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ON

RICE BIOTECHNOLOGY

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EDITORIAL

Rice is one of the most mportant cereal crops; which provides staple food for major population of the world. It is mainly grown in the developing world. In the increasing world's human population, rice consumption and demand is also increasing day by day. But now days due to competition from non-farming sectors, the land devoted to rice production is decreasing in many Asian countries. Hence, it is important the contributions of fragile environments such as the rainfed lowlands, uplands, and salinity-prone areas to rice productivity growth will be increase. Technologies, therefore, will be required not only for increasing rice productivity in these environments, but also for preventing resource and environmental degradation in marginal areas. Numerous biotechnological initiatives and strategies have been undertaken aiming for an enhanced rice production.

(Ashis Kumar Panigrahi)

IN THIS ISSUE:

Rice(*Oryza sativa*) is the most important food crop of the developing world, a major calorie source feeds more than half of the global population. It is used as staple food for over two billion people of the world. More than 90% of rice is grown and consumed in Asia. In direct proportion to the predicted rise in the global human population, the future demand of rice production should increase. Since the Green Revolution there is improvement in the agricultural sectors. The application of biotechnology in the genomics era offers unique scope for further improvement to attain environmentally friendly sustainable agriculture. Nutritious rice with high iron and beta carotene in polished seeds has been developed with genetic engineering technology.

GOLDEN RICE

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Introduction

A well-balanced, healthy diet is vital in providing our bodies with all the essential nutrients and energy to function properly. Those who deviate from a healthy diet soon notice the effects. An insufficient intake of certain nutrients can result in diseases, a weakened immune system or growth and developmental problems in children. For example, anemia arises from a lack of iron, whilst sight problems are the first sign of vitamin-A deficiency. Specific deficiencies can even result in death. The excess energy gain through over-consumption of nutrients results in obesity, which brings with it an increased risk of cardiovascular diseases, joint problems and diabetes.

Golden rice is a group of rice varieties that are genetically modified to counter **Vitamin-A** deficiency in developing countries (Tang et al., 2009). Many people in developing countries battle against vitamin-A deficiency due to an imbalanced diet including limited access to fresh fruit, vegetables and animal products. Persistent deficiency in this vital nutrient among children can result in blindness, illness and even death. Vitamin-A deficiency is the leading cause of childhood blindness and increases the risk of death from common childhood infections.

Golden rice differs from conventional rice having extra genes inderted genetic modification which produce more vitamin-A in the grain. Provitamin-A colors the grains yellow-orange, hence the name 'Golden Rice'. Once absorbed into the body, provitamin A is converted into vitamin A. Provitamin A is found in many fruits and vegetables; it is also what makes carrots orange, for example.

The Golden Rice nutritional trait was subsequently crossed into popular local rice varieties, using conventional breeding methods. These new rice varieties are currently being assessed in field trials across Asia.

The humanbeing mainly need around fifty essential nutrients through their food. This includes water and carbohydrates, as well as amino acids, fatty acids, and micronutrients such as vitamins and minerals.

If one of these nutrients is insufficiently absorbed, or not at all, our health will be undermined. This results in disrupted metabolism, which in turn results in sickness, poor health and potentially the impediment of children's development. One of these vital nutrients is beta-carotene, the pigment found in great quantities in carrots and responsible for their orange color. Also spinach, melons and maize are rich in this nutrient for example. Beta-carotene is the best known form of provitamin-A, and is converted into vitamin-A in our body. Vitamin-A deficiency can result in sight problems and even blindness. Animal products rich in vitamin-A, such as eggs, liver, cheese and butter, are often unaffordable for poor people of developing nation.

Vitamin-A deficiency is a global health problem, primarily in developing countries in Africa and South-East Asia. Children and pregnant women are particularly at risk. The World Health Organization states that each year between 250,000 and 500,000 children become blind as a result of vitamin-A deficiency. Half of those children die within a year. Vitamin-A deficiency also compromises the immune system, which means children die from common diseases including diarrhea, respiratory tract infections and measles. A research study that examined malnutrition among mothers and children, estimated that annually more than 100,000 children under five die due to vitamin-A deficiency. A significant populations of developing countries are primarily affected as this deficiency is the consequence of poverty related poor diet.

Fig. Golden rice

Development of Golden Rice

Golden Rice is the brainchild of Prof. Ingo Potrykus and Peter Beyer who in a collaborative effort were able to show that production of β-carotene could be turned on in rice grains using a minimum set of transgenes.

In the early 1990s two University scientists, Ingo Potrykus from the ETH institute in Zürich and Peter Beyer from the University of Freiburg, were well aware of the blindness caused by vitamin-A deficiency. They undertook the initiative to develop rice that produces provitamin-A in the grain.

Genetic modification offered an answer: adding two genes from the narcissus and one from the soil bacteria *Erwinia uredovora* to the DNA of rice enabled the production of provitamin-A in rice grains (Burkhardt et al., 1997, Ye et al., 2000 and Paine et al., 2005). More than one gene is needed because the production of provitamin-A is a multistep process. More than one gene is needed because the production of provitamin-A is a multistep process. For some of these steps no genes are active in white rice grains. It took eight years of laboratory work to develop such a rice plant. In 2000 this groundbreaking research was published in the Science journal.

To this end the researchers gained support from the Rockefeller Foundation, the European Commission, national governments in Asia and finally from the Bill and Melinda Gates Foundation. Because no rice varieties exist that produce provitamin-A in the grain, this trait couldn't be introduced via traditional breeding, such as was the case with the sweet potato.

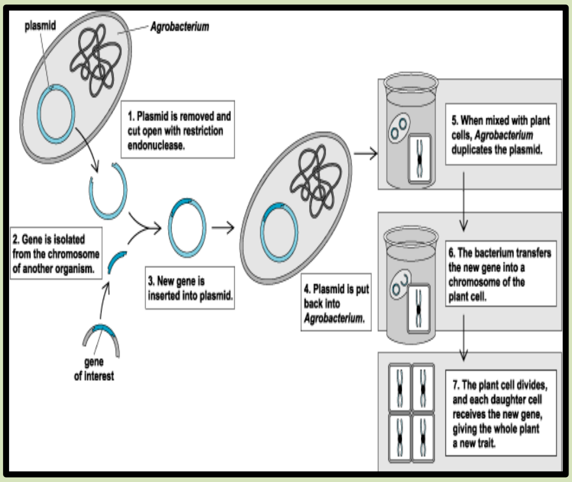

Fig. Steps of Golden Rice development.

In India vitamin-A deficiency could drop by 60%, if Golden Rice were to be generally cultivated and eaten. This could lead to 40,000 fewer deaths per year (WHO, 2009; Stein et al., 2008). Even with restricted cultivation thousands of lives could be saved each year. As vitamin-A deficiency is linked to poverty, the anticipated positive effects of Golden Rice will be greatest amongst deprived families. These families usually live in very remote areas, where the opportunity to obtain vitamin-A supplements is scarce. Moreover, Golden Rice would be a far more cost-efficient way in tackling health problems related to vitamin A deficiency.

Benefits of Golden rice

Golden rice has the promise to help prevent millions of deaths and to alleviate sufferings of children and adults afflicted with VAD and micronutrient malnutrition in developing countries. In addition, allowing further golden rice development may open up more possibilities of enhancing genetically modified, biofortified crops to combat micronutrient malnutrition in developing countries. This is the main benefit of golden rice. Supporters of the project believe that the improvement of public health in developing countries trumps the risks associated with golden rice.

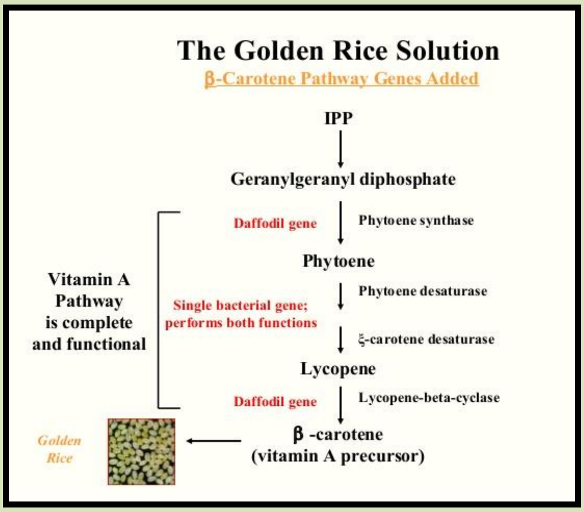

Fig. Golden rice technology

Conclusion

Rice is the staple having rich in carbohydrates and comprise a good source of energy but lack many essential nutrients, such as vitamins and minerals. For people who barely eat more than a portion of rice a day, those deficiencies can result in serious health problems. Tackling poverty, the lack of infrastructure and inadequate education are the greatest challenges. In attaining these goals the enrichment of staple food crops in developing countries can comprise a sustainable way of adding additional nutrients to people's diets. The development of Golden Rice is the first example of this. This rice contains provitamin-A, a substance that the body converts into vitamin-A.

Golden Rice offers great potential therefore in helping to combat vitamin-A deficiency in developing countries. Development of the plant has already made significant progress and meanwhile many varieties exist. The field trials and analyses however demand a great deal of time, and regulation surrounding GM crops is stringent. Moreover, Golden Rice also faces opposition that primarily arises through misconceptions. All these different factors mean Golden Rice still hasn't easily available in the market.

The Golden Rice is designed to ameliorate vitamin-A deficiency and comes free of technology costs through the Golden Rice Humanitarian Board. Even after a decade, there has been no progress in commercial release of Golden Rice. Both activist pressure and governmental apathy contributed to this delay affecting millions, particularly poor women and children in India, who suffer from vitamin-A deficiency disorders. The Indian varieties of rice with the Golden Rice Event, being developed in three public sector institutions (Tamil Nadu Agricultural University, Coimbatore, The directorate of Rice Research, Hyderabad and the Indian Agricultural Research Institute, New Delhi), need to undergo field trials for biosecurity clearance.

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RICE BIOTECHNOLOGY: PROGRESS AND PROSPECTS

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Inroduction

Rice is the second most important food crop of world. Over 150 million ha are planted to rice world with a production of around 375 million tones. Feeding more than one half of the world's population, over 90% of rice is produced and consumed in Asia. Average daily intake of rice provides 20-80% of dietary energy and 12-17% of dietary proteins for Asians. As there is no alternative to steady production growth of rice to keep pace with population growing at an alarming rate, high yielding varieties have been developed in mid sixties. Since then India emerges as the largest rice growing country in the world with special reference to yield which becomes more than doubled during the last five decades culminating in sustained self sufficiency and surplus for adequate buffer stocking and limited export.

The spectacular development of Molecular biological techniques with special reference to Molecular Plant breeding technologies solidly based on and blended with rich experience on plant biotechnology carefully handled more innovations in Rice improvement research. The mile stones of Rice biotechnology with special reference to crop improvement include:

A. Tissue culture in Rice Biotechnology.
B. DNA Marker in Rice Biotechnology
C. Omics in Rice Biotechnology
D. CRISPER-CAS9 in Rice Biotechnology
E. Molecular designing in Rice Biotechnology

A. Tissue culture in Rice Biotechnology

Plant tissue culture technique is an technique which is embracing the term which denote *in vitro* culture of gametic cells, tissues, organs and isolated protoplasts. The important tissue culture technique which have relevance to rice biotechnology includes:

(a)Anther culture for speed and efficiency in breeding a variety,
(b)Somatic cell culture for efficient screening of large cell population for variants resistant biotic and abiotic stresses,
(c)Embryo culture to rescue hybrid embryos in interspecific and intergenetic crosses for transfer of useful traits from taxa.
(d)Application of tissue culture for the recovery of novel genetic variants by somaclonal variation in regenerated plants of Tissue culture (Larkin and Scowcroft, 1981) and gametoclonal variation in anther/pollen derived plants (Evans, 1984).
(e)Development of monosomic Allien addition lines.
(f) Transgenic Technologies:

With advent of genetic engineering techniques, it has now become possible to identify, isolate and transfer genes to Rice plants. Among the various techniques, protoplast mediated DNA uptake, microprojectile electroporation, and *Agrobacterium* mediated gene transfer are commonly practiced.

Transgenic Rice among cereals was first developed by protoplast-based transformation system (Toriyama et al., 1988). Biolistic techniques are increasingly used for rice transformation as they circumvent genotype specificity (Christou et al., 1991). A major breack through in rice genetic engineering is successful *Agrobacterium* mediated transformation in Rice (Hiei et al., 1994).

After achieving the success of genetic engineering using *Agrobacterium*, biolistic particle gun and protoplasts, efforts were emphasized on to introduce traits previously unavailable to breeders. Transgenic rice carrying insecticidal protein gene from *Bacillus thuringensis*, chitase gene from bacterial sources and bacterial leaf blight resistance gene, X a 21 from *Oryza longistaminata* have been developed. Engineered rice with better nutritional quality (Vitamin-A and Iron), salinity tolerant genes obtained from *Avicenea* sp. are now available for the improvement of the crop.

B. DNA Marker technology in Rice biotechnology

Differences in the genomic DNA between potential parents in a genetic cross can be easily analyzed by comparing variations in the nucleotide sequence polymorphisms. There are several DNA markers viz. Restriction fragment length polymorphisms, Random Amplified Polymorphic DNA (RAPD) and Amplified fragment length polymorphisms (AFLP) markers are used detecting such variations. Polymorphisms can be used in gene analysis because at a given allelic locus they represent Mendelian Inheritance. This unique property makes it possible to estimate the genetic distance between each polymorphism and to construct a genetic map based on DNA markers.

Using RFLP markers genetic map have been constructed in Rice at the Cornell University, USA in collaboration with IRRL. (Causse et al., 1994). At the same time a rice genetic map using ca 2300 DNA markers including 1500 CDNA markers have been developed by the Rice genome Research programme in Japan (Kurat et al., 1994). The availability of comprehensive molecular genetic maps in rice has facilitated tagging of many genes of economic importance with DNA markers.

DNA marker technology has several potential application in rice biotechnology. These include germplasm characterization, assessment of genetic diversity, gene tagging and mapping of many agricultural important genes which from the basis of Marker assisted selection in Rice. Moreover, when a number of genes governing the expression of same phenotypes like resistance to disease or an insect pest, pyramiding of such genes facilitated successfully the use of MAS in rice for pyramiding of blast and BLB resistance genes.

In addition technology of high density molecular map in rice has facilitated on understanding structural genomics, marker aided selection and map based clone. Rice genome project become a model for plant genomics research for its small genome size, synteny with other monocots as well.

C. OMICS in Rice Biotechnology

As a model cereal crop, the complete genome sequence of rice has become fundamental for analyzing gene function with special reference to crop improvement. At present, rice researchers devoted much effort to generaling mutants and tagged lines or utilizing elite germplasms to clone important genes and identity their functions. Such process combines different Omic technologies including genomics, proteomics and transcriptomics. In the last 10 years Chinese scientists have made an important contribution towards integrated analysis of rice omics and biotechnological applications with particular reference to shotgun sequencing of Indica rice 9311, sequencing of chromosome 4 of Japanica rice Nipponbare, cloning and identifying 220 functional omics and using certain identified genes to improve rice agronomic traits through molecular breeding approaches.

D. CRISPER – CAS9 in Rice Biotechnology

Tool Quantitative and Plant genome editing in Rice biotechnology. Recently development in genome editing techniques have aroused substantial excitement among Agricultural Scientists. These techniques offer new opportunities for developing important trait lines addition of important traits or removal of undesirable traits. Increased adoption of genome editing has been geared by swiftly developing clustered regularly interspaced short polindromic repeats (CRISPR). CRISPER–CAS9 mediated genome editing is being used for raped, easy and efficient alteration of genes among diverse plant species including Rice. (Noman et al., 2016).

E. Molecular Dessigning Breeding in Rice biotechnology

The advances in rice omics and the enrichment of large scale marker data sets us with tools to determine the genetic basis of all important agronomic traits, breeders can design and combine all the most favorable alles in one variety. Such variety is considered to be included in a concept related to molecular design breeding which includes the design, construction of biological parts, devices, systems and redesigning also existing biological profile for crop improvement. One of the glaring examples of such designing is evidenced for introduction of BT gene in rice. Chen et al., (2005) and Tang et al., (2006) developed a transgenic rice with synthetic Cry 2A* rice and Cry 1C* which shows high resistance to lepidopteron pest in field. Fulfillment of this idea depends on several factors including QTL mapping of insect resistance, disease resistance and cold resistance and their interactions at gene environment level in different omics technology.

Conclusion

At present rice becomes a model species for plant omics research. With the rapid advancement and high throughput technologies about whole genome sequencing permits us to study in detail the parameters of genomics and transcriptomics of a population rather than an individual. In addition details analysis DNA marker technologies, functional genomics, protein expression profiling will represent a telescoping view on genetic basis of development in agronomic traits. The achievement of tissue culture technology with special reference to haploid culture, protoplast fusion, genetic engineering technologies with MAS & CRISPER will assist plant breeders to construct a molecular designing of gene pyramiding consisting of most favorable allies.

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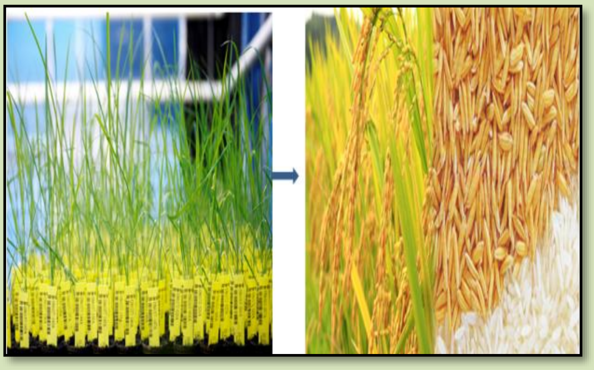

Fig. Golden rice development.

RICE BIOTECHNOLOGY: A CONSISTENT TECHNOLOGY TO FEED THE WORLD

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Introduction

Rice is the staple food for almost half of the world population. In Asia, near about 90% of rice is produced. Rice plays the key role in providing the daily micronutrient needs. Rice is the main energy source for 25 countries in Asia and Africa (Hegde and Hegde, 2013). Scarcity of agricultural land, drought, salinity, low protein content are the biggest threats for food security. To solve these issues different biotechnological approaches are helpful. Now a days, Rice has emerged as the model cereal for the study of genetic engineering (Upadhyaya et al., 2000; Datta et al., 2002; Bajaj and Mohanty, 2005). Different strategies have been applied by breeding methods and transgenic approaches to increase crop productivity, enhance different protein contents and stress resistance.

Different techniques used in Rice Biotechnology:

Agrobacterium-mediated Gene Transfer, Particle Bombardment, liposome mediated transformation, CRISPR-cas9 etc. are used in the field of biotechnology.

Resistance to abiotic stress:

Abiotic stresses such as drought, salt, sudden changes in temperature etc are some reasons behind lower crop production. Abiotic stress management can be obtained through genetic engineering.

1. **Drought Resistance-** Drought is one of the most dangerous threats to crop production. Low rainfall and poor irrigation system may induce drought in agricultural lands. Successful achievement was made by improving two rice cultivars Vandana and IR64 respectively through identification of large-effect QTLs and marker-assisted backcross breeding (Mishra et al., 2013; Swamy et al., 2013). Datta *et al*. (2012) introduced DREB1A gene from *Arabidopsis thaliana* gene into rice genome for successful drought and salt resistance.
2. **Salt Stress Resistance-** As rice seeds are somehow tolerant to salinity during seed germination stage but they are susceptible to high salinity during reproductive stage (Singh and Redoña 2010). Hossain et al. (2015) targeted chromosomes 1, 7, 8, and 10 of rice genome as they affect salinity tolerance by altering Na+ uptake, pollen fertility, and Na+/K+ ratio at the reproductive stage. LEA protein genes from barley enhanced growth and productivity under both saline and drought condition in two different rice cultivars (Babu et al., 2004).

Resistance to biotic stress:

Biotic stresses like- bacterial, fungal, viral pathogens and harmful insects are some disease causing agents.

i. Seedling blight (*Fusarium* spp., *Rhizoctonia solani, Sclerotium rolfsii*), Sheath sopt (*Rhizoctonia oryzae*), Stem rot (*Magnaporthe salvinii*), Downy mildew (*Sclerophthora macrospora*), Root rots (*Fusarium* spp., *Pythium* spp.) are different fungal diseases of rice. Kumar *et al*. (2003) improved Pusa Basmati 1 against sheath blight pathogen, *R. solani*. Rice blast/sheath blight resistance was obtained by Kalpana *et al.* (2006) using PR protein. Resistance to Sheath blight (Datta.,2001) was developed in IR72 and IR64 by particle bombardment and PEG method.

ii. Bacterial blight (*Xanthomonas oryzae*), Sheath brown rot (*Pseudomonas fuscovaginae*), Grain rot (*Burkholderia* *glumae*), Bacterial leaf streak (*Xanthomonas oryzae*) are bacterial diseases. Resistant to bacterial blight in IR72 was reported by Datta et al., (2002) by crossing of transgenic lines Bt-cry1AB and cry1AC.

iii. Rice mosaic virus (RMV), rice hoja blanca tenuivirus (RHBV), rice ragged stunt phytoreovirus (RRSV), rice wilted stunt virus (RWSV), rice dwarf phytoreovirus (RDV), rice gall dwarf phytoreovirus (RGDV) are the important viral pathogens (Abo & SY, 1997). In 1992, a rice variety (Kinuhikari rice) resistant to rice stripe virus was developed by electroporation method (Hayakawa et al., 1992). Huntley and Hall (1996) generated 'Taipei 309', an improved rice variety which is resistance to multiple viruses.

Protein Enrichment in Rice: Generally rice contains lesser amount and number of amino acids,proteins and enzymes. Introduction of rgMT gene in rice caused increase in cysteine content (Lucca and Hurrell, 2001). AMA1 protein gene obtained from Amaranthus having well balanced amino acids was introduced to improve nutritional quality in rice (Chakraborty et al., 2000). Over-expression of α subunit of anthranilate synthase gene in transgenic rice showed tryptophan accumulation in leaves and seeds (Wakasa et al., 2006).

Golden Rice: Vitamin-A is necessary to prevent night blindness, but it is lacking in rice. Provitamin A biosynthesis pathway was designed by Ye et al. (2000) in the rice endosperm via Agrobacterium mediated gene transfer using phytoene synthase (psy) and lycopene β-cyclase (lcy) gene obtained from Daffodil plant (*N. pseudonarcissus*) and phytoenedesaturase (crt1) from bacterium Erwinia uredovora respectively. It is intended to produce a fortified food to be grown and consumed in areas with a shortage of dietary vitamin A. In 2005, Golden Rice 2 was announced, which produces up to 23 times more beta-carotene than the original golden rice. In 2018, Canada, Australia, New Zealand, and the United States approved Golden Rice for cultivation.

Black Rice: Black rice is a source of iron, vitamin E, and antioxidants. Black rice (also known as purple rice) is a range of rice types of the species *Oryza sativa* L., some of which are glutinous rice. Black rice is known as chak-hao in Manipur. Higher anthocyanin production in black rice was obtained by rearranging Kala4 gene (https://www.downtoearth.org.in/news/food/rice-for-kings-57242).

Iron Containing Rice: Foods supplemented with low iron content may cause anaemia and other blood associated problems. Goto et al. (1999) increased iron content in the rice endosperm by transforming ferritin gene from Glycine max (Soyabean). Introduction of PvFERRITIN gene into rice from Phaseolus vulgaris showed increase in iron content many folds (Boonyaves et al., 2016).

Conclusion

Biotechnology has the potential to solve the nutrient deficiency and food scarcity. Rice containing provitamin-A, iron, vitamin-E, antioxidants and other trace elements is able to provide sufficient nutrition to the malnourished children. On the other hand reduction in agricultural fields can replace by higher crop productivity/ha area. Rice biotechnology also can provide tolerance against different biotic and abiotic stresses. Beside conventional breeding genetic engineering is the new horizon in crop improvement which employs various tools of gene editing. But wide scale cultivation of transgenic rice is restricted in our country due to various ethical and aesthetic issues.

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FORTHCOMING EVENTS

Events	Date	Place & Correspondence
World Conference on Disaster Management (WCDM-2020)	18-19 th Jan 2020	Bengaluru , Karnataka , India http://scienceplus.us/Conference2020/1/Bengaluru/WCDM/
International Conference on Forestry Food and Sustainable Agriculture (ICFFSA)	18th Jan 2020	Pune , Maharashtra , India http://wrfer.org/Conference2020/1/Pune/2/ICFFSA/
5th International Conference on Plant Science & Physiology	February 17-18, 2020	Osaka, Japan https://plantphysiology.conferenceseries.com/
ICAEB 2020: 14th International Conference on Applications of Environmental Biotechnology	February 20-21, 2020	Paris, France https://panel.waset.org/Support

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