characteristic of the insect species. Also, while some are polyphagous, some others are limited to one or a few crops only. Some can attack whole grains while some others prefer already damaged / infested grains. The highly complex nature of the "Insect Damage" [ID] issue is summarised in the following paragraphs.

1. The kind of damage to seed ranges from a mere scratch or bruise on the seed coat [testa] to

injuries of varying types and degrees as well as seed completely turning into powder and or the larvae forming seed web clusters.

2. Insect life-cycle comprises four stages *viz.*, Egg, Larva, Pupa and Adult. The duration of each stage and therefore the total span of the 'full life cycle' vary between insect species; and so also, the number of eggs laid on each occasion, features of the eggs, pattern of egg laying, survival rate of each of the four stages, the number of life-cycles in a year, etc. The actual location in /on the seed where eggs are laid and where the pupae are formed vary between insect species.
3. The eggs, larvae, pupae or adult may be alive or dead – the percentage varying depending on several factors. Further, more than one insect species may infest the seed at the same time or at different times during the storage period and again each one of them to varying degrees. Almost always, the larval stage is the actual 'damage causing' stage; while in some insect species with biting- cutting-chewing mouth parts, the adults do inflict injury to the seed. Of course, the egg and pupal stages per se do not directly injure the seed except for affecting the physical appearance of the seed mass and revealing the likely larval and adult populations, respectively that can be expected from them as also the possible extent of damages thereof by these stages.
4. The number of eggs laid by each adult female, the resultant population of larvae, pupae and adults; and the ratio of female / male adults, vary between insect species, the kind of crop seed, initial physical quality, status of seed, its moisture content, nature of packing / sealing, seed storage environment, storage duration, storage handling-management, etc.
5. The location of damage in or to the seed may be just the seed coat only or may extend into the endosperm, cotyledon[s], embryo etc – again to varying degrees in different locations in the seed. The insect's life cycle stage and the location and extent of damage, would influence the ability of the seed to germinate into a Normal Vigorous Seedling. Occasionally, if the insect damage has not affected the Embryo, the seed may not only germinate but also produce Normal Seedling in spite of the damage. So also, seed carrying eggs on its surface or with pupa as accompanying contaminant may still be producing Normal Seedling.
6. The damage may be visible to the naked eye or with magnification. It may not be visible externally, if the infestation is 'hidden'- with the larva, pupa or adult inside. This happens when the larva that has bored into the seed feeds on the inner contents and then seals the hole and pupates inside. The damage is visible only after the fresh adult emerges out from the pupa opening up the seal to come out.
7. The seed lot / seed sample may contain various forms of insect damage to varying extents, depending on the kind of host seed, insect species involved, initial seed quality, seed moisture level, storage environment, length of infestation duration, storage handling/ management practices adopted, packaging type etc. Broadly, the various forms or types of insect damage that may be seen in the seed lots or seed samples are as follows:
 - [a] only eggs -one or more on one or more seeds; all alive or all dead or some alive and some dead – [is there any technique to determine whether an egg is alive or dead?];
 - [b] only larvae – one or more, all alive or all dead or some alive and some dead;
 - [c] only pupae- one or more, all alive or all dead or some alive and some dead- [is there any technique to determine whether a pupa is alive or dead?];
 - [d] only adults- one or more, all alive or all dead or some alive and some dead;
 - [e] combination of two or more of [a] to [d];

[f] one or more seeds with varying degrees of damage of the varying types listed in [1] above compounded further by the situations mentioned in [2] to [6] above; and

[g] combination of two or more of [a] to [f] above.

The foregoing adequately explains the highly complex nature of the issue – ‘Insect Damage’.

8. Thus, the Seed Lot and the Submitted Sample can be expected to have the ‘Insect Damage’ with such complexities i.e., one or more of the types listed at 7[a] to 7[g] above, depending on the various factors already listed. This makes the Seed Analyst’s job quite difficult on how to proceed further with the analysis. The main issues on which the Analyst needs clear guidance are listed below:

[a] Working Sample [WS] for each type of damage listed in 7[a] to 7[g]: Whether to analyse the entire Submitted Sample [SS] itself or derive a WS from the SS; if to be derived - then what should be the size of WS for each type;

[b] Classifying the ‘Damage’ into the types listed in 7[a] to [g] above: Classification becoming complicated with the presence of more than one type of Damage among 7[a] to [g];

[c] Identification of the ‘hidden infestation’ mentioned in [6] above, estimating and expressing the same in Numbers and or Weight per Unit weight of WS or converting into percentage terms on weight basis;

[d] Limitations involved in Applying the ‘Half Seed Rule’ and classifying those seeds with half or more of the seed damaged as Inert Matter. Unlike other “less than half seeds “due to immaturity, mechanical damage, etc and therefore classified as Inert Matter. The seed with insect damage has another dimension to ‘Quality’ i.e., insect incidence too. Therefore, application of ‘Half Seed Rule’ should not stop with merely classifying such seeds as Inert Matter, but also take into account the ‘ID’ aspect

of seed quality and assess and quantify ID;

[e] Estimating the ‘Damage’ in quantity terms: Quantifying the damage in terms of Numbers [Counts] and or Weight; the Estimation & Quantification getting complicated with the presence of more than one type of Damage among 7[a] to [g];

[f] Expressing / Reporting the ‘Damage’: In terms of Numbers or Weight as such or converting into percentage terms; and, if so the precise method for conversion to percentage;

[g] Application of tests such as ‘Ninhydrin test’, ‘Dyeing tests e.g., Malachite green test, Alkali or Glycerine method, Lactophenol method etc., to detect ‘hidden infestation’ and the standard procedure[s] thereof; and

[h] Identification of the actual insect species involved in the ID and reporting their names in the Results-if possible, quantify the damage with reference to each species.

9. ISTA Rules, even the latest 2021 edition, does not have any exclusive testing procedure for insect damage; except for applying the ‘Half Seed Rule’ during Physical Purity Analysis, to classify the seed as Inert Matter [along with any other Inert Matter], if half or more of the seed is damaged, irrespective of the cause of damage. The limitation with applying ‘half seed rule’ is explained in para 8[d] above as it does not reflect the presence of insect damage. However, the Hand Book of Seed Testing [1993] published by the Dept of Agriculture, Govt of India has dealt with this aspect in some detail in Chapter 10. However, critical review of the same will bring out the gaps needing attention, clarification, elaboration and guidance with reference to the issues listed in paras 7 & 8 above.

10. In this context, the IMSCS-1998 & 2013 prescribe the standard [i.e., maximum permissible limit] for Insect Damage as follows:

“A seed lot under certification shall not have apparent or visible evidence of damage by insects for both F&C Classes

in excess of 1% for the seeds of maize and legumes and 0.50% for other seeds unless otherwise prescribed. “

11. The prescription in IMSCS is based more on arbitrary general opinion rather than on any research data- results. Yet, the prescription is given in IMSCS as an ad-hoc arrangement so as to have some form of maximum permissible limit for seed certification purposes rather than having none. This specification, hopefully, also is adopted by the Seed Sector for the Labelled seed produced outside official certification. In any case, the Standard is silent regarding whether the prescribed 0.5% and 1% are by weight or by number. This needs clarity. Also, how to determine the quantum of damage of the various types listed in [7a] to [7g] so as to confirm the ‘evidence of damage’ mentioned in the Standard. This also needs clarity. Above all, the prescribed Standard on the one hand and the Testing procedure, Result expression pattern/unit and Reporting and Interpretation of the result on the other should match, and be in tune with each other.
12. Further, even though the above-mentioned Standards for Insect Damage have been in vogue for more than 32 years since 1988, [same continued in the revised edition of 2013], it has not undergone any form of review or validation by a regular / suitable research experiment. Therefore, it may be worth that the Standard is verified / validated by research [may be under
- Seed Technology Research (STR) component of AICRP-Seed], at least now. If the results justify any revision in the Standard already prescribed, suitable proposal to the Central Seed Certification Board [CSCB] will be needed. Even if any STR Project is not envisaged, at least some form of deliberation among the research groups [involving the seed testing group at some stage] should prove useful in bringing the required clarity with authenticity.
13. The Seed Analysts or STLs in the country adopt some form of locally devised method to analyse insect damage and express their observations. Considering the complexity, occasionally a Physical Examination Committee [PEC] is formed within the STL to evaluate the ID. If possible, personnel from production, processing or certification are co-opted into the PEC to enable better judgment.
14. Hope, the foregoing sufficiently explains the highly complex nature of the ID aspect of seed quality justifying the urgent need for elaborating and standardising the testing procedure to cover all the aspects listed in paras 7 and 8 for uniform adoption by the Seed Analysts. It is earnestly believed that this will stimulate further thinking on the crucial issue of *Insect Damage Assessment During Seed Testing* and pave the way for elaborating and standardizing the testing procedure for uniform adoption by Seed Analysts in the STLs in the country.

NOTE:

All the ISST members are requested to contribute to various columns of Seed Tech News by providing information on a) Awards and Honours received; b) Upcoming trainings/ events; c) Recommendations of scientific gatherings; d) Latest research findings etc. In addition, brief technical notes may also be submitted for fast dissemination.

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