

Seed Tech News



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The United Nations General Assembly has declared 2022 the International Year of Artisanal Fisheries and Aquaculture (IYAFA 2022). Its main aim is to raise awareness about the importance of small-scale fisheries and aquaculture for our food systems and environment.

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

Dear Members,

Greetings

Building an 'Atma Nirbhar Bharat' or the self-reliant India, and promoting entrepreneurship in every sector, are the two most dynamic government campaigns. Agriculture being the single most important occupation in India, supporting entrepreneurship in agriculture and allied sectors has also attracted due attention of the government, and continuous efforts are being made to improve the social and economic status of the rural people by encouraging local products and supporting agri-based enterprises. One of the popular schemes that the Indian government has sanctioned in 2015 in this regard, is ASPIRE, a Scheme for Promotion of Innovation, Rural Industries and Entrepreneurship (ASPIRE), under the Ministry of Micro, Small and Medium Enterprises (MSME). In addition, many programmes have also been started by the different ministries, specially the Ministry of Agriculture & Farmers' Welfare; Rural Development; Commerce; Food Processing; Science and Technology, etc. to backstop such drives. Notable among these is the AGRI-UDAAN programme which aims to promote innovation and entrepreneurship in agriculture. It also aims to support startups and help them connect with potential investors. An agri-business accelerator programme has been launched to help convert innovative ideas into viable agri-businesses through mentorship, training and help in raising funds, which is managed by the Indian Council of Agricultural Research (ICAR) through 25 Agribusiness Incubators (ABIs) in its institutes. Notable among these are ICAR-IARI, New Delhi; ICAR-NAARM, Hyderabad; ICAR-IIHR, Bangalore, etc. Under this programme, start-ups get incubation space to start their business, have access to research laboratories and libraries and get the benefit of guidance on technical matters from the experts. This programme also helps the most promising start-ups with regulatory services.

A Central Sector Scheme on "Formation and Promotion of Farmer Produce Organizations (FPOs)" was launched to form and promote 10,000 new FPOs with a total budgetary provision of Rs. 4496 crore for five years (2019-20 to 2023-24). A further budget outlay of Rs. 2369 crore has been made for 2024-25 to 2027-28 for handholding of each FPO for five years from its aggregation and formation. Considering that availability of quality seed and planting material is one of the most vital components in agriculture, there are many opportunities in the production and service-based agri-business in the seed supply chain, not restricted to only multiplication of seeds of new improved varieties. It is therefore surprising to not find many seed-supply FPOs and startups, specially in difficult and hilly regions, where these are needed most.

The NSP (Crops) and ICAR Seed Projects (Crops and Vegetables) in agricultural universities and the ICAR institutes, are engaged in producing Breeder Seed; production of certified or labelled seeds in participation with the farmers; and imparting trainings of different levels in seed production, storage, quality testing and seed enhancement to farmers and others. Now there is a need to support establishing FPOs, specially in difficult regions for the production of quality seed of field crops and planting material of horticulture crops. Seed technologists can play a big role in it.

Malavika Dadlani

AWARDS AND HONOURS

Woman Achiever in Seed Sector, 2022



At the 3rd Annual Conference of 5F Farming on 'Realigning Indian Agriculture for a Sustainable Future', organized by the Foundation of Advanced Training in Plant Breeding (ATPBR) in partnership with OUAT, Bhubaneswar, Institute of Life Sciences (ILS) and Indian Council of Agricultural Research (ICAR) held at Kalinga Institute of Industrial Technology (KIIT) during 10-12 March 2022, in Bhubaneswar, Odisha, ATPBR conferred the Award of "Woman Achiever in Seed Sector 2022" to Dr. Malavika Dadlani, Former Joint Director (Research),

ICAR-IARI, New Delhi recognizing her contributions in the field of Seed Technology and its dissemination to farmers in India.

Best performance centre awards, 2021-22 under AICRP on Seed (Crops)

- **Mahatma Phule Krishi Vidyapeeth, Rahuri** received best performance award (SAU Category) in Quality Seed Production Component for 2021-22 during AGM of AICRP on Seed (Crops) held during 12-13 May, 2022.
- **ICAR- Indian Institute of Wheat and Barley Research, Karnal** received best performance award (ICAR Category) in Quality Seed Production Component for 2021-22 during AGM of AICRP on Seed (Crops) held during 12-13 May, 2022.
- **Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal** received best performance award in Seed Technology Research Component for 2021-22 during AGM of AICRP on Seed (Crops) held during 12-13 May, 2022.

SCIENTIFIC BREAKTHROUGHS (Collated from various sources)

Editing of 1 aminocyclopropane 1 carboxylate oxidase genes negatively affects petunia seed germination

Ethylene production in the seeds of Petunia hybrid cv. 'Mirage Rose' was associated with expression of 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACO) genes (PhACO1, PhACO3 and PhACO4). Suppression of their expression by ethylene inhibitor silver thiosulphate (STS) significantly reduced ethylene production and inhibited seed germination. When it was combined with ethylene precursor ACC, ethylene production was re-promoted via activation of the genes and higher seed germination was restored. This was confirmed using the mutants editing the genes and WT. In this study, compared with wild type plants, three different mutants (phaco1, phaco3, and

phaco4) showed significantly decreased germination percentages as well as delayed germination time and seedling growth. These reductions were associated with lighter seed weight, lower ACO transcript levels, and lower ethylene production in mutants. Inhibited seed germination owing to reduced ethylene production was further verified by the supplementation of exogenous ACC and gibberellic acid (GA3) to growth medium, which restored high seed germination activity in all mutants via enhanced ethylene production. In this study, researchers reported a key regulatory role of ethylene in seed germination mechanisms in petunia. Further, they highlighted the need to consider the negative effects of ethylene reduction in seed germination and plant growth when editing genes in the ethylene biosynthesis pathway for the maintenance

of postharvest fruit, vegetable, and flower quality. The research was published in 'Plant Cell Reports' during January 2022 and can be obtained at <https://doi.org/10.1007/s00299-021-02802-5>

Phytoglobin-NO cycle and AOX pathway play a role in anaerobic germination and growth of deepwater rice

An important and interesting feature of rice is that it can germinate under anoxic conditions. Though several biochemical adaptive mechanisms play an important role in the anaerobic germination of rice but the role of phytoglobin-nitric oxide cycle and alternative oxidase pathway is not known, therefore in this study researchers investigated the role of these pathways in anaerobic germination. Under anoxic conditions, deepwater rice germinated much higher and rapidly than aerobic condition and the anaerobic germination and growth were much higher in the presence of nitrite. The addition of nitrite stimulated NR activity and NO production. Important components of phytoglobin-NO cycle such as methaemoglobin reductase activity, expression of Phytoglobin1, NIA1 were elevated under anaerobic conditions in the presence of nitrite. The operation of phytoglobin-NO cycle also enhanced anaerobic ATP generation, LDH, ADH activities and in parallel ethylene levels were also enhanced. Interestingly nitrite suppressed the ROS production and lipid peroxidation. The reduction of ROS was accompanied by enhanced expression of mitochondrial alternative oxidase protein and its capacity. Application of AOX inhibitor SHAM inhibited the anoxic growth mediated by nitrite. In addition, nitrite improved the submergence tolerance of seedlings. This study revealed that nitrite driven phytoglobin-NO cycle and AOX are crucial players in anaerobic germination and growth of deepwater rice. For more details, refer to the published paper in 'Plant, Cell and Environment' during January 2022 and can be obtained at <https://doi.org/10.1111/pce.14198>

Cold-induced secondary dormancy and its regulatory mechanisms in *Beta vulgaris*

The dynamic behaviour of seeds in soil seed banks depends on their ability to act as sophisticated environmental sensors to adjust their sensitivity thresholds for germination by dormancy mechanisms. Here researchers show that prolonged incubation of sugar beet fruits at low temperature (chilling at 5°C, generally known to release seed dormancy of many species) can induce secondary nondeep physiological dormancy of an apparently nondormant crop species. The physiological and biophysical mechanisms underpinning this cold-induced secondary dormancy include the chilling-induced accumulation of abscisic acid in the seeds, a reduction in the embryo growth potential and a block in weakening of the endosperm covering the embryonic root. Transcriptome analysis revealed distinct gene expression patterns in the different temperature regimes and upon secondary dormancy induction and maintenance. The chilling caused reduced expression of cell wall remodelling protein genes required for embryo cell elongation growth and endosperm weakening, as well as increased expression of seed maturation genes, such as for late embryogenesis abundant proteins. A model integrating the hormonal signalling and master regulator expression with the temperature-control of seed dormancy and maturation programmes is proposed. The revealed mechanisms of the cold-induced secondary dormancy are important for climate-smart agriculture and food security. The research paper was published in 'Plant, Cell and Environment' during April 2022 and can be obtained at <https://doi.org/10.1111/pce.14264>

Changes in genotoxic stress response, ribogenesis and PAP (3'-phosphoadenosine 5'-phosphate) levels are associated with loss of desiccation tolerance in overprimed *Medicago truncatula* seeds

Re-establishment of desiccation tolerance is essential for the survival of germinated seeds facing water

deficit in the soil. The molecular and ultrastructural features of desiccation tolerance maintenance and loss within the nuclear compartment are not fully resolved. In this study, the impact of desiccation-induced genotoxic stress on nucleolar ultrastructure and ribogenesis was explored along the rehydration–dehydration cycle applied in standard seed vigorization protocols. Primed and overprimed *Medicago truncatula* seeds, obtained through hydropriming followed by desiccation (dry-back), were analysed. In contrast to desiccation-tolerant primed seeds, overprimed seeds enter irreversible germination and do not survive dry-back. Reactive oxygen species, DNA damage and expression profiles of antioxidant/DNA Damage Response genes were measured, as main hallmarks of the seed response to desiccation stress. Nuclear ultrastructural features were also investigated. Overprimed seeds subjected to dry-back revealed altered rRNA accumulation profiles and up-regulation of genes involved in ribogenesis control. The signal molecule PAP (3'-phosphoadenosine 5'-phosphate) accumulated during dry-back only in primed seeds, as a distinctive feature of desiccation tolerance. The presented results show the molecular and ultrastructural landscapes of the seed desiccation response, including substantial changes in nuclear organization. For more details, refer to the published paper in 'Plant, Cell and Environment' during May 2022 and can be obtained at <https://doi.org/10.1111/pce.14295>

Abscisic acid-induced cytoplasmic translocation of constitutive photomorphogenic 1 enhances reactive oxygen species accumulation through the HY5-ABI5 pathway to modulate seed germination

Seed germination is a physiological process regulated by multiple factors. Abscisic acid (ABA) can inhibit seed germination to improve seedling survival under conditions of abiotic stress, and this process is often regulated by light signals. Constitutive

photomorphogenic 1 (COP1) is an upstream core repressor of light signals and is involved in several ABA responses. Here, researchers demonstrate that COP1 is a negative regulator of the ABA-mediated inhibition of seed germination. Disruption of COP1 enhanced *Arabidopsis* seed sensitivity to ABA and increased reactive oxygen species (ROS) levels. In seeds, ABA induced the translocation of COP1 to the cytoplasm, resulting in enhanced ABA-induced ROS levels. Genetic evidence indicated that HY5 and ABI5 act downstream of COP1 in the ABA-mediated inhibition of seed germination. ABA-induced COP1 cytoplasmic localization increased HY5 and ABI5 protein levels in the nucleus, leading to increased expression of ABI5 target genes and ROS levels in seeds. Together, the results reveal that ABA-induced cytoplasmic translocation of COP1 activates the HY5-ABI5 pathway to promote the expression of ABA-responsive genes and the accumulation of ROS during ABA-mediated inhibition of seed germination. These findings enhance the role of COP1 in the ABA signal transduction pathway. The research paper was published in 'Plant, Cell and Environment' during May 2022 and can be obtained at <https://doi.org/10.1111/pce.14298>

SWI2/SNF2 chromatin remodeling ATPases SPLAYED and BRAHMA control embryo development in rice

In plants, SPLAYED (SYD) and BRAHMA (BRM) encode chromatin remodeling ATPases that use the energy derived from ATP hydrolysis to restructure nucleosomes and render certain genomic regions available to transcription factors. However, the function of SYD and BRM on rice growth and development is unknown. Here, researchers constructed *ossyd* and *osbrm* mutants using CRISPR/Cas9 technology and analyzed the effects of mutations on rice embryo development. Researchers discovered that the *ossyd* and *osbrm* mutants exhibited severe defects during embryonic development, whereas endosperm development was

normal. These results indicated that the development of the embryo and endosperm is independent of each other. Consequently, the *ossyd-* and *osbrm-* null mutants did not germinate due to the abnormal embryos. Furthermore, researchers observed the embryos of *ossyd-* and *osbrm-* null mutants, and they indeed had distinct differentiation defects in shoot establishment, acquired during embryogenesis. To verify the function of OsSYD and OsBRM in embryogenesis, researchers measured the transcript levels of marker genes at different stages. Compared with wild type, the expression levels of multiple OSH genes were significantly reduced in the mutants, which was consistent with the defective shoot establishment phenotypes. The interaction between SYD and RICE FLORICAULA/LFY (RFL) was revealed using a yeast two-hybrid screening system, suggesting that the interaction between the LFY homolog and chromatin remodeling ATPases is ubiquitous in plants. Collectively, their findings provide the basis for elucidating the function of OsSYD and OsBRM during embryo development in rice. The research paper was published in 'Plant Cell Reports' during June 2022 and can be obtained at <https://doi.org/10.1007/s00299-022-02864-z>

TECHNICAL ARTICLES

Seed Microbiome Conservation: A Unifying Perspective

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Multicellular life forms such as plants and animals have existed in symbiotic relationships with microbes throughout the course of evolution. This means that a "unit of life" can be considered more than a single organism containing a single nuclear

genome. Lynn Margulis, an American evolutionary theorist and biologist, coined the term 'holobiont' to represent the life-long symbiotic relationship between a multicellular host and a vast diversity of microbial counterparts. The complete genetic content of the holobiont, which includes the genome of the host organism as well as the genomes found inside organelles (like mitochondria) and symbiotic microbes, is referred to as the 'hologenome'. The idea of a hologenome has now been a hot topic of study and discussion among scientists. For example, a change in the human gut microbiome may predict how well you age, the new research published in the journal *Nature Metabolism* found that as people get older, the composition of this complex community of microbes, collectively known as the gut microbiome, tends to change and decides the health status of a person. Similarly, the Human Microbiome Project (HMP) took a research initiative to understand the microbial flora involved in human health and disease. In this context, it is also important for us to know about the seed microbiome and its conservation.

Seed Microbiome:

The seed is considered as a plant reproductive unit that harbors a diverse microbiome. Microbes communicate with seeds throughout the entire plant development cycle. These contacts may be friendly or hostile, but they all have an impact on the seed microbiome, which is constantly changing and may affect later stages of development. This should not come as a surprise given the prevalence of microbes and the several desirable microbial habitats that plants offer. There may be strong links between the formation of the seed microbiome and each of these microbial habitats that contribute to the plant microbiome.

Seed-associated microbiomes are ubiquitous and thus potentially important for plant growth and sustainable crop production. Throughout the domestication period, wild plants were transformed into food crops as a result of conscious and unconscious genetic selection of important traits,

such as grain size, shape and seed hull elimination etc. As the plants evolved, their associated microbiomes are supposed to have undergone substantial changes too. Evolutionary processes affect the overall composition of microbial communities, whereas dramatic changes in environments (climate change) during domestication contribute to the loss of many important microbial world in seeds. Mutualistic co-evolution of host plants and their seed endophytes in response to distinct stresses will probably provide alternative solutions to promote modern crop cultivation.

When talking about seed microbiomes, it is crucial to distinguish between endophytic microbiota (i.e., microbial species that live inside of seed tissues and are vertically transmitted to progeny seedlings) and epiphytic microbiota (i.e., those microbial species that colonize seed surfaces and may or may not become internalized within seed tissues and transmitted either vertically or horizontally). Despite the fact that endophytes can transform into epiphytes and vice-versa, this distinction is made because the endophytic microbiota frequently comes from different seed tissues or environmental sources than the epiphytic microbiota. In contrast to those associated with the seed coat which are likely to be far more diverse and transported horizontally, microbes associated with the embryo and endosperm, for instance, are more likely to be transmitted vertically and contribute to both horizontal and vertical resistance against biotic and abiotic stress in plants.

Seed endophytes are adapted for a symbiotic life cycle inside plants, vertically transmitted via seed at the cost of competitiveness and ability to survive in most environments outside the plant. "Vertical transmission" is the direct transfer of the endophyte from parent to offspring. This type of transmission should always favor mutualism against pathogenicity, while the endophyte remains dependent on host for survival and reproduction. It is still very difficult to differentiate at initial stages whether a seed inhabiting microbe is endophyte or pathogen. True seed endophytes will result in a healthy seedling, perhaps providing prenatal care to

the plant. Seed endosymbiosis is a vital relationship that confers enhanced seed vigor, germination and resilience thus improving growth under stressful conditions.

Microbiomes of maternally born seeds that are buried in threshing yards:

Plant domestication at least in cereals, is associated with the loss of the shattering allele. In such species, grains are manually harvested and threshed, leaving behind naked seeds. This could have led to the loss of maternally borne seed microbiomes and their associated benefits in modern-day domesticated species (Figure 1). In wild species, the dispersal unit is the spikelet that shatters from the abscission zone located at the rachis. When the spike is released from the panicle, the seeds are dispersed with the accessory seed structures including the lemma and palea and the rachis segment. Here, as the seeds germinate, the microbiome present in the seed accessory structures enters the growing seedling. Whereas, in the domesticated species where shattering does not occur, seeds are harvested and threshed manually. This process cleanses the seed of all accessory structures except the seed coat, leading to what could be referred to as naked seeds. Seedlings growing from naked seeds will be devoid of seed accessory tissue-borne seed microbiome.



Fig. 1: Seed Dispersal in Wild and Domesticated Grass Species. (a) In wild species, the dispersal unit is a spikelet that shatters at the abscission zone and is dispersed with the seed accessory structures including the lemma and palea and the rachis segment. (b) In the domesticated species where shattering does not occur, seeds are forced out of the plant through manual or machine threshing.

Changing Microbe Partners Over the Course of Evolution:

For example, Maize did not exist 10,000 years ago, it descended from a weedy grass with tiny

hard-shelled seeds that we would not recognize as maize kernels. That wild ancestor of maize, called “teosinte”, grew in mixtures of many other plants. Big changes between teosinte and maize that we can see above ground lead to think that there have been changes below ground as well. Scientists found that microbes near the roots of teosinte are different than microbes that live around maize roots. Studying maize’s roots and its microbes can help us make farming more efficient. The first step is to identify desirable traits of the roots and microbes. We may find that teosinte roots are better at getting nutrients or modern maize might have microbes that provide more nitrogen. Once we find those good traits, we can trace them to the plant genes that control them. Plant breeders can combine these genes to make maize plants that are better at getting nutrients from soil and from microbes. These plants would need less chemical fertilizer and other plant protection inputs. However, we still have many questions, but research on how maize’s roots and microbes have changed will lead to develop maize varieties that use less inputs and that would be better for the environment.

Seed Microbiome Pool Inside the World’s Biggest Seed Bank:

The Millennium Seed Bank, nestled in the grounds of Wakehurst Place in the UK countryside, coordinated by Kew, is the largest seed bank in the world with over 2.3 billion seeds from almost 40,000 species, as well as a global partnership dedicated to the collection and conservation of seeds worldwide. Here, the survey on banana wild relatives suggested that the microbiome of wild relatives could also have a role to play in protecting our crops that we can potentially introduce endophytes from wild relatives into crops to pass on useful properties, such as stress tolerances. Protecting wild relatives and their microbiomes can be seen as a safeguard for the future of the crops we all rely on for food security.

A New Definition for Seeds:

A seed may be defined as a dormant embryonic plant supplied with nutrients enough to fuel early seedling growth and contained in a hardened protective coating. However, it is becoming increasingly clear that this definition is not complete; seeds also carry a community of symbiotic microbes that provide multiple critical functions for developing seedlings and without these symbiotic microbes’ seedlings are less likely to survive. In this respect, a seed is like “Noah’s ark” containing the plant and the microbes that are needed for the growth and survival of the seedling as it colonizes in a new place.

However, the current knowledge on the seed microbiota allows drawing some conclusions for several applied aspects which could be deliberated or researched.

- The structure of the seed microbiota can be used as a novel biomarker in breeding strategies. Moreover, joint breeding strategies of the plant and the indigenous plant-associated microbiota are promising.
- Breeding strategies can be successfully combined with bio-control strategies. The bio-control and stress-protecting agents can be designed and applied as seed treatments.
- Seed cleanings and assessments can be evaluated using microbial diversity as a criterion.
- Conservation strategies for seeds to preserve genetic diversity, which already exists, should include conservation strategies for seed microbes as well.
- The co-evolution of plants and microorganisms resulted in genotype-dependent seed microbiomes, which need to be better understood.

Since multi-omics technologies allow us deeper insights into the functioning of the holobiont, one

should intensively focus on the following issues:

- ⊙ How does native seed microbiota perform under stressful conditions?
- ⊙ How stable is the seed microbiome?
- ⊙ How does horizontally transmitted seed microbiota overcome the plant's defense strategies to become endophytes?

Conclusion:

The indiscriminate use of pesticides and fertilizers adversely affects the agro-ecosystem and causes threats to consumers also; second, an increasing population and higher rate of environmental degradation are further points of concern for scientists in various fields. In order to improve soil and human health, the application of endophytes as biological agents either alone or as a component of IPM packages will be a better option and remedial alternative against chemical farming that has been gaining importance in recent years due to their potential properties in managing abiotic and biotic stresses imposed by the surrounding environment and destructive insect pests and/or pathogens, respectively, on host plants by various mechanisms. In addition to that, endophytes have shown great potential in promoting crop growth and productivity. Endophytes have been proven to be a safe and cost-effective option for attaining sustainable farming owing to their ability to produce growth hormones and other essential compounds that help in promoting the growth of host plants. Such microorganisms are of immense importance and attempts would be taken towards their application in sustainable agriculture in the near future.

Here we propose a 'back to the future' approach for conserving and reintroducing missing beneficial seed microbes to improve plant biotic and abiotic tolerance through using wild relatives of crops/landraces. Saving seeds with their indigenous microbes offers an enormous potential to safeguard,

explore and exploit microbial diversity and their untapped metabolic potential for plant, human and environmental health. Therefore, the endophytes seed treatments can be designed, which allow better functioning of the crop holobiont to cope with pathogen pressure and even climate change which may lead to better crop productivity and sustainable growth in agriculture.

Nanofiber Seed Coating - An Innovative Approach for Smart Delivery of Functional Molecules

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Application of exogenous materials on the surface of the seed to improve the quality of seed by delivering active compounds such as plant growth regulators, micronutrients, fungicides, insecticides and microbial inoculants which protects the seed against phytopathogens and increase germination is termed as "seed coating". For a longrun, techniques like dry coating, seed dressing, film coating, encrustments, and seed pelleting were used for seed coating. In recent decade, nanofiber seed coating with the polymers (nature derived biopolymers/synthetic polymers) were investigated as an alternate approach due to its efficiency.

Nanofibers from the desired polymer solution are produced by a facile and convenient, yet a versatile technique known as electrospinning. In electrospinning process, high voltage (HV) electric current is applied to the polymer solution/spin dope solution (Fig2). The HV charges the polymer solution which undergoes physicochemical reaction and ultimately generates nanofibers with diameter ranging from few nanometer to 500 nm that gets deposited on the oppositely grounded collector. The virtues of nanofiber such as high surface to volume ratio, high porosity, tunable release rate, gaseous exchange, water permeability draws the attention

to use it as seed coating over other conventional techniques. By modulating the choice of polymer and hydrophilicity of nanofiber, the delivery of functional molecules or the agrochemical inputs can be altered. Various functional molecules or the agrochemical inputs including but not limited to hormones, pesticide, fertilizer, beneficial microorganisms were successfully loaded in the nanofiber and used as seed coating for its sustained as well as smart delivery with regards to improve seed germination and seedling growth.

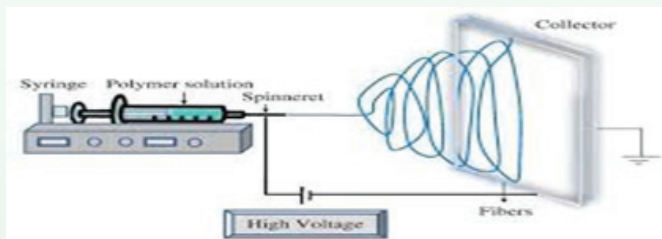


Fig.2. Electrospinning Process

Hormone encapsulated nanofiber seed coating

Plant hormones are one of the major factors influencing the important process in crop growth i.e., seed germination and dormancy etc., Collection of plant hormones significantly affect the seed germination by interacting with each other. With the understanding of plant hormones importance in germination of seeds, research has been conducted to encapsulate the hormones in nanofiber and precisely deliver it to the seeds at right time. Gibberellic acid (GA₃) and Indole acetic acid (IAA), and they were successfully infused in the electrospun nanofibers and tested as seed coating material on groundnut and blackgram seeds. The results of the study showed that the hormones were delivered at optimum level at critical time, thus improving the seed quality parameters which directly improved seed germination and seedling vigor compared to the control uncoated seeds. Under in vitro condition, the germination and seedling vigor were improved by 10 and 20% in groundnut (Table 1) while in black gram it was 8 and 16% (Table 2) (Raja et al., 2021).

Table 1. Effect of hormones loaded E-spun fibre coating on seed quality in groundnut

Treatment	Germination (%)	Seedling length (cm)	Vigour index
Uncoated seeds	68	31.9	2169
PVA Coating	74	33.8	2501
GA ₃ infused PVA nanofibre	78	38.3	2987
IAA infused PVA nanofibre	88	39.3	3458
Mean	77	35.8	2779
SEd	0.774	0.247	32.63
CD (P=0.05)	1.642	0.524	69.17

Table 2. Effect of hormones loaded E-spun fibre coating on seed quality in blackgram

Treatment	Germination (%)	Seedling length (cm)	Vigour index
Uncoated seeds	70	31.9	2233
PVA Coating	74	32.1	2375
GA ₃ infused PVA nanofibre	78	38.3	2987
IAA infused PVA nanofibre	86	39.9	3431
Mean	77	35.5	2756
SEd	0.854	0.283	49.0
CD (P=0.05)	1.831	0.609	98.12

Agrochemicals incorporated nanofiber seed coating

Controlled release of agrochemicals including but not limited to fertilizer, pesticide, fungicide ensures that, it is available to the plant at the right time at the appropriate place and in optimum dosage. In order to improve the germination rate and seedling vigour, seeds are coated with the nanofibers incorporated with the agrochemical inputs. Urea, an important nitrogen fertilizer was encapsulated in the polyvinylpyrrolidone (PVP) polymer by electrospinning. Using it as seed coating material improved the nitrogen availability significantly. Similarly, micronutrients like copper, cobalt were also incorporated into polymeric nanofibers and tested as seed coating material. This technique precisely delivered the micronutrients close to the seed at miniscule dose. Fungicides tebuconazole was effectively loaded in PVA nanofibers and coated

over blackgram seeds and tested for evaluating seed quality. The results revealed significant improvement in seed germination and seedling growth, besides reduction of root rot disease incidence caused by *Macrophomina phaseolina* (Fig.3) (Latha 2019). Combination of nutrients (micro and macro nutrients) was successfully loaded in the PVA nanofiber and coated on green gram seeds for ready availability to the crops at necessary time. Significant positive result in the inhibition of fungal disease was observed in the crop seeds coated with contact fungicide Vitavax, Thiram incorporated ethylcellulose nanofiber. Notably, the nanofiber seed coating approach exhibited better performance than conventional film coating approach. Also, slow release of the active ingredient from the seed coat is a cost-effective and environment-friendly approach as it can reduce the need for multiple applications.

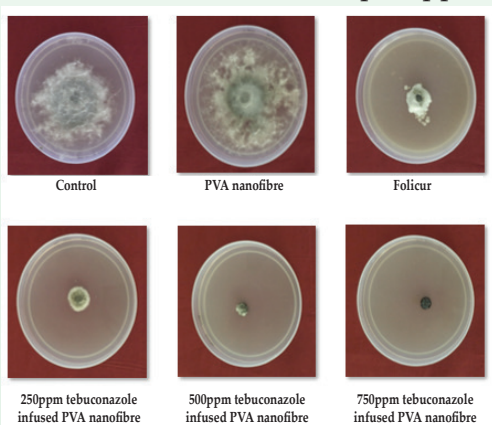


Fig.3. Bio-efficacy of Tebuconazole infused PVA nanofibres on *Macrophomina phaseolina* (Poisoned food technique)

Bioinoculants immobilized nanofiber seed coating

Incorporation of beneficial microbes in the soil cause a positive effect on plants through direct and indirect mechanisms. It is also considered as an alternative promising strategy to decrease the use of chemicals. Nevertheless, innovations needed to reduce the rapid degradation of microbial cell and to maintain the viability of these microbial cells in seed treatment as well as in the storage is quite challenging. Hence polymers are used to

immobilize bacteria thereby protecting them from the adverse environmental conditions. To augment the efficacy and quality of bioinoculants, single and composite polymer formulations from natural or synthetic polymers were studied as carrier materials. Moreover, microbes in polymers can be dry stored at room temperature for extended period. Polymers for immobilization of bioinoculants are chosen on the basis of good biocompatibility, electro spinnability and biodegradability. Hydrophilicity/hydrophobicity of the polymer chosen, distribution of inoculants in the nanofiber are factors that significantly contributes to the delivery of the inoculants. Poly (ethylene oxide) and poly (vinyl alcohol) are highly preferred synthetic polymers while polysaccharides and proteins in the natural polymers. Research has been conducted to immobilize *Methylobacterium aminovorans* in the PVA nanofibers and after successful immobilization it was tested as seed coating on groundnut. Besides extending shelf life of microbes in the nanofibers, the microbial colonization in the rhizosphere was enhanced resulting in improved germination, seedling vigor and plant growth in groundnut (Chinna *et al.*, 2022 & Helen Rani, 2021). In many studies conducted, seed coating with nanofiber-immobilized microbes improved its survivability on stored seeds and contributed to the successful colonization on the rhizosphere.

S.No	Microbe	Polymer	Crop	Result
1.	<i>Bacillus subtilis plus Seratia marcescens</i>	Poly (vinyl alcohol)/ poly (vinyl pyrrolidone)	Canola	Improved nutrient acquisition by the plant
2	<i>P. agglomerans</i>	Poly (vinyl alcohol) (PVA)	Soybean	Increased germination, length and dry weight of the root.
3.	<i>B. caribensis</i>	PVA	Soybean	Increased leaf number and dry weight of the shoot
4.	<i>Methylobacterium aminovorans</i>	PVA	Groundnut	Increased nodule number and fresh nodule weight

S.No	Microbe	Polymer	Crop	Result
5.	<i>Trichoderma viridie</i>	Poly (ethylene oxide) PEO	Capsicum	Inhibited the growth of <i>Fusarium and Alternaria</i> .

Considering all these results, it is found that nanofiber seed coating technique with desired ingredients has higher potential of increasing crop yield by improving the seed germination, inhibiting the pathogen propagation, smart delivery of nutrients and fertilizers during crop establishment. This technique has the flexibility to incorporate various types of active ingredients and/or nutrients to improve the crop production as well as protection. Choice of non-toxic solvents and bio-based polymers to develop nanofiber is under exploration to move towards the eco-friendly and green approach of producing nanofibers as a smart and effective carrier for delivering of agricultural input through seeds.

Suggested Readings:

- Raja, K., Prabhu, C., Subramanian, K. S., & Govindaraju, K. (2021). Electrospun polyvinyl alcohol (PVA) nanofibers as carriers for hormones (IAA and GA3) delivery in seed invigoration for enhancing germination and seedling vigor of agricultural crops (groundnut and black gram). *Polymer Bulletin*, 78(11), 6429-6440.
- Latha, M (2019). Thesis on “Nanofibre seed encapsulation for smart delivery of tebuconazole fungicide in blackgram (*Vigna mungo*)”, Department of Nanoscience and technology, Tamilnadu Agricultural University, Coimbatore, India.
- Chinna mukiri., Raja, K., Senthil kumar murugaiya., & Subramanian, K.S (2021). Immobilization of beneficial microbe *Methylobacterium aminovorana* in electrospun nanofibre as potential seed coatings for improving germination and growth of groundnut *Arachis hypogaea*. *Plant Growth Regulation*. 97(2), 419 – 427
- Helen rani, R (2021). Thesis on “Groundnut seed invigoration of Methylobacterium infused nanofibre to enhance productivity”, Department of Nanoscience and technology, Tamilnadu Agricultural University, Coimbatore, India.

Seed Nanopriming – Switching To A Greener Approach

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In the interest of assisting plant growth and reinforce their strength to deal with environmental cues, priming has been revealed to play a remarkable role through the induction of several metabolic and physiological alterations. Priming of seeds has been of great interest for researchers in recent years which bring about changes in seed metabolism to support the successful establishment of plants in their surrounding environment through faster and more prominent seed germination and further growth of developing seedlings. The conspicuously displayed rise in applications of nanotechnology in agriculture has also stirred up research on the nanopriming of seeds. The method of synthesizing nanoparticles (NPs) is generally chemical reduction. However, most of the reducing agents are highly toxic chemicals that have unintended effects such as environmental pollution, large energy consumption, and potential health problems. The nanoparticles prepared by the chemical methods are not suitable for biological activities due to their toxicity and unstable nature. In response to these challenges, plant substitutes can replace such toxic reductants because they are simple, cost-effective, eco-friendly and gained a lot of importance in the recent past. Hence green nanopriming of seeds has arisen as an emerging tool in the seed sector.

Synthesis of green nanoparticles

In the biosynthesis of green NPs, different parts of plants (leaves, stems, roots, etc.) can be used. They are washed with distilled water, and then boiled in a general solvent (distilled water) to obtain the extracts. The extract filtrate is simply mixed with the nanoparticle precursor (concentration depends on plants and their species) and boiled at a specific temperature. Nanoparticles are thus synthesized within minutes in an eco-friendly method (Fig. 4). This method, which is different from chemical synthesis, is called green synthesis. In structure, chemically synthesized NPs are naked, while green synthesized NPs are wrapped with a layer of plant active substances. It is important to ensure that the bioactivity of the liquid extract is retained during the extraction from selected plant materials. Therefore, using green materials to obtain NPs and to prime or invigorate seeds is called green nanopriming.

However, the biosynthesis of green NPs depends on factors, namely plant species used, temperature, reaction/incubation time and pH values, which influences the yield, size, or shape of the resulting green NPs. They are more stable and have low toxicity.

Green nanoparticle entry inside seeds

The entry mode of green nanoparticle inside seed can be proposed by several mechanisms.

- Green NPs are small molecules that can easily cross the plasma membrane by a direct diffusion process. The passage is concerned with numerous features like size, hydrophobicity, constitution, charge and shape of the particles.
- Green NPs are actively transported into the cell by engulfing its cell membrane, a process called endocytosis.
- They can enter the seed by means of trans-membrane proteins or through channels that regulate the movement of NPs into cells..

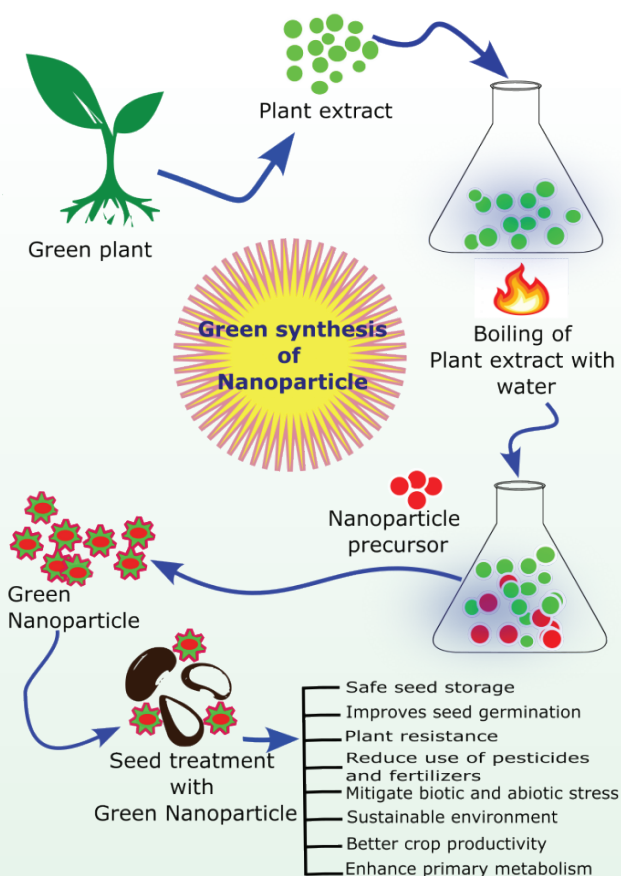


Fig.4. Green synthesis of nanoparticles and their application to seeds

Mechanism of Stimulatory Effects of Green NPs on Seeds

Seed germination:

Green nanopriming can increase the activity of metabolic enzymes in seeds, which further improve the water absorption and make full use of nutrients inside, resulting in increased rate and higher germination with vigorous seedlings.

The possible mechanisms of promoting seed germination after green nanopriming are:

1. The hydroxyl radicals can be produced to loosen the cell wall and form little pores on the surface of seeds to increase water absorption.
2. Furthermore, seeds undergo various physiological and biological changes during germination and organic materials such as starch,

fat and protein undergo a series of metabolic transformations including physiological hydrolysis, transportation and reconstruction.

Reactive oxygen species (ROS) homeostasis:

Reactive oxygen species homeostasis is a balance between ROS production and processing. This involves several enzymatic and non-enzymatic mechanisms, including antioxidant metabolites and major redox buffers that allow antioxidant cycles to process an excess of ROS produced because, when ROS levels are too high, the resulting oxidative stress would damage cellular structures.

Green nanoprimed seeds exhibit a stronger antioxidant system during germination and early seedling growth. They protect chloroplasts from aging and extend the photosynthetic time of chloroplasts by increasing the activity of antioxidant enzymes and therefore, green nanopriming can improve the integrity of plasma membrane by providing more stable lipids to participate in the integrity of the cell membrane, thus playing an important role in plant resistance to both biotic or abiotic stresses.

The increased ROS will be balanced by the seed's antioxidant network to maintain ROS below the basal level during signal transduction. Green nanoprimed seeds and the resulting seedlings demonstrate an organized Reactive Oxygen Species (ROS) homeostasis and antioxidant system to protect seeds from oxidative damage and maintain seed longevity during storage.

Hormonal cross-talk:

The binding proportion between seeds and priming agents in nanoprimed seeds are found to be high compared to other agents' primed seeds, such as PEG, water, and vitamins. In relation to this, the seed coat phenolic substances are endogenously regulated by the hormonal balance of ABA and GA, helping

in nutrient passage across seed compartments, simultaneously the phytohormone auxin (IAA) produced in the endosperm get transported to seed coat in crosstalk with GA. There are evidences showing that IAA has a role in nanoparticle internalization and transport across tissues.

Aquaporins (AQPs):

AQPs are membrane proteins and act as channels for water transfer across the cell membrane. In addition, small solutes or molecules including green NPs may enter seeds as well as cell-to-cell contact through AQPs. The aquaporins play an important role in triggering the germination of seeds. In addition to uptake of water, aquaporins also mediate the diffusion of H₂O₂ or ROS beyond biological membranes, consequently, the mobility of water into seeds get shifts after nanopore formation and aquaporin genes activation, thereby facilitating the dissipation of ROS (H₂O₂) via the cell membrane.

Growth promotion and acceleration in photosynthesis:

Green NP treatment enhances plant growth and development, possibly through protein-coding, miRNA gene expression regulation and blocking ethylene signaling pathways in seedlings. They promote growth by accelerating cell division, notably improving elongation of root cells and greater activity of dehydrogenase enzyme.

They enhance the photosynthetic efficiency in seedlings via increasing the CO₂ fixation. Synthesis of chlorophylls and the activity of carbonic anhydrase will be improved. Green NPs acts as a photocatalyst and improve the absorbance of light and convert light energy into chemical energy, thereby leading to greater CO₂ assimilation. They act at the gene level by inducing the marker gene expression for RuBisCO activase mRNA, which results in the greater synthesis of proteins, thereby enhancing RuBisCO carboxylation.

Conclusion:

Nanopriming is a considerably more effective method compared to all other seed priming methods. The salient features of nanoparticles (NPs) in seed priming are to develop electron exchange and enhanced surface reaction capabilities associated with various components of plant cells and tissues. Nano-priming with green nanoparticles can produce safe, high-quality agricultural products, protect the natural environment, and improve the socio-economic status of farmers. Using green synthesis, we can synthesize different varieties of nanoparticles that can further be applied in a diversity of applications without affecting the ecosystem. This technology can promote the germination of seeds effectively without poisoning seedlings. Therefore, green nanopriming has huge potential for enhancing crop productivity. For example, most green NPs have the dual effect of seed sterilization and stimulation of germination, and can thus reduce the use of harmful chemicals, such as fungicides, besides their safe usage. Despite the many advances in green nanotechnology for agricultural applications, the production of large volumes of nanoformulations remains an imperative challenge. In most laboratories, the volume prepared will be usually few litres. Furthermore, producing large amounts and maintaining the same physicochemical properties is often challenging. Of course, the emergence of new technology needs to demonstrate value in many ways, and green nanopriming has a long way to go for its effective utilization in the interest of bio-safety. Further, we need to explore the utilization of 'weed flora' to synthesize green NPs instead of domesticated crop plants, although a critical analyses of the published information reveals that plants such as *Aloe vera*, *Avena sativa*, *Azadiracta indica*, *Chenopodium album*, *Jatropha* and *Pelargonium graveolens* have been extensively used to produce NPs and found to mediate NP synthesis more efficiently than other plant species. However, an in-depth insight is needed to assess the real impact, causes and to know the mechanism of seed invigoration due to green NPs treatment and their influence on soil health.

Pure Seed, Germination and Pure Live Seed- True Planting Value

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1. The True Planting Value of any seed lot essentially depends on the actual "PURE SEED CONTENT" and "GERMINATION STATUS" of the lot. In "Seed Testing" and in "Seed Standards", Pure Seed is a "Physical Purity Component"; also known as "Mechanical Purity". It refers to seed of the Kind / Crop concerned, with no relation/ reference to the variety. Thus, any varietal admixture is still Pure Seed from Physical Purity angle. However, by further quality analysis, reporting etc it is reflected separately as ODV [Other Distinguishable Variety].
2. Germination refers to "Normal Seedlings [NS]". From certification and seed lot clearance angle, the minimum standard is referred to as "Germination", because only NS can be expected to result in a plant to give proper field stand. In those species in which Hard Seeds are known, the standard is referred to as "Germination including Hard Seeds". Therefore, "Hard Seeds [HS]" reported by the Lab are also added to the NS to arrive at the Germination%. In general, HS are noticed in some species /crops belonging to *Leguminosae*, *Malvaceae* etc. As of now, it is believed that HSs will result in Normal Seedlings. This is an aspect for furthermore research to confirm whether HSs always result in NSs.
3. In Seed Testing Rules /Procedures, the Terms "Pure Seed" [PS] and "Normal Seedling" [NS] have several definitions for different Botanical Families and various crop species. In brief:
[a] Pure Seed means intact seed unit which is larger than one-half the normal/original size of the genotype/crop; and
[b] Normal Seedling means intact seedling with all the essential structures which are well developed, complete, in the expected proportion and reasonably healthy, showing the capacity for continued development into a satisfactory plant when grown under favourable conditions.

4. Accordingly, strictly speaking, by definition, immature, shrivelled, undersized, discoloured, insect attacked, and diseased seed is also included as “Pure Seed” provided it is clearly identifiable to the crop, is intact and is more than half the original/normal seed size for the crop concerned. On the same basis and logic, even if the seed is intact, but is half or less than half the normal / original size expected for the genotype/crop, it is considered as “Inert Matter”. Similarly, immature, shrivelled, under-sized, deformed, discoloured and diseased seed which is half or less than the original size is also “Inert Matter”.

5. Thus the term Pure Seed does not necessarily mean ‘Good True to Type Viable and Germinable Seed’. External Seed Morphological features vary between crops and sometimes between species and even among varieties too. Accordingly, Seed Testing Rules contain “Pure Seed Definitions” which should be consulted for correct identification and assessment.

6. As per Seed Testing Rules/Procedures, Germination evaluation is made using only the Pure Seeds randomly taken from the Pure Seed fraction of Purity Analysis or Pure Seed randomly taken from the Submitted Sample. Thus, any Normal Seedling is first a Pure Seed and then only a Seedling. This is the importance of “Pure Seed” content of a seed

lot. Also, Pure Seed content is usually visible in the overall “Physical Appearance” of the seed stock /lot. Hence, any compromise on Pure Seed content is not acceptable and can be risky even if Germination is satisfactory.

7. Pure Seed estimation is on “weight basis” and reporting is on % by weight up to one decimal place; without rounding off. Germination is on “count basis” and reporting is on % by number, after rounding off to the nearest whole number, as no seedling can exist as fraction.

8. From quality viewpoint, any seed lot should have the highest possible Pure Seed content and maximum possible Germination. Therefore, the standards prescribed for these factors are in terms of the minimum required. The interaction between PS and Germination per cent together give the “Pure Live Seed” [PLS] value [a number without any unit of measurement] which is calculated as follows:

$$\text{Pure Live Seed [PLS]} = (\text{Pure Seed \%} \times \text{Germination \%}) / 100$$

9. In Summary, based on the minimum standards prescribed in the IMSCS-2013 [as amended from time to time] the crops in the current product range in the Indian Seed Sector fall into different PS and Germination standard levels as shown below in descending order:

Pure Seed std [min%]	Crops with the PS std given in col 1	Germination std [min %]	Crops with the Germination std given in col 3
1	2	3	4
99.0	Bhindi	90	Maize
98.0	All crops other than those listed here in this column	85	Wheat, Gram, Mustard
97.0	Mustard, Jute, Coriander	80	Paddy, Safflower, Jute
96.0	Palak, Beetroot	75	Jowar, Bajra, Red gram, Black gram, Green gram, Cotton hybs, Peas, French beans
95.0	Carrot	70	Soybean, Sunflower, Castor, Brinjal, Tomato, Radish, Turnip, Onion, Cabbage, Cauliflower, Guar
Less than 95%PS	No crop	65	Cotton vars, Bhindi, Coriander
----	--	60	Chillies, Capsicum, Carrot, Cucumber, all Gourds & Melons, Palak, Beet root
--	--	Less than 60% G	No crop

10. The PLS values obtained / derived using the minimum prescribed PS & Germination standards are summarized below in ascending order:

PLS Value	Crops with PLS value ie [PSx G / 100] indicated in col 1	Std for PS	Std for G
1	2	3	4
57.00	Carrot [95x60]/100	Very Low	Very Low
57.60	Palak, Beet root [96x60]/100	Very Low	Very Low
58.80	Capsicum, Chillies, Cucumber, all Gourds & Melons [98x60]/100	High	Very Low
63.05	Coriander [97x65]/100	Low	Low
63.70	Cotton varieties [98x65]/100	High	Low
64.35	Bhindi [99x65]/100	Very High	Low
68.60	Castor, Sunflower, Soybean, Guar, Brinjal, Tomato, Cabbage, Cauliflower, Radish, Turnip, Onion [98x70]/100	High	Low
73.50	Jowar, Bajra, Red gram, Black gram, Green gram, Cotton hybrids, Peas, French bean [98x75]/100	High	Medium
77.60	Jute [97x80] /100	Low	High
78.40	Paddy, Safflower[98x80]/100	High	High
82.45	Mustard [97x85]/100	Medium	High
83.30	Wheat, Gram [98x85]/100	High	High
88.20	Maize [98x90]/100	High	Very High

11. Thus, the PLS value which is the ultimate true planting value of any seed lot is a function of both PS & Germination and one does not compensate/ substitute for the other. Thus, in spite of meeting the prescribed standard, because of low standards fixed for carrot for both PS and Germination [ie 95.0% and 60%, respectively], its PLS is only 57; while on the other end, for maize it is 88.20 because of high standards fixed for both PS and Germination [ie 98.0% and 90%, respectively]. Hence, meeting the minimum standards for both PS & Germination is important to achieve the desired PLS.

12. Hope this information gives an insight into the concept of PLS and would enable all concerned to recognize the importance of both PS & Germination for achieving high PLS in the seed stocks handled.

NOTICE:

Secretary, ISST has informed that Dr Malavika Dadlani has excused herself from the responsibilities of President, ISST w.e.f 21.03.2022 due to personal reasons.

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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