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Global Hunger Crisis
In the midst of all the conflicting headlines you see each and every day, you’ve probably heard about hunger, food insecurity and malnourishment as a crisis in sub-Saharan regions of Africa and Asian subcontinent. Hunger and malnourishment (Hidden Hunger) is one of the most extreme manifestations of poverty and human deprivation. The World Food Programme (WFP) estimates that there are 821 million undernourished people in the world, which means that one in nine people do not get enough food to be healthy. According to recent Food and Agriculture Organization of the United Nations (FAO) statistics, the vast majority of the world’s hungry people live in developing nations i.e., approx 520 million people in Asia, 243 million people in Africa and 42 million people in Latin America and the Caribbean. Within any country, the majority of all hungry people live in rural areas. These people depend heavily on agriculture and often have no alternative source of getting food and nutrition.

Millions on the brink of starvation: A Report
The 2018 Global Report on Food Crises provides the latest estimates of severe hunger in the world. An estimated 124 million people in 51 countries are currently facing Crisis food insecurity or worse. Conflict and insecurity continued to be the primary drivers of food insecurity in 18 countries, where almost 74 million food-insecure people remain in need of urgent assistance. Last year’s report identified 108 million people in Crisis food security or worse across 48 countries (OXFAM International, 2019). Thus, the report reveals an increase of 11 million people in the number of
food-insecure people across the world that requires urgent humanitarian action.

Millions of people are experiencing unprecedented food crisis. A combination of conflict, reoccurring severe drought and high food prices are to blame as one of the reason of this crisis. For about 100,000 people in South Sudan, it’s so bad the United Nations officially declared famine in this country. Large areas of Somalia, Nigeria and Yemen are on the brink of famine, too. About 1.4 million children are severely acutely malnourished in these countries. Severe drought and widespread food insecurity also are ravaging entire communities in Kenya, Ethiopia, Niger, Chad, Cameroon and parts of the Southern Africa region. In East Africa alone, South Sudan, Kenya, Ethiopia and Somalia approx 22 million people need urgent food assistance. More than 3.5 million children in the region are suffering from malnutrition, well above globally acceptable rates for hunger. In Kenya and Ethiopia, about 700,000 children younger than five are facing starvation. Crop and livestock losses and water shortages in neighbouring Somalia have caused more than 440,000 people to leave their homes, which are worse hit by food insecurity (World Vision, 2019). About 10.8 million people need humanitarian assistance in these regions. More than 500,000 children are suffering severe levels of malnutrition. It will only get worse if nothing is done to help. Children, especially those younger than five, are the most vulnerable, because they need critical nutrients to build strength and immunity against disease (OXFAM International, 2019).

India’s Hunger Crisis: Very Serious Concerns
In a list of 119 countries, India ranked at a poor 100 in the Global Hunger Index (GHI) of the International Food Policy Research Institute (IFPRI). The report puts India at the high end of “very serious” category. Data from the report showed that India’s rank (100) was lower than all its neighbours viz., Nepal (72), Myanmar (77), Bangladesh (88), Sri Lanka (84) and China (29) except Pakistan (106) having higher GHI score. Even North Korea (93) and Iraq (78) fared better in hunger parameters and GHI rankings, shows the report. “India’s GHI score is at the high end of the serious category,” it said (Global Report on Food Crises, 2018).

Food Crisis: Causes, Effects and Solutions
Hunger (Food crisis) is a serious problem facing the world and is prevalent in sub-Saharan Africa. The scarcity of food is caused by economic, environmental and social factors such as crop failure, overpopulation and poor government policies are the main cause of food scarcity in most countries. Environmental factors determine the kind of crops to be produced in a given place, economic factors determine the buying and production capacity and socio-political factors determine distribution of food to the masses. Hunger has far reaching long and short term negative impacts which include starvation, malnutrition, increased mortality and political unrest. There is need to collectively address the issue of food insecurity using both emergency and long term measures. The crisis is also the result of prolonged drought, violence and insecurity. Consecutive years of poor rains and harvests have decimated crops across South Sudan, Somalia, Ethiopia, and Kenya. Ongoing fighting in countries such as in Syria, Afghanistan, Yemen, others prevent humanitarian workers from reaching many of the children, women, and men who need lifesaving assistance (OXFAM International, 2019).

For people to be food secure, food must be available in sufficient quantities - either home grown, locally grown or imported from elsewhere. Food must be accessible - in other words, people must be able to acquire it regularly in adequate quantities and diversity whether through purchase, home production, barter, gifts, borrowing or food aid (Care, 2019). And finally, the food that is available and accessible needs to have a positive nutritional impact on people. This refers to the way it is utilised by households, for instance, household storage, cooking, hygiene and sharing practices (Global Report on Food Crises, 2018).

Conclusion
It is very clear from these discussions that food crisis/shortage is a serious global problem given its devastating impact on the population and government, and this calls for an urgent remedial measure by players in the food sector. The effect of food scarcity can be short and long term. Short terms impacts are more concentrated on women, children and the
elderly population who cannot withstand hunger for longer period. Causes of food crisis/shortage are well known and can be solved if appropriate measures to solve the problem are taken and effectively implemented. Environmental causes of food crisis/shortage are changes in climatic and pollution due to human activities such overgrazing and deforestation which can be controlled through legislation.

Reference
Care, United States of America (2019).


2. ENTOMOLOGY

Bondar’s nesting whitefly, *Paraleyrodes bondari* Peracchi: a new Invasive Alien Species (IAS) in India

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Bondar’s nesting whitefly, *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae) is one of 17 species in the genus *Paraleyrodes*. It causes highly visible wax and sooty mold. It is native to Brazil, but has been reported from several countries, including China in 2010, Florida in 2011 on *Ficus* and Uganda in 2018 on cassava. In India, it has been reported by ICAR-CPCRI from Kerala during December 2018 on coconut.

**Description:** Adults are 1mm long, and have a dull yellow body with white wings (Fig. 2). Each forewing possesses two oblique grey bands that form an “X”-pattern with the center of the “X” missing. The nymphs are flat and possess wax filaments on dorsum. Pupae are 1mm long, oval and translucent yellow. The lateral margin is fringed with short wax filaments (Fig. 1). The white shiny wax filaments of more than 1mm long are projected dorsally that break and adhere to the leaf surface to give nest pattern. The dorsum of the pupa possesses large compound pores aligned along the submargin (Stocks, 2012).

**Host:** Yet it is not reported as economic important pest on any crop. It has been reported on more than 25 hosts including banana, custard apple, citrus, avocado, cassava and ornamental figs (Chowdappa, 2018).
Natural enemies: There is no record of predators or parasitoids on this species. However, various coccinellid beetles, *Chrysoperla* and *Encarsia variegate* Howard (Aphelinidae) are natural enemies of other species of genus *Paraleyrodes* (Stocks, 2012).

References


3. AGRICULTURE ECONOMICS

Concept of Yield Gap in Agriculture

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Introduction
It is always common that some farmers get higher yields and many get comparatively lower yield in same season for same crop even same variety. Yield gap is the difference between the maximum attainable (or potential) yield and actual farm yield. Maximum attainable yield is the crop yield of experimental/on farm plots with no physical, biological, and economic constraints and with the best management practices at a given time and in a given ecology. The Farm level yield is the average farmers yield in a given target area at a given time and in a given ecology. The factors responsible for yield gap known are as yield constrains.

Components of Yield Gap: According to (Pandey, 1978) total yield gap has been divided into three components such as Yield Gap-I, Yield Gap-II and Yield Gap-III.

Yield Gap-I: The difference between yield attained at research stations and yield that could be demonstrated on farmers fields. This theoretical gap has been termed as “Research Level Yield Gap”. The responsibility of reducing this gap is of agricultural scientists in agrobiological fields.

Yield Gap-II: It is the difference between demonstrations plots yield and yield obtained at best farmers field. This has been termed as the “Field Level Yield Gap”. This gap is matters of concern for researchers, machinery & inputs support mechanism and extension workers.

Yield Gap-III: This is the gap between the best farmers yield and yield obtained by the average farmers (average for the area). This is termed as “Farm Level Yield Gap”. Minimization of this yield gap is the basic concern of both the planners and extension workers. In other words minimization of farm level yield gap in productivity aims at bringing the average farmers closer to the best yield at farmer’s level through extension education and increasing input supply. This gap is because of individual farmers personnel managerial skills and lack of Transfer of Technology (ToT) agents on individual farmers field. The total gap has been broken down into three components. The details of these three types of yield gap are as follows:

\[
\begin{align*}
\text{Yield Gap-I:} & \quad (Y_1) = Y_R - Y_D \\
\text{Yield Gap-II:} & \quad (Y_2) = Y_D - Y_B \\
\text{Yield Gap-III:} & \quad (Y_3) = Y_B - Y_A
\end{align*}
\]

Total Yield Gap: \( (Y_T) = Y_1 + Y_2 + Y_3 \) or \( Y_R - Y_A \)

Where; \( Y_R = \) Yields at research farms or ultimate potential yield, \( Y_D = \) Yields at demonstration plots, \( Y_B = \) Yields at best farmer’s field and \( Y_A = \) Yields at average farmers field.

Before releasing a variety and associated practices, quite a few experiments are conducted at more than one research stations by a team of scientists. Later adoptive trails are
conducted. Subsequently this variety and package of practices for different agro-climatic situations are released/standardized, which also indicate the potential yield. These yields were considered as research yield (Yr). Demonstrations on farmer’s fields are conducted by the Department of Agriculture, Directorate of Extension of Agricultural University and input supply agencies like IFFCO, KRIBHCO, etc. under various schemes.

The data on yield for various crops have mainly taken by averaging the yield of demonstration laid by Department of Agriculture and Agriculture University. These yields were considered as yield at demonstration plots (YD).

The yields obtained by the best farmers of each district were obtained by discussion with SMS and district officials of the Department of Agriculture. Thus, yields were considered as yield at best farmer’s field (Yb). The average yield at farmers’ field reported by revenue board for each district was known as average farmer’s yield (Ya). Both absolute and percentage gap in yield at various stages can be estimated. According to (Datta, 1981) the yield gaps have at least two components. One of them is mainly owing to factors that are generally not transferable, such as the environmental conditions and some of the built-in technologies that are available at research station. This component of the gaps (Gap-I in Figure-1) cannot, therefore, be narrowed and is not exploitable.

<table>
<thead>
<tr>
<th>Research</th>
<th>Best farmers</th>
<th>Actual farmers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yields</td>
<td>Yields</td>
<td>Yields</td>
</tr>
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</table>

**Figure-1** View on narrowing yield gaps
Source: Datta, 1981

**Gap-I:** Environmental differences and non-transferable factors cannot be narrowed.

**Gap-II** Differences in crop management can be minimized

The second component of yield gaps (Gap-II in figure-1) is mainly the result of differences in management practices. Gap-II arises when farmers use suboptimal doses of inputs and cultural practices. Gap-II is manageable and can be narrowed by deploying more efforts in research and extension services as well as by appropriate government intervention, particularly on institutional issues.

The International Rice Research Institute (IRRI), 1988 have developed methodology to estimate the magnitude of yield gaps, wherein the yield gap is divided into two components. According to them, the Yield Gap-I is the difference between yield attained on research stations average attainable maximum potential yield (at least two different locations of varieties under cultivation) and on farm experiments average maximum yield (i.e. demonstration plots). This yield gap arises from differences in environments that cannot be managed in the farmer’s field. Yield Gap-II is the difference between yield attainable on farm experiments (i.e. demonstration plots) and the average actual farm yield at farmer’s fields. This gap reflects the effect of biological, soil & water, physiological, genetic and socioeconomic constraints.

**Factors causing yield gap:** There are several factors which causes yield gap has been created. The factors causing yield gap (RAP, 1999) could be classified as:

1. **Physical factors:** problematic soil, poor water management, drought, flash floods and temperature stress.
2. **Biological factors:** climate/weather, insects, diseases and other pest caused by inadequate crop management-post harvest losses.
3. **Technical/managerial factors:** tillage, varieties/seed selection, water nutrient, weeds, pests and post harvest management.
4. **Socio-economic factors:** social economic status, family size, household income/expenses/investment, labour shortage, cost-benefit, farmers traditions, knowledge, skills and welfare conditions.
5. **Institutional policy factors:** government policy, price, credit, input
supply, land tenure, market, agricultural research, development and extension.

6. Technology transfer and linkages factors: competence of extension staff, research, development and extension integration, farmers resistance to new technology, knowledge and skills, weak linkages among public, private and non-government extension staffs.

Reference

4. AGRICULTURE MICROBIOLOGY

Microbial Degradation of Organophosphorus Compounds (Ops)
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Introduction:
Organophosphorus compounds (OPs) are most widely used around the world and have been used as pesticides in agriculture, plasticizers and chemical warfare agent. Although OPs play important roles in protecting agricultural crops from insect pests and weeds and in controlling disease-transmitting vectors. Organophosphate Pesticides are also called "non-persistent pesticides" because they break down fairly rapidly in the environment (within days or weeks), reducing their potential to accumulate in the tissues of plants, animals or humans. Organophosphorus pesticides irreversibly inactivate acetyl-cholinesterase (AChE), which is essential to nerve function in insects, humans and many other animals.

Organophosphorus pesticides such as chlorpyrifos, parathion, methyl parathion are most extensively used in many agricultural practices (Barthidasan et al., 2014). Tabun (GA), Sarin (GB), Soman (GD) and VX are used as chemical warfare agents (nerve agent). Continuous and excessive use of OPs has led to the contamination of several ecosystem in different part of the world and cause serious environmental pollution problem (Musa et al., 2011). Use of microorganisms in detoxification or decontamination of OPs in considered a viable and environmental friendly approach.

Mode of action:
The mode of action of OP compounds can be attributed to the inhibition of the enzyme acetylcholinesterase (AChE). This enzyme is essential for the central nervous system, and being present in both humans and insects. The normal function of AChE is the hydrolysis of acetylcholine neurotransmitter in the synaptic membrane to prevent its accumulation, and as a result forming acetylated enzyme and releasing choline. The high percentage of released choline is transported back into the nerve ending for reconversion to acetylcholine and storage. This degradation process results in a lowered level of acetylcholine, andultimately the termination of nerve impulses.

OP compounds covalently block the active site of serine residue of AChE by undergoing nucleophilic attack to produce a serine-phosphoester adduct. This irreversible inactivation leads to an excess accumulation of acetylcholines in the peripheral and central nervous system causing cholinergic manifestations. At high doses, there is depression of the respiratory centrein the brain, followed by peripheral neuromuscular blocked causing respiratory paralysis and death. The pharmacologic effects and toxicity of these OP compounds are dependent on their stability, rate of absorption by various routes, distribution ability to cross the blood-brain barrier, rate of reaction with AChE.

Microbial metabolism of ops:
Microbial degradation of OP insecticides has
been recognized as the most important process controlling their environmental fate. However, the extensive and repeated use of soil-applied OP compounds on certain occasions has led to reduced biological efficacy due to microbial adaptation. This phenomenon was named as enhanced or accelerated biodegradation, and was attributed to the development of a soil microbial population that was able to rapidly mineralize the OP pesticides.

The significance of enhanced biodegradation depends on use of the pesticides, frequency of use, interval between successive applications and stability of the active microflora without the presence of pesticides. There are four major reactions involved in OPs metabolism: hydrolysis, oxidation, alkylation and dealkylation. Hydrolysis of the phosphoesteric P–O–C or phosphothioesteric P–S–C bonds present in the OP molecules is considered the initial step in their metabolism.

Microbial enzymes:

Microbial enzymes that can hydrolyze organophosphorus compounds have been identified and characterized from different microbial species. Several bacterial and fungal isolates with novel enzyme/gene systems are reported (Hsu et al., 2008).

1. Organophosphorus hydrolase (oph):
   This enzyme was isolated from bacteria, P. diminuta. It has the widest range of substrate specificity. It hydrolyzes P–O, P–F, and P–S bonds to different extents. The lowest specificity is for the P–S bond.

2. Organophosphorus acid anhydrolase (OPAA):
   It has been isolated from A. radiobacter and was found to have 90% homology to OPH at the amino acid level and a very similar overall secondary structure. Despite these similarities, the two enzymes have different substrate specificities. This enzyme which detoxify the organophosphorus nerve agents. Highly active OPAA from Alteromonas undina was isolated and purified and is composed of a single polypeptide with molecular weight 53 kDa. It possesses low catalytic activity against P–O but high activity against P–F bonds.

3. Laccase: Phenol oxidase:
   It is an broad spectrum fungal enzyme which degrade organophosphorusphosphorothiolates. It is isolated from a white-rot fungus P. ostreatus. This enzyme attacks P–S bond, which is comparatively resistant to OPH and OPAA cleavage. Several white-rot fungi are capable of organophosphorus degradation and it will be interesting to know if the degradation capability of all white rot fungi towards organophosphorus compounds is mediated by the presence of laccase, or whether different fungi possess different enzyme systems.

Conclusion:

Even though OPs degrade rapidly, upon accumulation cause contamination. Physical and chemical methods are in use but they also have adverse effect on environment. As alternative, microorganisms are put to use, since they efficiently degrade OPs without harming the environment. Further research works are needed to improve the microbial strains and enzyme catalytic activity.

References:


5. POSTHARVEST TECHNOLOGY

Extrusion Technology: A Novel Approach in Food Processing

Ayeeshya Hasansab Kolhar
Ph.D. Scholar, College of Horticulture, Bagalkot

Introduction
Increasing in number of single person households and gender equality rights has lead to changes in food preparation and consumption habits. i.e., modernization and globalization made peoples very hurry and no time to cook their own food. Now a day’s consumers do choose for nutritionally rich healthy snacks. As we aware of fruits and vegetables are the most utilized commodities among all horticultural crops. The United Nations Food and Agriculture Organization (FAO) have estimated that losses and waste in fruits and vegetables are the highest among all types of foods, and may reach up to 60%. The processing operations of fruits and vegetables produce significant wastes of by-products, which constitute about 25% to 30% of a whole commodity group. The phytochemicals present in these can be utilized in different industries including the food industry, for the development of functional or enriched foods.

Now a day’s novel technologies are gaining more popular among these, extrusion technology can be used for production of such healthy snacks. It is a modern high-temperature short-time (HTST) processing technology in which material is pushed through an orifice or a die of given shape, the pushing force is applied using a piston or a screw. As it is a HTST process, which reduces microbial contamination and inactivates enzymes, the main method of preservation of both hot and cold extruded foods is by the low water activity of product (0.1-0.4). It is an energy efficient continuous process, which combines a number of unit operations i.e. mixing, cooking, shearing, puffing, shaping and drying.

Raw materials used: The most used raw materials in the extrusion process are starch and protein based materials. The structure of the extruded products may be formed from starch or protein polymers. Most products, such as breakfast cereals, snacks and biscuits are formed from starch, while protein is used to produce products that have meat-like characteristics and that are used either as full or partial replacements for meat in ready meals, dried foods and many pet food products.

Physico-chemical changes during extrusion
In extrusion cooking the prevailing high temperature and low moisture contents (12-22%) leads to starch gelatinization, hydrolysis which forms the continuous fluid melt one the this material passes the die then the final product expands as starch polymer becomes glassy as moisture is removed, forming a hard brittle texture. Extrusion cooking leads to denaturation of protein due to breaking of the peptide and disulfide bonds. The enzymes which are also protein in nature lose their activity. Lipids have a powerful influence in extrusion cooking processes by acting as lubricants, because they reduce the friction between particles in the mix and between the screw and barrel surfaces and the fluid melt. The prevailing high temperature leads to conversion of insoluble fibre to soluble fibre. Due to rupture of covalent and noncovalent bonds between carbohydrates and proteins associated to the fibre, resulting in smaller molecular fragments that would be more soluble. It destroys natural toxins and anti-nutrients, thereby improving safety of the food product.

Influence of extrusion cooking on quality traits:
Nutritional quality: Improved nutrional quality due to increased bioavailability of proteins, carbohydrates and antioxidant phytochemicals.

Microbiological quality: As extrusion cooking involves high temperature which kills the pathogenic micro organism and the end product formed is having least water activity which is not favorable for the growth of spoilage.
causing organism. Thus it favors both safety and quality of the processed product.

**Product quality:** Extrusion cooking facilities seasoning of the processed product thus facilitate the improvement in sensory attributes of the processed product.

**Conclusion**

Extruded food products can be successfully blended with fruits, vegetables and their by-products up to the level of 10% for added nutritional qualities, reduced anti-nutritional factors with no significant difference in acceptability. Thus this technology can offer healthy nutritious snacks to the today’s busy world.

**References**


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### 6. EXTENSION EDUCATION

**Role of Attracting and Retaining Youth in Agriculture (ARYA) for Agriculture Development**

**Dr. Jeewan Ram Jat**

*Senior Research Fellow, Department of Animal Production, RCA, MPUAT, Udaipur*

**Introduction:**

India is primarily an agricultural country where more than 50% of the population is involved in agricultural activities. However climatic changes, frequent drought and floods makes agriculture extremely risky. Youth are the primary productive human resource of socio-economic development. Youth minds are creative and they are capable of handling risk factors such as monsoon management, climatic change adaptation and poverty in an efficient way, using various technologies.

In order to create interest and confidence among rural youth in agriculture, there is need to make agriculture more profitable. Retaining youth in agriculture and making agriculture more profitable are thus, big challenges. There is a continuous increase in migration of rural youth to urban areas. On the other hand, small holdings are on the rise which possess challenge to food security for increasing population. Thus, it was felt to bring a comprehensive model for the development of rural youth in general and agricultural youth in particular. Thus, realising the importance of rural youth in agricultural development especially from the point of view of food security of the country, ICAR has initiated a programme on "Attracting and Retaining of Youth in Agriculture (ARYA)".

**Objective of ARYA Project:**

To attract and empower the Youth in Rural Areas to take up various Agriculture, allied and service sector enterprises for sustainable income and gainful employment in selected districts.

To enable the Farm Youth to establish network groups to take up resource and capital intensive activities like processing, value addition and marketing.

To demonstrate functional linkage with different institutions and stakeholders for convergence of opportunities available under various schemes/program for sustainable development of youth.

**ARYA Project Implemented:**

In first phase, ARYA project has been implemented in 25 States in 25 selected districts through KVKs. In one district, 200-300 rural youths are identify for their skill development in entrepreneurial activities and establishment of related micro-enterprise units. Training given in Apiary, Mushroom, Seed Processing, Poultry, Dairy, Goatry, Soil testing, Carp-hatchery, Vermi-compost, nursery management etc., KVKs involved the Agricultural Universities and ICAR Institutes as Technology Partners. At KVKs also one or two enterprise units will be established so that they serve as entrepreneurial
training units for farmers. The purpose is to establish economic models for youth in the villages so that youths get attracted in agriculture and overall rural situation is improved. Skill development of rural youths will help in improving their confidence levels and encourage them to pursue farming as profession, generate additional employment opportunities to absorb under employed and unemployed rural youth in secondary agriculture and service related activities in rural areas.

**FIG.:** Entrepreneurship development among youth.

**Advantages:**
- Improves Agro- productivity in the country.
- Controls rural migration of Youth.
- Income of rural youth become sustainable and meaningful.
- Develops service sector in the rural area.
- Business and Entrepreneurial skills of youth get developed.
- New scheme would help develop service sector in rural area by building entrepreneurial skills.

**Disadvantage:**
- Youth would remain confined to rural areas. The disguised unemployment and technology barriers should be tackled first as involving more people does not going to affect productivity.

**Conclusion:**
Agriculture is the backbone of India. ARYA scheme has all the potential to keep the educated youth within the rural areas. However, its success depends upon the improvement in the quality life of the rural area.

**Reference**
file:///H:/ARYA/ARYA%20II%20proceedings.pdf
file:///H:/ARYA/ARYA.pdf
file:///H:/ARYA/Attracting%20and%20Retaining%20Youth%20in%20Agriculture%20(ARYA)%20Scheme%20-%20Bank%20Exams%20Today.pdf
INTRODUCTION:

Present era of molecular biology is witnessing revolutionary developments in sequencing technology. This advancement has considerably influenced plant virology in the field of diagnostics and host virus interaction. Next generation sequencing technology (NGS) has made it possible to directly detect, identify and discover novel viruses in several plants in an unbiased manner without antibodies or prior knowledge of the virus sequences.

During earlier days genetic information encoded in DNA was achieved through Capillary Electrophoresis (CE) based Sanger sequencing. The NGS technology based on the Capillary Electrophoresis (CE) principle which involves rapid sequencing of large stretches of DNA base pairs spanning entire genomes, with the latest instruments capable of producing hundreds of giga bases of data in a single sequencing run. NGS techniques are powerful tool for metagenomics based strategy for identification of unknown disease associated Viruses and discovery of novel plant viruses. NGS enables the direct detection, identification and discovery of viruses in an unbiased manner without requiring antibodies or prior knowledge of the pathogen macromolecular sequence.

Methods of next-generation DNA sequencing technologies:


<table>
<thead>
<tr>
<th>Sequencing platform</th>
<th>Amplification method</th>
<th>Sequencing chemistry</th>
<th>Read length (bp)</th>
<th>Sequencing Speed/h</th>
<th>Maximum Output Per run</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>454 Pyrosequencing</td>
<td>Emulsion PCR</td>
<td>Pyrosequencing</td>
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<td>700 Mbp</td>
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<td>75-85</td>
<td>21-28 Mbp</td>
<td>80-300 Gbp</td>
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</tr>
</tbody>
</table>

**454 Pyrosequencing**

In pyrosequencing, each incorporation of a nucleotide by DNA polymerase results in the release of pyrophosphate, which initiates a series of downstream reactions that ultimately produce light by the firefly enzyme luciferase. The amount of light produced is proportional to the number of nucleotides incorporated. The library fragments are mixed with a population of agarose beads whose surfaces carry oligonucleotides complementary to the 454-specific adapter sequences on the fragment library, so each bead is associated with a single fragment. Each of these fragment:bead complexes is isolated into individual oil:water micelles that also contain PCR reactants, and thermal cycling (emulsion PCR) of the micelles produces approximately one million copies of each DNA fragment on the surface of each bead. These amplified single molecules are then sequenced. First the beads are arrayed into a picotiter plate (PTP; a fused silica capillary structure) that holds a single bead in each of several hundred thousand single wells. Enzyme containing beads that catalyze the downstream pyrosequencing reaction steps are then added to the PTP and the mixture is centrifuged to
surround the agarose beads.

The PTP is seated opposite a CCD camera that records the light emitted at each bead. The first four nucleotides (TCGA) on the adapter fragment adjacent to the sequencing primer added in library construction correspond to the sequential flow of nucleotides into the flow cell. This strategy allows the 454 base-calling software to calibrate the light emitted by a single nucleotide incorporation.

**Illumina sequencing-by-synthesis**

The single molecule amplification for the Illumina starts with an Illumina-specific adapter library, takes place on the oligo-derivatized surface of a flow cell, and is performed by an automated device called a Cluster Station. The flow cell is an 8-channel sealed glass microfabricated device that allows bridge amplification of fragments on its surface, and uses DNA polymerase to produce multiple DNA copies, or clusters, that each represents the single molecule that initiated the cluster amplification. A separate library can be added to each of the eight channels, or the same library can be used in all eight, or combinations thereof. Each cluster contains approximately one million copies of the original fragment, which is sufficient for reporting incorporated bases at the required signal intensity for detection during sequencing.

The Illumina system utilizes a sequencing by-synthesis approach in which all four nucleotides are added simultaneously to the flow cell channels, along with DNA polymerase, for incorporation into the oligo-primed cluster fragments. Specifically, the nucleotides carry a base-unique fluorescent label and the 3-OH group is chemically blocked such that each incorporation is a unique event. An Imaging step follows each base incorporation step, during which each flow cell lane is imaged in three 100-tile segments by the instrument optics at a cluster density per tile of 30,000. After each imaging step, the 3 blocking group is chemically removed to prepare each strand for the next incorporation by DNA polymerase. This series of steps continues for a specific number of cycles, as determined by user-defined instrument settings, which permits discrete read lengths of 25–35 bases. A base-calling algorithm assigns sequences and associated quality values to each read and a quality checking pipeline evaluates the Illumina data from each run, removing poor-quality sequences.

**Sequencing-by-ligation: SOLiD**

The SOLiD platform uses an adapter-ligated fragment and uses an emulsions PCR approach with small magnetic beads to amplify the fragments for sequencing. Read lengths for SOLiD are user defined between 25–35 bp, and each sequencing run yields between 2–4 Gb of DNA sequence data. Once the reads are base called, have quality values, and low-quality sequences have been removed, the reads are aligned to a reference genome to enable a second tier of quality evaluation called two-base encoding.

**Advantage and disadvantage of NGS**

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>454 Pyrosequencing</td>
<td>Error rate with polybase more than 6. Low throughput. Long run: Reduction in enzyme efficiency or loss of enzymes (resulting in a reduction of the signal intensities).</td>
</tr>
<tr>
<td>Illumina sequencing-by-synthesis</td>
<td>Some time incorporated nucleotides may also fail to be correctly terminated allowing the extension of the sequence by another nucleotide in the same cycle. Short read assembly.</td>
</tr>
<tr>
<td>Sequencing-by-ligation: SOLiD</td>
<td>Beads carrying a mixture of sequences and beads in close proximity to one another create false reads. Short read assembly.</td>
</tr>
</tbody>
</table>

**Concluding Remarks**

These technologies have been a beneficial tool to detect and diagnose virus and virus like and other pathogens present in a sample. It has been successfully demonstrated particularly when prior knowledge of the causal agent of the disease is not known. It will unfold many mysteries of virus-host interaction. The etiology of several important diseases has been established using high NGS. Viral derived sRNA sequencing is emerging as a promising strategy for exploration of viruses and this can be a valuable part of the virologist’s tools for diagnosis and discovery of new viruses in plants and other organisms.
8. SOIL SCIENCE

Digestion Methods for Soil and Plant Analysis

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Introduction

Digestion is the part of sample preparation for elemental analyses in plants, soils, sediments and sludges. It is an oxidation process in which organic matter destruction and decomposition of tissues and complex materials into solution form takes place.

Purpose

Quantitative determination of elements in solids generally through the employment of following spectroscopic technologies viz: ICP-AES, ICP-MS, AAS, etc.

Aim

1. Complete solution of the elements
2. Complete decomposition of the matrix
3. Avoiding losses and contamination and
4. Reduction of handling and processing time.

Methods:

1. High temperature thermal digestion
2. Chemical digestion
3. Pressure digestion
4. Microwave digestion
5. Total digestion and
6. Pseudo-total digestion

High temperature thermal digestion

It refers to the ignition of plant samples and the procedure of ashing varies with temperature. Temperature of 500°C and 2-4 hour time is sufficient to digest the plant samples completely. Both conditions (>500°C/<500°C) result in low elemental recoveries. This is also called dry-ashing or thermal oxidation. This process is not suitable if N and S are to be determined. Boron can be determined by dry-ashing only.

Procedure

Add 0.5 gm of dried plant tissues into a 50 ml high porcelain or quartz crucible. Place crucible rack in a cool muffle furnace and set furnace temperature to reach 500°C in about 2-4 hours. After 4-8 hours of muffle at 500°C, remove the crucible rack from furnace and let cool. Then add 5 ml of 6N HCl to dissolve it. After complete dissolution, filtered it and make 50 ml volume.

Chemical digestion (wet digestion)

It refers to the process in which organic matter is destroyed by oxidation in liquid medium. This process utilises various mineral acids (HCl, HF, HNO₃, H₂SO₄) with or without H₂O₂ and other reagents such as potassium peroxide sulfate, boric acid and is carried out in either an open or closed vessels described by Tolg. The selection of the specific reagents or preparation of the reagent mixture depends on the samples to be digested. Organic samples are generally decomposed with the aid of oxidising acids primarily nitric acid and hydrogen peroxide and completely mineralized. Inorganic samples are also to be completely mineralized or dissolved. To do this primarily acid mixtures of HF or HCl are employed. The solubility of resulting salts must be considered. This method has been written to provide two separate digestion procedures: -One for the preparation of sediments, sludges and soil samples for analysis by FLAA or ICP-AES and other for the preparation of sediments, sludges and soil samples for analysis by GFAA or ICP-MS. The extracts from these two procedures are not interchangeable and should be only used with analytical determination. The recommended determinative technique for each element is listed below:

<table>
<thead>
<tr>
<th>FLAA/ICP-AES</th>
<th>GFAA/ICP-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al, Mg, Cr, Tha</td>
<td>As, Pb</td>
</tr>
<tr>
<td>An, Mn, Co, Va</td>
<td>Be, Mo</td>
</tr>
<tr>
<td>Ba, Mo, Cu, Zn</td>
<td>Cd, Se</td>
</tr>
<tr>
<td>Be, Ni, Fe</td>
<td>Cr, Tha</td>
</tr>
<tr>
<td>Cd, K, Pb</td>
<td>Co</td>
</tr>
<tr>
<td>Ca, Ag, Na</td>
<td>Fe</td>
</tr>
</tbody>
</table>

This method is further of following types:
Open acid digestion, closed acid digestion, Di-acid digestion and Tri-acid digestion

<table>
<thead>
<tr>
<th>Open acid digestion</th>
<th>Closed acid digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Maximum temperature limited by the solution boiling point</td>
<td>Maximum temperature is 260-300°C</td>
</tr>
<tr>
<td>2. Permits large sample weighing</td>
<td>Amount may be large or small</td>
</tr>
<tr>
<td>3. High acid consumption</td>
<td>Reduced acid consumption</td>
</tr>
<tr>
<td>4. Digestion quality is frequently unsatisfactory</td>
<td>High digestion quality</td>
</tr>
<tr>
<td>5. Loss of volatile elements (e.g. Hg, Pb)</td>
<td>No loss of volatile elements</td>
</tr>
<tr>
<td>6. Contamination risk</td>
<td>No contamination risk</td>
</tr>
<tr>
<td>7. Digestion duration (2-15 hr)</td>
<td>Digestion duration: Microwave-20-60 minutes, Tolg bomb-2-5 hr</td>
</tr>
</tbody>
</table>

Di-acid digestion
It refers to the oxidation of plant samples which is carried out in the presence of HNO₃ and HClO₄ in the ratio of 4:1. This process is applicable for those dried sample, in which P, K, Ca, Mg and micronutrients are to be determined. Di-acid digestion mixture can be prepared by: - a. HNO₃ and HClO₄, b. HNO₃ and 30 per cent H₂O₂ and c. H₂SO₄ and 30 per cent H₂O₂.

Procedure
Weigh 0.5 gm dried plant tissue into a beaker or digestion tube. Add 5-6 ml of conc. HNO₃ and cover the beaker with a watch glass or place the funnel into the mouth of digestion tube. Let stand overnight. Placed covered beaker on hot plate or digestion tube in a port of digestion block and digest at 80°C for 1 hr. After that remove beaker or digestion tube and let it cool. Add 1.5 ml HClO₄ and heat at 180-200°C for 2-3 hour or until the digest is clear. During digestion brown fumes of nitrous oxide is evolved and after that white fumes coming out and reaction completed. Let it cool and make the volume 50 ml and filtrate.

Tri-acid digestion
It refers to the oxidation of dried plant tissues in the presence of triple acid mixture (HNO₃, HClO₄ and H₂SO₄) in the ratio of 9:3:1. N is determined from only triple acid digested sample.

Procedure
All are same but difference is only the addition of digestion mixture (CuSO₄ and K₂SO₄) along with H₂SO₄.

Pressure digestion
This process is followed by mixing of samples with acid mixtures in a stainless steel digestion vessel at maximum operating pressure of 200 bars and temperature of 260°C. Pressure digestion can be done in Tolg bomb or microwave oven. BERGHOF introduced a series product based on the pressure digestion method developed by Prof. Tolg.

Procedure
Take a suitable amount of sample in vessel and heating takes place in a special heater block, not in a laboratory oven. Digestion is generally carried out at specific external temperature. Final operating temperature is typically reached after 45-60 minutes. Due to the high maximum operating pressure of 200 bars and the maximum operating temperature of 260°C, these systems are capable for completely digesting of any samples and transferring them into solution. This allows even the hardest samples (e.g. Sic, alpha-Al₂O₃) to be completely dissolved.

Total digestion
For total elemental analysis, samples are digested by total digestion or pseudo total digestion process. Total digestion is normally done with HF acid because it can dissolve silicates. The entire material is solubilized and the total metal contents are released for measurement. Special precautions are required for handling HF. It cannot be stored in glass vessel. Fume woods have to be designed to avoid corrosive vapors and use of appropriate personal protection equipments including provision of cogluconate-HF antidote gel is considered.
9. AGRICULTURAL ENGINEERING

Surface Drainage Systems – A Solution for Crop Cultivation in Water Logged Area

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It is a well known fact that Indian agriculture is a gamble with monsoons flanked by the extremities of calamitous droughts and catastrophic floods. Water logging adversely affected agricultural lands creating doubts about the sustainability of irrigated agriculture. Later on, it was recognized that negligence of drainage component in the irrigation project plans was one of the major mistake in the earlier planning processes. Simultaneously, there was pressure for resource conservation in terms of land and water resources. Economic justification, need to sustain profitable crop production system, positive socio economic impacts on rural masses and regional economy promoted the planners to think seriously about better drainage facilities in irrigation commands.

Surface drainage

Surface drainage is the diversion or orderly removal of excess water from the surface of land by means of improved natural or constructed channel, supplemented when necessary by shaping and grading of land surface to such channels. Basically the surface drainage is adopted on flat areas having slow infiltration and low permeability to eliminate ponding and prevent prolonged saturation.

Adverse effects of absence of surface drainage methods

Retard crop growth due to inundation.
Deficient oxygen in the root zone thereby resulting in poor germination and the uptake of nutrients.
Sowing operations are delayed.
Low soil temperature in temperate regions.

Surface drainage system for flat area slope less than 2%

1. Bedding system: The soil is formed into beds and that are separated by constructing parallel dead furrows oriented in the direction of greatest land slope. The water drains from the beds into the dead furrows which discharge into field drain. This system is not recommended for new crops due to crop loss in and adjacent to the dead furrows. However it is acceptable for grass land in some areas. The bed width should not be more than 10 m. the soils with low infiltration and low permeability require narrower beds than soils with better characteristics.

Disadvantage of this system

Reduction in crop yield as the top soil is moved from the sides of the bed to the middle.
The system restricts mechanized farming.
The dead furrows require regular maintenance to prevent weed growth.

1. Parallel field drain system: It is the most effective method of surface drainage when it is adopted in combination with land forming. The function of the field drains is to collect surface runoff and discharge it into field laterals which further transmit water to the main drainage system. The spacing of the field drains primarily depends on water tolerance of crops and the amount of land grading involved, it is essential for efficiency of the system. Land planning is usually applied in conjunction with land grading which is the process of smoothing the land surface with land plane to remove minor depressions and irregularities without changing the general topography. The flow velocities in furrows should not exceed 0.5 m/s to avoid erosion. The row length should not exceed 150 m for highly erodible soils whereas it is...
recommended up to 300 m for slightly erodible soils.

2. **Random field drainage system**: The random field drainage system connects the depressions distributed at random over a field by means of a field drain and directs the stagnant water into field lateral. Generally, these depressions are large and shallow for which land forming is not economical. The field drains are shaped so that these are crossable by farm machinery.

3. **Parallel open ditch system**: It is the combination of surface drainage system combined with subsurface drainage system. This combination system is useful for peat and muck soils. For critical waterlogged areas where water table is within 1.5 m from ground surface, this method is essential for getting sustainable crop production.

**Surface drainage systems for sloping areas greater than 2%**

Bench type or step type terraces are constructed by forming a number of horizontal steps. Basically Cross slope drainage and standard erosion control terraces system are constructed for drainage and erosion control in the area greater than 2% slope.

1. **Cross slope drainage system**: The cross slope drainage system is a channel type graded terrace also called a Nichols terrace. It is suitable on lands with a slope of up to 4%. It resembles the parallel field drainage system. It is effective on soils with poor internal drainage and where overall slopes are long and regular but many minor depressions occur. The drains should run approximately parallel to the contours of the land with a uniform or variable grade of between 0.1 and 1% depending on the topography. The soil surface between the drains must be smoothened and all farming operations should be done parallel to the drains.

2. **Standard erosion control terrace**: The Standard erosion control terrace is a ridge type graded terrace also called a Mangum terrace and is suitable for land having slope as much as 10%. In this method soil from the channels is used to build up a relatively higher ridge on the down slope side. In such channels only 50% of the water is contained below the original land surface. The channels of the erosion control terraces should run approximately parallel to the contours of the land with a uniform grade between 0.1 and 0.6% depending on the topography. The maximum length of a terrace channel draining to one side only is about 350 or 450 m. The depth of channel varies from 25 cm for terraces 60 m long to 35 cm for 300 m long. Channel can be either triangular or trapezoidal with cross sectional area between 0.35 to 0.90 m². A freeboard of about 10 cm is also recommended for design channel.

**Advantages of surface drainage system**

Agricultural land drainage consists of a set of technical strategies and hydraulic structures allowing the removal of excessive water and/or salts present in the soil volume occupied by root crops, to provide an adequately oxygenated environment, suitable for root normal development, keeping adequate water and air relative proportions according to crop physiological needs, to enable soil sustainability for crop productive conditions.

**Reference**

10. AGRICULTURAL

Introgressiomics: A Novel Approach For Crop Improvement

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Introgressiomics was first defined by Anderson and Hubricht (1938) as the infiltration of germlasm from one species into another through repeated backcrossing of the hybrids to the parental species. Edgar Anderson’s 1949 book *Introgressive Hybridization* stated introgression as a potentially important process for introducing adaptive variation into a population. The evolutionary impact of hybridization is mediated through introgression.

Types of introgression

I. Based on the nature of gene flow, *introgression is of four types. They are:*

Adaptive introgression
Localized introgression
Dispersed introgression
Assymetric introgression

1. **Adaptive introgression:** It refers to transfer of genes from a well adapted variety to a less unadapted one.

2. **Localized introgression:** Gene flow is restricted to short distance. It is more common.

3. **Dispersed introgression:** Gene flow is widespread. It is rare.

4. **Asymmetrical introgression:** It is gene transfer between parental taxa that differ in abundance.

II. Based on the nature of occurrence, *introgression is of two types. They are:*

Natural introgression
Artificial introgression

1. **Natural introgression:** Gene flow due to natural out crossing is called natural introgression.

2. **Artificial introgression:** Gene transfer mediated through various conventional and molecular techniques is called artificial introgression.

Steps for introgressiomics:

Identifying the target wild species
Interspecific hybridization
Production of F1 hybrid
Backcrossing
Development of introgression lines
Use of these in further breeding programmes

Introgression and its consequences in plants:

1. Increased adaptation of the species.
2. Origin of New Plant Types.
3. No transferability limits between species
5. Reduction in genetic diversity

Limitations of introgressiomics:

1. Linkage drag
2. Prebreeding often takes more time.
3. Reduced availability markers for identification and confirmation of introgressed lines.
4. Ploidy differences between crossed species that hampers transfer of genes.
5. Information on already existing pre-bred materials is less.
6. Limited access to germplasm by breeders.

References


Biofortification: An approach to increase micronutrient status in staple foods

Deepshikha1, Elangbam Premabati Devi2 and Jai Prakash Jaiswal3

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Biofortification

Biofortification is a strategy currently being researched and developed for increasing the bio-absorbable content of micronutrients in the edible portion of staple food crops. The micronutrients currently being targeted by the bio fortification program are iron, zinc, and pro vitamin A. In many parts of the world, micronutrient deficiency is a more widespread problem than poor dietary quality and low energy intake (Stewart et al., 2010), and about 20% deaths in children under five can be attributed to vitamin A, Zn, Fe and/or I deficiency. In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of daily diet. Biofortified staple foods may not deliver equally high levels of minerals and vitamins per day, compared to supplements or fortified food products, but they can increase micronutrient intake for the resource poor people who consume them daily and therefore complement existing approaches (Bouis et al., 2011). World Health organization (WHO) has estimated that bio fortification of iron could help in curing 2 billion people suffering from iron deficiency which induced anemia. It is being done by genetic or agronomic techniques, so that when consumed regularly will generate measurable improvement in vitamin and nutritional status.

Genetic biofortification

It is a strategy which uses plant breeding techniques (selective breeding or genetic modification) to produce staple food crops with higher micronutrient levels, reducing levels of antinutrients and increasing the levels of substances that promote nutrient absorption (Bouis, 2003). It offers a sustainable solution to malnutrition problems by exploring natural genetic variation to develop mineral dense crop varieties (Pfeiffer and McClafferty, 2007). Plant breeders screened thousands of crop varieties and stored in global seed banks to discover varieties with naturally higher amounts of essential micronutrients. Then, through collaborations with various breeding centers of the Consortium of International Agricultural Research (CGIAR), and national agricultural research systems, these nutrient rich varieties are used to breed bio fortified varieties that are also high yielding, disease and pest resistant and climate smart in local agro ecological conditions.

Agronomic biofortification

As compared to the breeding approach, agronomic biofortification represent a short term solution to the problem (Cakmak, 2008). The micronutrient density in plant based food is increased by agricultural methods of crop cultivation, like by adding the target nutrients with fertilizers or by applying amendments that can increase their uptake from pools which are already in the soil but not sufficiently available (Graham et al., 2001). Agronomic approaches such as application of Zn fertilizers to soil or foliar spray seem to be a practical tool for Zn biofortification in wheat. Zn concentration in wheat grain remarkably increases by foliar application of Zn as compared to application of Zn in soil. The Zn concentration could be further increased by optimizing the timing and the solute concentration of foliar Zn application not only in whole grains but also in the endosperm of wheat grain.
HarvestPlus

HarvestPlus, a major NGO in the development of biofortified crops primarily use conventional breeding techniques. It was launched in 2004 with funding from the Bill and Melinda Gates Foundation the UK Department for International Development (DFID) and others. Now it has 15 funding institutions with more than 200 scientists and researchers collaborating with universities, institutions and many organizations with which they have formal agreements. It improves public health by developing and promoting biofortified for crops that are rich in vitamins and minerals.

It developed Biofortification Priority Index (BPI) to help stakeholders assess for which crop and in which country their investments will have the greatest impact in reducing micronutrient deficiencies. The BPI ranks seven staple crops according to their suitability for biofortification investment in 127 countries in Africa, Asia, and the Latin America and Caribbean region. The seven micronutrient-rich crops includes iron beans, iron pearl millet, vitamin A cassava, vitamin A maize, vitamin A sweet potato, zinc rice, and zinc wheat. According to preliminary monitoring data estimates, by the end of 2017 approximately 30 million people were benefitting from biofortified crops in HarvestPlus. By 2020, HarvestPlus aims to reach 20 million farming households with biofortified planting material, benefiting at least 100 million people, and, by 2030, one billion people are expected to consume biofortified foods globally. More than 180 varieties of eleven biofortified crops have been officially released in over 30 countries. As advances in research for crop development, the nutrient density of crops is further increased and biofortified varieties are better adapted to the changing climate and consumer preferences. Most recently, zinc maize was released in Honduras and will be released in additional Latin American countries in 2018. Thus, genetic and agronomic biofortification which are complementary and synergistic solutions which offer sustainable solutions to the increasing micronutrient related malnutrition problems.

Benefits of biofortification

It improves the nutritional status of staple food and reaches the rural communities without the access of fortified food
Vulnerability to social and economic changes is less
The potential to impact a large number of people at a low cost per person
Biofortification in staple foods can provide profound health benefits to the whole family

References

Hydropriming: A valuable method of seed treatment for better germination of plants

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²G.B. Pant University of Agriculture and Technology, Pantnagar, 263 145, Uttarakhand

Introduction
Poor germination and improper seedling establishment are one of the reasons of soil salinity and it is an enormous problem which adversely affects growth and development of plants and ultimately resulted into less production. Besides, under drought condition seed germination and seedling establishment were inhibited due to reduce amount of water potential, which lead to decline in water uptake by the plants (Farooq et al., 2009). Moreover, another major problem when plants are exposed to drought stress is oxidative damage due to the overproduction of reactive oxygen species (ROS). Seed priming can be a simple solution towards expected good plant stand establishment in biotic and abiotic stress conditions.

Seed priming is the pre-sowing treatments of seeds either in water or in an osmotic solution which allows seeds to imbibe water to proceed for the first stage of germination, but it restricts protrusion of radicle length by seed coat. Different types of seed priming treatments were reported to enhance drought tolerance in many plants. The process of seed germination are induced by either soaking seeds in water or in solutions containing exogenous molecules like salts, metals or hormones etc. followed by surface drying of seeds. The beneficial effects of seed priming have been demonstrated in many field crops like maize, barley, mung bean, lentil, cucumber etc. (Sadeghian and Yavari, 2004).

Hydropriming as seed treatment
Hydropriming involved soaking of seed in water before sowing. When seeds are imibed, the lag period before radical emergence is considerably reduced and improved the rate and uniformity of germination. Hydro primed seeds could able to achieve earlier and more uniform germination, higher germination index and increased vigour index. On-farm seed priming by soaking seeds overnight in water followed by surface drying and then sowing has markedly improved the plant stand establishment and early vigour of maize plants in field condition. Rapid establishment and high vigour has also resulted with faster development, earlier flowering and maturity and provide higher yield. Seed treatment of KNO₃ and hydropriming increased sunflower germination and seedling growth under salt and drought stress conditions (Kaya et al., 2006).

Similarly, hydropriming improved seed germination and seedling emergence of lentil (Saglam et al., 2010). Seed priming with PEG and water increased drought stress tolerance in seeds of rice cultivars at the germination stage. These positive effects of priming are associated with a wide range of metabolic and physiological improvement as well as activation of various protective enzymes such as superoxide dismutase, peroxidase, catalase etc. and accumulation of osmoprotectants, like proline, soluble sugar and soluble protein which are the typical stress avoidance responses (Farhad et al., 2011).

Soaking of maize seed in water for 18 hr followed by 2 hr surface drying was found effective with the highest germination percentage, germination index and lowest mean germination time of seed (Ahmad et al., 2014). Hydropriming generally enhance rate of seed germination and seedling emergence under both saline and non-saline conditions as well as enhanced beneficial effect on various enzyme activity required for rapid germination of seed. Moreover, Rehman et al. (2011) suggested that seed priming is a very cost effective technology which can enhance early crop growth leading to earlier and more uniform establishment of plant with increasing in yield associated benefits.
many field crops including oilseeds crops. Singh (1995) observed that hydro priming practically ensured rapid and uniform germination accompanied with low abnormal seedling percentage. They further noted that hydropriming had high potential in improving field emergence and ensured early flowering and harvesting under stress condition, especially in dry areas. It is being used to reduce the germination time in order to get synchronized germination, improve germination rate and better seedling in many crops such as maize, soybean, wheat, lentil, chickpea, mungbean, cowpea, tomato, onion etc. (Singh, 1995).

**Advantages of hydropriming**

- Increase germination percentage of seed
- Enhance seedling growth and vigour of plant
- Ensure early flowering
- Increase water use efficiency (WUE)
- Easily practicable
- Provide higher yield
- Cost effective and safe to environment
- Ensure more yield production
- Increased tolerance to biotic and abiotic stress conditions

**Conclusion**

Hydropriming of seed is a very cost effective technology which is very simple to apply and it can enhance early crop growth leading to more uniform establishment of seedlings which can resulted into increase in yield associated benefits in many field crops and oilseeds crops. Thus, hydropriming could be a valuable approach to attain more food production towards the sustainable agriculture.

**References**


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13. **HORTICULTURE**

**Nursery Techniques of Papaya**

**Ravi Kondle*  

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**Introduction**

Papaya (Carica papaya L.) is a tropical fruit belonging to the family Caricaceae having commercial importance because of its high nutritive and medicinal value. It is one of the few fruit crops that flowers and fruits throughout the year giving early and steady returns. Papaya cultivation had its origin in tropical America, perhaps in Southern Mexico.
and neighbouring Central America. Introduced from Philippines through Malaysia to India in the later part of 16th century, papaya cultivation has now spread widely in tropical and subtropical regions of our country. Besides table purpose, fruits are also used for making jam, syrup, tutti-frutti and ready to serve beverages. Papain, the latex extracted from immature papaya fruit is a rich source of proteolytic enzyme which is used in tanneries, pharmaceutical and also beer industry. Fruits are rich source of carbohydrates (6.0-9.5 g/100g), minerals and vitamins and highly valued for its curative properties. India is one of the largest producers of papaya in the world, ranks second position in area (73.1 thousand hectares) and fourth (2.37 million tones) in production of papaya (Singh, 2008). In India, papaya is mainly cultivated in Andhra Pradesh, Gujarat, West Bengal, Karnataka, Tamil Nadu and Assam. Productivity of papaya is highest (41.7t/ ha) among the fruit crops, which has attracted the growers for its commercial cultivation and intern contributed for increase in area and production in last few decades.

**Propagation of papaya**

Papaya is mainly propagated by seeds and conventional methods of vegetative propagation like cuttings, grafting, budding etc, have not proved successful for commercial cultivation. Seeds are used for raising the seedlings should be fresh as their viability is short. The seedlings can be raised both on raised beds as well as in polythene bags.

**In vitro propagation**

Leaf bits, apices and axillary buds are suitable for mass multiplication through in-vitro culture. Murashige and Skoog medium with benzyl amino purine at 2.0 μM + 0.5 μM NAA produce rapid proliferation of tissues.

**Seed extraction**

Fruit size or weight has no association with seed quality except that the seed content is more in large fruits and less in small fruits. The seeds from different fruit weight or size classes did not differ in their quality. Hence, all ripened fruits can be used for seed extraction.

**Washing**

Washing of seeds increased the seed germination and seedling growth of papaya. Okeyo and Ouma (2008) reported that papaya seeds should be washed and soaked in water before sowing to enhance the seed germination. Rathinavel and Selvaraj (1996) stated that, water soaking of papaya seeds for 16 hour prior to sowing was found to be good for seed germination and seedling growth of papaya.

**Invigoration of old seeds**

Stored seeds can be invigorated by soaking them in dilute solution of disodium phosphate (10 M) adopting 1:8 seed to solution ratio for 4 hours followed by drying back to original moisture content.

**Preparation of Seed-bed and Raising of Seedlings**

The seedbeds are prepared on well drained soil where the farmyard manure is thoroughly mixed. Generally 40 sq. Meters area is sufficient to raise seedlings for transplanting one acre and for this much area one quintal of well rotten farmyard manure is recommended. The seedlings can be raised in sed beds 3m long, 1m wide and 15cm high above the ground level.

The seeds are sown in April and seedlings are transplanted in the month of July. However, the nurserymen should sow the seed in two, three lots of 15 days interval. This will help in regulating the plant sale.

Seeds before sowing are treated with 3g of Captan per kg., of seed. About 150g seed is enough to raise seedlings for one acre.

The seeds are sown 1-2cm deep in rows 10 cm apart, row to row distance also kept 15cm and covered with fine compost of leaf mould. Light watering should be done with water can in the morning. The nursery beds may be covered with polythene sheet of dry paddy straw to protect seedlings.

**Aftercare and Transplanting of Seedlings**

The seeds germinate in 2-3 weeks. When the seedlings have emerged, drench the nursery beds with 012 per cent Captan (200g in 100 litres of water) to prevent the damping off of
young seedling. Repeat the drenching of nursery beds after 4 days, if necessary.

One month after the emergence, the seedlings attain the height of about 15cm and they are recommended for transplanting in the field.

Top dressing of seedlings in nursery with urea or ammonium sulphate should be avoided as this encourages damping off disease and the development of tall and lanky seedling which are not suitable for transplanting.

Raising the Seedlings in Polythene Bags

Papaya seedlings raised in polythene bags can stand transplanting better than those raised in seed beds.

Perforated polythene bags of 22.5 X 15 cm size with 100 gauge thickness can be used for sowing the seed. These bags are filled with a mixture of farmyard manure, soil and sand in equal proportion. Two or three seeds are sown in bag and after germination only one seedling is retained. These seedlings in the polythene bags should also be treated with Captan (0.2 per cent) after their emergence. The transplanting in this case is done along with polythene bags. Only care taken in this case is that the bags should be ruptured at the bottom while transplanting.

References


14. BIOTECHNOLOGY

Cadherin-Bt Receptor: An overview

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Summary

Cadherin is a calcium-dependent adherent protein, constitute a large family of cell surface transmembrane glycoproteins, having cytoplasmic domain and an extracellular ectodomain. The presence of Ca2+ is necessary for cadherin adhesive function and hence their name arose as an approximate contraction of “calcium-dependent adherent protein”. Mammalian genomes have over 100 genes belonging to the cadherin superfamily, lancelet, a sea anemone and a placozoan have 30, 16 and eight cadherin genes. Removal of the 12-cadherin- domain protein would remove a toxin binding site but did not explain how this could confer resistance in the presence of toxin-binding aminopeptidases that remained.

Introduction.

Cadherin is a calcium-dependent adherent protein, constitute a large family of cell surface transmembrane glycoproteins, having cytoplasmic domain and an extracellular ectodomain. The presence of Ca2+ is necessary for cadherin adhesive function and hence their name arose as an approximate contraction of “calcium-dependent adherent protein”. Structural information from the analysis of several cadherin domains indicates that calcium ions bind at sites between adjacent cadherin repeats (CRs), forming a rigid rod. Cadherins are responsible for maintaining the integrity of selective cell–cell recognition and adhesion properties (Gumbiner, 1996). Cadherin plays fundamental role in development, morphogenesis, cell sorting and migration, cell signalling and the maintenance of structural integrity. Specific functions of cadherins include embryonic cell layer separation and the formation of tissue boundaries, synapse formation, neuron growth and connectivity. The establishment of cell polarity,
mechanotransduction, cell adhesion, cell signalling and physical homeostasis. Cadherins are defined by the presence of an extracellular region consisting of cadherin repeat (CR) domains, a transmembrane domain and an intracellular cytoplasmic (IC) domain. The extracellular region includes a variable number of CRs, which contain the conserved motifs that include alanine (Ala), arginine (Arg), asparagine (Asn), aspartate (Asp), glutamate (Glu), phenylalanine (Phe) and proline (Pro), as well as variable amino acids (X): Asp-Arg-Glu; Asp-X-Asn-Asp-Ala-Pro-X-Phe; and Asp-X-Asp. Each of these CRs consists of about 110 amino acids and forms a unique immunoglobulin-like -sandwich fold. The interface between these CR domains harbours calcium-binding sites that are important for the adhesive properties of cadherin.

**Cadherin genes and their organization**

Mammalian genomes have over 100 genes belonging to the cadherin superfamily, lancelet, a sea anemone and a placozoan have 30, 16 and eight cadherin genes, respectively. The *Caenorhabditis elegans* genome encodes 13 cadherins. Genomic organization of cadherin superfamily genes. Many of the cadherin superfamily genes have been mapped to specific human and mouse chromosomes. The genomic DNA structures are divided into two large groups, the classic and desmosomal cadherin group, and the CNRs (Pcdha), Pcdhb, Pcdhg, and Pcdh8 group. The classic and desmosomal cadherins in general consist of 12-17 exons, and share a remarkable degree of conservation in intron positions. On the other hand, CNRs (Pcdha), Pcdhb, Pcdhg, and Pcdh8 genes contain long first exons that encode the extracellular domain (EC), the transmembrane region (T), and a part of the cytoplasmic tail (CP). The first exons of all of these genes with the exception of Pcdhb are linked by small exons, which encode the remainder of the cytoplasmic region.

**Bt Receptor**

BtRs are transmembrane proteins of 175-250 kDa composed of four domains

- An extracellular domain consisting of repetitive CRs
- A membrane proximal region (MPR)
- An IC domain

Cadherin binding sites for Cry toxins map primarily to the CR domains adjacent to the membrane-proximal regions of the protein.

*FIG.1. Structure of Cadherin receptor* A) Cadherin repeats B) Vertebrate Cadherin cis and trans domain C) cadherin bridge the intermembrane space between cells and cytoplasmic domain of cadherin.

*FIG.2. Role of lepidopteran BtRs (Bacillus thuringiensis Cry toxin cadherin receptors) in the mode of action of Cry1 toxin*

Cry1Ac binds to recombinant peptides corresponding to extracellular regions of a cadherin protein (BtR) in a major cotton pest,
pink bollworm (*Pectinophora gossypiella*) (PBW). Cadherins binds to Bt toxins, BtR has at least two Cry1Ac-binding domains in cadherin-repeat regions 10 and 11, which are immediately adjacent to the membrane proximal region. Protoxin and activated toxin forms of Cry1Ac bound to recombinant BtR fragments, suggesting that Cry1Ac activation may occur either before or after receptor binding (Fabrick and Tabashnik, 2007)

**Cadherin-mediated resistance**

Studies with the 12-cadherin domain proteins from susceptible strains of *Manduca sexta* (Vadlamudi et al., 1995) and *Bombyx mori* (Nagamatsu et al., 1999) showed that they bound Cry1A toxins, whether present in the native brush border membrane or on the surface of cells heterologously expressing them. Disruption of the 12-cadherin-domain protein would remove a toxin binding site but did not explain how this could confer resistance in the presence of toxin-binding aminopeptidases that remained. Nor was it anticipated that the absence of this protein could be tolerated by the organism and not result in lethality, even though it has been found in the midgut of every lepidopteran investigated so far (despite being absent from the fully sequenced genomes of *Drosophila melanogaster* and *Caenorhabditis elegans*). Indeed, the precise role of the 12-cadherin-domain protein in the mechanism of toxicity is still controversial. Binding sites for Cry1A toxins have been mapped to the membrane-proximal region of the protein (Nagamatsu et al., 1999) suggesting a role in increasing toxin concentrations at the membrane surface which may promote oligomerization and pore formation. By using alanine-scanning mutagenesis on peptide fragments overlapping this region, Xie et al. (2005) have shown that single amino acid substitutions can reduce toxin binding, although whether this could confer resistance *In Vivo* is unknown. have hypothesized that cadherin binding by the toxin results in an additional processing step whereby two alpha-helices from domain 1 are clipped off, promoting toxin oligomerization and subsequent binding to aminopeptidases eventually resulting in pore formation (Bravo et al. 2004). Zhang et al. (2006) cell-killing mechanism in cultured cells heterologously expressing the cadherin is not pore formation but rather a signaling pathway initiated by toxin binding to the cadherin and mediated by adenyl cyclase and Protein Kinase A, although data on this mechanism in midgut cells is lacking Strains from Arizona, recessively inherited resistance to Cry1Ac is associated with mutations in the cadherin gene called *BtR* (Morin et al. 2003, Tabashnik et al. 2004).

**Conclusion**

Cadherins are transmembrane proteins that mediate cell–cell adhesion in animals. By regulating contact formation and stability, cadherins play a crucial role in tissue morphogenesis and homeostasis. Cadherin superfamily genes have been mapped to specific human and mouse chromosomes. The genomic DNA structures are divided into two large groups, the classic and desmosomal cadherin group, and the CNRs (Pcdha), Pcdhb, Pcdhg, and Pcdh8 group. BtRs are transmembrane proteins and plays central role in Bt's mode of action.

**References**


Tabashnik, B. E., Liu, Y.B., Unnithan, D. C.,
Plant development is plastic and subject to modulation by environmental cues such as light, water, and gravity. A dramatic example is the regulation of seedling development by light. A number of candidate phototransduction mutants that develop as light-grown plants in the dark have been isolated. One of them is De-Etiolated Homolog 1 (DET1) a negative regulator of light-mediated responses in plants, plays pleotropic role in plant development through complex interactions with target proteins which affect the photo regulated gene expression. DET1 plays roles in plant development through complex interactions to target proteins for proteolysis and to affect chromatin remodeling for photoregulated gene expression.

DET1 is a nuclear protein and recent findings suggest that the functional form of DET1 is within an approximately 350 kDa complex that also contains the plant homologue of UV-damaged DNA binding protein 1 (DDB1). Benvenuto et al (2002) have shown that DET1 binds to hypo-acetylated amino-terminal tails of the core histone H2B and have proposed that DET1 may be involved in chromatin remodelling around photoregulated genes. For example, DET1 may limit access of positive regulatory factors to the promoters of light responsive genes. DET1 is a transcriptional repressor affects the activity of a repressor that interacts with target genes such as cab, rbcS, or chs. DET1 is a negatively acting molecule near the top of a regulatory cascade that is used in common by the light stimulus transduction pathway and by temporal or spatial regulatory signals. Although molecular biology of light-regulated developmental pathways in higher plants is unknown, many studies developed det1, det2, and det3, mutants of Arabidopsis and have shown the gross morphology of light-grown plants, including the development of chloroplasts and leaf mesophyll tissue.

Mutations at any one of several DET or COP loci cause dark-grown plants to develop short hypocotyls, open and expanded cotyledons, and true leaves. Light-regulated nuclear- and chloroplast-encoded genes, such as the light-harvesting chlorophyll A/B-binding protein (CAB) genes or the ribulosebisphosphate carboxylase/oxygenase genes RBCS and rbcL, are expressed at (or nearly at) their light-grown levels. Many studies found that det and cop mutations are recessive suggests that these genes mediate negative regulation of a signal transduction pathway coupling light perception to leaf and chloroplast development and photoregulated gene expression.

The enhancement of nutritional quality is an important objective of modern plant breeding. Conventional molecular breeding and genetic modification (GM) technologies have been employed to generate better nutritional quality including marker-assisted screening.
The DET-1 gene is involved in light perception and its down-regulation results in the plant believing it receives a greater quantity of incident light, thus leading to the simultaneous, increased production of antioxidants. It has been shown that, constitutive and fruit specific silencing of DET1 resulted in simultaneous elevation of secondary metabolites viz., carotenoids, flavonoid, isoflavones, tocopherol and saponins. The genes encoding most of these bioactives/antioxidants are light regulated. In the last decade, genetic manipulation of light signal transduction components has been an effective means to improve nutritional quality, because it enables simultaneous enhancement of phytonutrients such genes may therefore represent promising genetic tools to improve nutritional value.

Examples

Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes

Davuluri et al in 2005 attempted to increase tomato fruit nutritional value by suppressing an endogenous photomorphogenesis regulatory gene, DET1, using fruit-specific promoters combined with RNA interference (RNAi) technology. Molecular analysis indicated that DET1 transcripts were indeed specifically degraded in transgenic fruits. Both carotenoid and flavonoid contents were increased significantly, whereas other parameters of fruit quality were largely unchanged. These results demonstrate that manipulation of a plant regulatory gene can simultaneously influence the production of several phytonutrients generated from independent biosynthetic pathways, and provide a novel example of the use of organspecific gene silencing to improve the nutritional value of plant-derived products (Davuluri et al., 2005).

Increased in the total content of anthocyanidins present in mature tomato green fruit for all DET1 mutants.

Analysis of the anthocyanidins present by liquid chromatography–tandem mass spectrometry revealed the sole presence of delphinidin-3-(coumaroyl)-rutinoside-5-glucoside. At the level of gene expression, they quantified several key transcripts relating to biosynthetic genes involved in phenylpropanoid, flavonoid, and anthocyanidin formation. In both the mature green and ripe fruit, the levels of several transcripts encoding enzymatic steps within these phenolic pathways were dramatically increased (e.g., CHS up to 37.5-fold), other notable increases mainly occurred were related to anthocyanidin biosynthetic transcripts (e.g., DFR, ANS, RT, and 3-GT were induced 5- to 35-fold) (Davuluri et al., 2004).

RNAi-Mediated Suppression of DET1 Alters the Levels of Carotenoids and Sinapate Esters in Seeds of Brassica napus

Carotenoids and sinapate esters in Brassica napus affect the nutritional value of the seed. The levels of 1,2-di-O-sinapoylglucose in seed in both set of transgenic plants were lower compared to non-transgenic seeds. The result revealed that DET1 suppression in B. napus can increase the levels of carotenoids and reduce the levels of sinapate esters simultaneously in the seeds, thus enhancing their overall nutritional value (Wei et al., 2009).

References


DE - ETIOLATED1 downregulated tomato fruit.

Plant Cell 22(4): 1190–1215

16. PLANT BREEDING AND GENETICS AGRICULTURE SCIENCE
Applications of Casein in Food Industry

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Casein is derived from Latin word Caseus means cheese, where casein is one of the main chemical substances in cheese, casein belongs to family of phospho proteins, which commonly found in all mammal milk. In British terminology, term caseinogens derived for un-coagulated protein and casein for the coagulated protein. As it exists in milk, a salt of calcium and is chief protein present in milk, comprises about 80 % of total protein in milk. Buffalo milk and Ewe milk contains highest amount of casein in their constituents compared to other mammal milk. As a food source casein supplies carbohydrates, amino acids and chiefly composed of two minerals calcium and phosphorous, basically casein is obtained in four forms namely αS1, αS2, β, κ or Kappa casein. Casein is obtained in form of claim phosphate complex mixture. The most common form of casein found in nature is Sodium caseinate. Casein is insoluble in water and neutral salt solutions and readily dispersible in dilute alkali and aqueous salt solutions. Iso-electric p[point of casein is 4.6, which provides an negative charge in milk. Casein resembles in tertiary structure to a small extent, contain high number of proline residues. Casein is partially hydrophobic and contains a suspension of particles known as casein micelles which show only limited resemblance with surfactant type micelles and usually appears in spherical shape.

Applications and benefits
Being a protein, in nature casein has several uses

1. A1/A2 beta caseins in milk: A1 and A2 beta-casein are genetic variants of the beta-casein milk protein that differ by one amino acid; a proline occurs at position 67 in the chain of amino acids that make up the A2 beta-casein, while in A1 beta-casein a histidine occurs at that position. It is said to be these A1/A2 beta caseins are helpful in prevention of many chronic diseases. The A1 beta-casein type is the most common type found in cow’s milk in Europe (excluding France), the United States, Australia, and New Zealand.

2. Cheese making: Cheese consists of proteins and fat from milk, usually the milk of cows, buffalo, goats, or sheep. It is produced by coagulation that is caused by destabilization of the casein micelle, which begins the processes of fractionation and selective concentration. Unlike many proteins, casein is not coagulated by heat. During the process of clotting, milk-clotting proteases act on the soluble portion of the caseins, κ-casein, thus originating an unstable micellar state that results in clot formation.

3. Food source: Casein has a wide range of use in different food products, it is used as stabiliser, stabilisation of fat, binding agent, retention of taste etc., Several foods,
Creamers, and toppings all contain a variety of caseinate. Sodium caseinate acts as a greater food additive for stabilizing processed foods. Many industries use this calcium caseinate for increasing of fortifying calcium content in foods that are low in calcium.

4. Food powders: The main food uses of casein are for powders requiring rapid dispersion into water, ranging from coffee creamers to instant cream soups.

5. As a Glue: Casein-based glues, formulated from casein, were popular for wood working, including for aircraft, laminating fireproof doors and labelling of bottles, manufacturing transformer board etc.,

6. Paintings: Casein paint is a fast-drying, water-soluble medium used by artists. Casein paint has been used since ancient Egyptian times as a form of tempera paint, and was widely used by commercial illustrators as the material of choice until the late 1960s when, with the advent of acrylic paint, casein became less popular. It is still widely used by scene painters.

7. Plastics and fiber: Fiber can be made from extruded casein, Lanital, a fabric made from casein fiber was particularly popular in Italy during the 1930s.

8. Protein supplements: An attractive property of the casein molecule is its ability to form a gel or clot in the stomach, which makes it very efficient in nutrient supply. The clot is able to provide a sustained slow release of amino acids into the blood stream, sometimes lasting for several hours. Now a days, for fast growth, muscle development, casein incorporated powders as protein supplements playing a vital role in world. Due to their natural solubility, availability in different flavours, composition, versatility, protein supplements had made good demand in market. It is said to compatible, can used by all stages of people with minimal side effects.

9. Medical and dental uses: Casein-derived compounds are used in tooth remineralization products to stabilize amorphous calcium phosphate (ACP) and release the ACP onto tooth surfaces, where it can facilitate re-mineralization.

10. Autism: Although research has shown high rates of use of complementary and alternative therapies for children with autism, including gluten or casein exclusion diets, as of 2015, the evidence that such diets have any impact on behavior or cognitive and social functioning in autistic children was limited and weak.

**TABLE NO. 1 Casein content in Processed food products**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Food product</th>
<th>% Caseinate</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bakery products</td>
<td>1-15</td>
<td>Water binding</td>
</tr>
<tr>
<td></td>
<td>Cheese</td>
<td>3-28</td>
<td>Matrix formation, fat, and water binding</td>
</tr>
<tr>
<td></td>
<td>Ice Cream</td>
<td>1-7</td>
<td>Texture and stabilizer</td>
</tr>
<tr>
<td></td>
<td>Meat and poultry products</td>
<td>2-20</td>
<td>Texture and increase nutritional value</td>
</tr>
<tr>
<td></td>
<td>Whipped toppings</td>
<td>2-11</td>
<td>Fat stabilization</td>
</tr>
<tr>
<td></td>
<td>Pasta</td>
<td>2-18</td>
<td>Texture, nutrition, and taste</td>
</tr>
</tbody>
</table>

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17. AGRICULTURE

Water harvesting in drylands

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Introduction

Drylands cover approximately 40% of the world’s land area and support more than two billion people, 90% of whom live in developing countries, with relatively low amounts of precipitation in the form of rainfall or snow (UN 2011). In these regions precipitation is insufficient to support low-risk crop production. As it is poorly distributed over the growing season and often comes in intense bursts, it usually cannot support economically viable farming. The non-uniform distribution of precipitation, in arid and semi-arid regions, usually results in frequent drought periods during crop growth which severely stress growing crops thereby reducing yields, sometimes to crop failure. Even this water is mostly lost in evaporation and runoff, leaving frequent dry periods during the growing season. Although social and cultural factors may be implicated, the loss of water without benefit for agricultural or domestic use, and the mismanagement of land are significant factors in the process of desertification and the increasing poverty of dry areas. For agriculture to have a chance of success rainwater must be husbanded and land must be properly managed. Water harvesting can alleviate drought stress in arid and semi-arid environments and significantly contribute to water livelihood and environmental management by augmenting domestic water supplies, stabilizing crop yield by increasing the amount of water availability per unit of cropping area, uses runoff beneficially and supporting fragile ecosystems. As water shortage in the dry areas is a recurrent crisis, people have a great need for information on how to capture and use every available drop of water efficiently. Water harvesting is an effective and economical means of achieving this objective and information on its various systems and techniques is in great demand. Water harvesting is based on the principle of depriving part of the land of its share of rain, which is usually small and non-productive, and adding it to the share of another part. This brings the amount of water available to the latter area closer to crop water requirements and thereby permits economic agricultural production. This amount may be enough to support drought-resistant crops. Such concentration of rainwater is called water harvesting, which may be defined as “the process of concentrating precipitation through runoff and storage, for beneficial use.”

Water harvesting may occur naturally or by intervention. Natural water harvesting can be observed after heavy storms, when water flows to depressions, providing areas for farmers to cultivate. Water harvesting by intervention involves inducing runoff and either collecting or directing it, or both, to a target area for use. Besides being applied to agriculture, water harvesting may be developed to provide drinking water for humans and animals as well as for domestic and environmental purposes.

How Can Water Harvesting Help?

Water harvesting is particularly advantageous in the following circumstances:

In dry areas, where low and inadequately distributed rainfall normally makes agricultural production impossible. Provided other production factors such as soils and crops are favorable, water harvesting can make farming possible despite the absence of other water resources.
and help towards economic stability by reducing the uncertainty of human life in arid ecosystems and help in combating desertification by tree plantation.

In rainfed areas, where crops can be produced but with low yields and a high risk of failure. Here waterharvesting systems can provide enough water to supplement rainfall and thereby increase and stabilize production. In areas where water supply for domestic and animal production is not sufficient. These needs can be satisfied with water harvesting.

In arid land suffering from desertification, where the potential for production is diminishing, due to lack of proper management. Providing water to these lands through water harvesting can improve the vegetative cover and can help to halt environmental degradation.

Components of Water-Harvesting Systems

The main components of water harvesting systems are:

**Catchment area**: the part of the land that contributes some or all its share of rainwater to a target area outside its boundaries. The catchment area can be as small as a few square meters or as large as several square kilometers.

**Storage facility**: the place where runoff water is held from the time it is collected until it is used. Storage can be in surface reservoirs, subsurface reservoirs such as cisterns, in the soil profile as soil moisture, and in groundwater aquifers.

**Target area**: where the harvested water is
used. In agricultural production, the target is the plant or the animal, while in domestic use, it is the human being or the enterprise and its needs.


Declaration: I hereby declare that the work contained in article is my original contribution, and has not been published anywhere and the sole responsibility in this respect lies with the corresponding author. Reader self magazine will not be responsible for that.

18. AGRICULTURE

Agricultural schemes and programmes in India over last five years

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Indian is an agriculture based nation, where more than 50% of population is depend on agriculture. Agriculture is the most important sector of and backbone for Indian Economy. Indian agriculture shares around 18 per cent in India’s gross domestic product (GDP) and provides employment to 50% of the countries workforce (Madhusudhan, 2015). However, income of Indian farmers is low due to shortage of resources and land, climatic factors, pest attack and low price at the time of harvesting (Upadhyaya, 2018). Moreover, farmers commit suicide on crop failure. Therefore, Indian government has launched several schemes and programmes to boost agricultural output and socio-economic status of farmers (Maher, 2018).

Soil health card: This scheme was launched in February 2015. Under this scheme, soil health cards are issued to the farmers for providing information on nutrient status of their soil and recommendations regarding nutrient dosage to be applied.

National Mission for Sustainable Agriculture (NMSA): This mission has been made operational from the year 2014-15 which aims at encouraging sustainable agriculture against climate change by integrated farming, soil health management and synergizing resource conservation in rainfed areas to increase crop productivity.

Neem Coated Urea (NCU): It aims at inhibiting misuse of urea in chemical factories and slowing down the release of nitrogen in soil for better availability to the crop. It reduces the cost of application, cost of cultivation and improves soil health also.

Pradhan Mantri Krishi Sinchai Yojana (PMKSY): It was launched in 2015 along with motto 'Har Khet Ko Paani' by creating water sources and protective irrigation by harnessing rain water at micro level through ‘Jal Sanchay’ and ‘Jal Sinchan’. Per drop-more crop is ensured by micro irrigation.

Paramparagat Krishi Vikas Yojana (PKVY): This scheme was launched in April 2015 which aims at promoting organic farming, improving soil health and organic matter content and increasing net income of the farmer.

National Agriculture Market (e-NAM): This was launched in 2016 with view ‘One Nation One Market’. It facilitates the better price discovery and brings in transparency and competition to enable farmers to get improved remuneration for their produce.

Pradhan Mantri Fasal Bima Yojana (PMFBY): It was introduced in January 2016 to provide financial support to farmers suffering crop loss/damage due to unforeseen events. For this, farmers have to give premium of 2% for Kharif, 1.5% for Rabi and oilseed crops, and 5% for annual commercial/horticultural crops. This scheme is to facilitate prompt claims settlement within two months of harvest.

Conclusion

Over last five years, Indian government has launched several programmes and schemes
from time to time to assist the farmers. These schemes benefit the farmers by improving soil health, reducing cost of cultivation, increasing crop productivity, availability of fertilizers, water management, providing market information, and providing financial assistance on crop damage/failure. Moreover, integration of different schemes will increase the farmer’s income in future.

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19. AGRICULTURE EXTENSION
Role of Information and Communication Technology in Agricultural Extension Process
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Introduction
Modern agricultural education in India has interconnected form relevant to teaching, research and extension functions. Moreover, the link between these three major strands is weak, particularly because extension is still not well recognized as the other two academic functions of the Agricultural Universities (Abrol and Johri, 2005). Technology, such as Information and Communication Technology (ICT) intermediates to strengthen the links between research, teaching and extension. ICTs are the persuasive force for economic, social, political and educational reforms. ICT assisted agriculture focuses on enhancement of agriculture and rural development and includes the use of computers, internet, Remote sensing, Global Positioning System, Geographic Information System, Bio-Informatics, mobile phones, RFID, automated machines, intelligent systems, as well as traditional medium such as radio or TV. Although it is a relatively new phenomenon, evidence of the contribution of ICT to agricultural development and poverty alleviation is becoming increasingly available.

Need of ICT in Agriculture Extension
Farmers require various kind of information at different stages of crop cultivation like best seed for sowing and its available sources for procurement, water management, fertilizer application, pest management, harvesting, post-harvest handling, transportation, packaging, preservation, storage, marketing and insurance. This information must be availed to farmers in user-friendly form, easy to access, cost-effective and must be accurate. With ICTs in picture, these information will be in digital format i.e. photographs, audio, video, process descriptions and many more. Agriculture extension worker’s main job is to transfer information and knowledge rapidly over large distances through communications networks. They need to achieve greater interactivity in communicating, evaluating, producing and sharing useful information and knowledge. In some situations they need to collect feedback and even analysis it. ICT has many potential applications in agricultural extension (Ismail et al., 2013; Zijp, 1994).

Applications of ICT in Agriculture Extension
Web Sites/portals
Farmers Portal (http://farmer.gov.in): This portal provides information for farmers related to various diversified agricultural fields and activities and covers areas like package of practices (POP), agromet advisory, post-harvest information/guidance and risk management. It also
provides advisory pertaining to livestock like Pashu-Palak calendar, small ruminants, fodder, feed analytical rate charges and use of antibiotics in food producing animals. Videos available on this portal also provide valuable information (www.farmer.gov.in, 2019).

e-Krishi Kiran (Soil Health Card Portal) http://shc.gujarat.gov.in: This application provide worth full information about soil nutrients of farmer’s land and also suggest a suitable crop for getting best yield. It is developed by Agriculture & Co-operation Department (Govt. of Gujarat) for farmer’s welfare and has won several awards (Anonymous, 2019).

State government’s websites: The three websites i.e. ikhedut (https://ikhedut.gujarat.gov.in)/ kisankerala (http://www.kisankerala.net/home.jsp)/ mahaagri (http://mahaagri.gov.in/Index.aspx) are very popular sites updated by state government of Gujarat, Kerala and Maharashtra respectively. The two sites i.e. ikhedut and mahaagri are in regional language. These sites provides information like farming practices, schemes and subsidies, market rates, projects and plans, insurance, finance and weather information. Most of these sites provide mobile apps (www.ikhedut.gujarat.gov.in, www.kisankerala.net/home.jsp, www.mahaagri.gov.in/Index.aspx (2019)).

Delhi Kisan Mandi (http://delhikisanmandi.in/): It promoted and supported by Small Farmer’s Agri-Business Consortium (SFAC). Objective of this site is to provide online trading platform to small farmers (www.delhikisanmandi.in, 2019).

Mobile based services

mkisan: It provides a tool for 2 way agricultural extension in which not only information/advisory services are provided to farmers as per his/her need in a broadcast mode (in keeping with selection of crop / agricultural practice, requirements and location) but they can also raise specific queries through Pull SMS in their own language. It’s a integrated portal to ensure proper storage in previous advisories/messages and also effective monitoring at various levels (www.mkisan.gov.in, 2019).

ikhedut Mobile Apps: An android based mobile application developed by Govt. of Gujarat provides updated information regarding package of practices of various crops and livestock management in regional Gujarati language. Farmers can get solutions of their problems by interacting with experts via Farmer’s Agriculture related Query and solution section (www.ikhedut.gujarat.gov.in, 2019).

WhatsApp: A mobile based social networking application has created a revolution and is used by small farmers or their children. Some Subject Matter Specialist (SMS) and KVK’s have created a group of farmers, through which a farmer can share his experience, can raise queries via text, voice or photographs and anybody within group can provide solution. SMS or KVK’s monitor these groups and avail timely updates regarding weather, government policies, diseases - remedies related to a crop and other relevant information.

Television

From many decades programs related to updates in farming practices, advisory and warning are broadcasts by national or regional channels. Regional channel’s programs are telecast in regional language and have concentrated on local region or market. An innovative step has taken by Indian government by starting a 24X7 DORDARSHAN KISSAN channel. Its role is to broadcast current issues and solution, precautionary & AGROMET advisory and market updates of commodities in national language. The channel has been dedicated to agriculture and allied sectors, which disseminates real-time inputs to farmers on new farming techniques, water conservation and organic farming, etc (www.wikipedia.org, 2019).

Kisan Call Centres

The Kisan Call Centres (KCCs) are proving a boon for the farming community. Farmers can dial the toll-free telephone number-1551-to get
specialists, positioned at 84 call centres across the country, to answer a repertoire of questions related to agriculture and allied fields. The KCCs consist of three operational levels: Level One - the basic Call Centre interface - which has a high quality bandwidth and a local language-proficient agriculture graduate; Level Two - this has subject-matter specialists - connected through good bandwidth telecom and computers; and Level Three - the management group that ensures the ultimate response and resolution of all queries not resolved at Level-II (Swaminathan, 2005).

Conclusion
From few decades ICT has been best option for easing the task of imparting knowledge and will definitely be a helping hand for knowledge dissemination pertaining to latest trends and technology for Agricultural Extension. Till last decade, websites was the only prominent ICT tool for supporting Agriculture extension but as usage of mobile has escalated in past few years, new ICT tools like mobile apps and call centres have opened doors to satisfy individual farmer's needs and queries. A dedicated 24x7 television based free to air KISSAN channel allows farmers to update their knowledge at their convenience.

References

20. AGRIBUSINESS MANAGEMENT

Innovative Strategies for Rural Marketing
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Introduction
In India, leaving out a few metropolitan cities, all the districts and industrial townships are connected with rural markets (Jain and Saini, 2012). Typically, a rural market will represent a community in a rural area with a population of 2500 to 30000 (Ashu, 2015). On account of green revolution, the rural areas are consuming a large quantity of industrial and urban manufactured products. The Indian rural market generates about 50 per cent of the country’s gross domestic product (GDP) (Nisha, 2016). In this context, a special marketing strategy, namely, rural marketing has emerged. The rural market in Indian economy generates almost more than half of the country’s income (Kaur, 2015).

The Indian rural market with its vast size and demand base offers great opportunities to marketers (Kaur, 2015; Sirisha, 2016). 'Go Rural' is the slogan given by the marketing guru’s after analyzing the socio-economic changes in Indian villages (Anand and Tyagi, 2017). With the urban markets getting saturated for several categories of consumer goods and with rising rural incomes, marketing executives
are fanning out and discovering the strengths of the large rural markets as they try to enlarge their markets. Marketers and manufactures are increasingly aware of the burgeoning purchasing power, vast size and demand of the consumers. Efforts are now on to understand the attitude of rural consumers (Meenakshi and Takkar, 2015).

**INNOVATIVE RURAL MARKETING STRATEGIES**

**Rural Market segmentation and Targeting:** Market segmentation means divide the market into different segmentation according to homogeneous features of rural consumers. Right segmentation and targeting policies are keys to success in rural market. There are various variable for segmentation rural markets (Arora, 2015).

**People Oriented:**
- Geographical: Region, Density and Climate.
- Demographic: Age, Gender, Marital status.
- Socio-economic: Income, Occupation, Education, Culture.

**Product Oriented:** Brand loyalty, buyer attitude, Personality, Benefit pattern.

**Others:** Land holding pattern, cropping pattern, irrigation facilities, proximity to cities and occupation (labour, farmer, employees etc).

**Product Strategies:** For rural consumers, companies have to make market survey to understand their needs/wants and then choose product strategy. Rural people spend money for basic needs and think twice while purchasing so some of the points must be kept in mind when developing products for rural markets: 1. Product must be in small size packing, 2. It must be simple in design, operate and maintain, 3. Price should be with in economic competence, 4. Innovation in product design, 5. Utility oriented products and 6. Brand name should suit rural background.

**Pricing strategies:** It must be linked with packaging and product strategy for rural segments. Different things like low income level, poor cash flow in rural sector must be kept in mind for decision of fixing price. But it is wrong to presume that rural people always buy cheap or low priced product. But they purchase utility oriented products so low pricing cost saving in packing, competition premium product value are carefully through in case of rural pricing (Arora, 2015).

**Distribution strategy:** Product should be available in rural market through an effective distribution system. The company has to establish an appropriate channel of distribution consisting whole seller, stockiest, village level retailer. The company should extend credit facilities to rural distributor to motivate them to stock and sell in rural areas. Many distribution problems are faced in rural areas like availability of dealers, badly damaged roads, poor communication, banking facilities. Strategies for various regular segments are: agro input dealers, activating co-operative societies, utilizing public distribution stores/petrol pumps, towns as feeder centres (mandis) and potential village linkage.

**Promotion Strategies:** Promotion is the process of marketing communication to inform, persuade, remind and influence consumer to purchase the product. It includes advertising, publicity, personnel selling and seller promotion. There is different way of approach in urban and rural markets for e.g. in urban markets, e-shopping, TV shopping, E-mail, voice mail are main ways of direct selling but in rural markets, there are direct talk with traders, co-operative societies, village fair hoarding in towns/high ways etc. The marketer has to pass message about product through rural media like use of audio-visual van, group meeting huts, melas, wall paintings, direct mailers in regional languages. The salesperson also plays important role in promoting product. In rural market, salesperson has to identify potential market and carry field activities such as house to house/farm to farm visit, group meeting, film shows, product demonstration in village fairs/festivals. In additional, TV, radio, print media, hoarding are other promotional ways in rural markets.

Other strategies in Indian rural markets adopted by different companies:

- Decentralizing rural market by detaching them from urban bases and replacing one-way exploitation.
- Salesman should be selected from educated on employed villagers and trained as stationary salesman.
- Client and location specific promotion.
Joint or co-operative promotion among marketing agencies and client. Brand promotion through rural youth (Arora, 2015).

Analysis
The rural marketing strategies and approaches calls for innovation and substantive changes. The innovation should be carried out within the framework of what can best be characterized as the 4-R principle: Relevance, Reliability, Reach and Reincarnate innovation. If the Indian advertising industry is to reach out to rural India in an effective and efficient manner, it has to be grounded firmly in rural perceptions, value and traditions. It has to immerse itself in local colours, customs and modes of communication in order to make itself relevant to the needs and desires of rural society. It has to gain the trust of the masses by undercutting its own excessive dependency on western styles of advertising, on the one hand and on its use of deceptive and manipulative claims, on the other. It has to reach out to rural consumers and relate to them at an appropriate level, so that it can bring about the desired behavioural changes. Finally, it has to find ways to reincarnate innovation (Kumar and Naruka, 2015).

Conclusion
Rural marketing plays a vital role in the development of country’s economy. Indian rural market is undoubtedly complex but there are some simple truths that we need to accept. The rural consumers are very value-conscious. They may or may not have purchasing power, but they can make a difference to the company’s growth if concentrated. A small increase in rural income, results in an exponential increase in buying power. The growing power of the rural consumer is an opportunity for the companies to flock to the rural markets. The market share of urban market when compared to the rural market is low; hence if business organizations concentrate on rural markets their sales and market share will get increased.

References

21. SOIL SCIENCE

Seaweed - Acts As Soil And Plant Conditioner

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Seaweeds “The Multicellular Marine Macroscopic algae” are one of the most important marine renewable resources of the world with tremendous commercial applications that belong to primitive group of non flowering plant (Thalophyta), which grows largely in
intertidal, shallow and deep sea areas up to 180 m depth, estuaries and backwaters on the solid substrates such as rocks, dead corals and pebbles. The Seaweeds are totally different from higher plants as they neither have true leaves, stems and roots or vascular systems none specialized sex organs. The total seaweed production of the world was increased from 9.7 to 30.4 million tonnes from the year 2001 to 2015 (FAO, 2018). Of all the species, red algae species population was predominantly higher in the world (6500 species) followed by brown algae (1800 species) and green algae (1500 species). In India, 200 species of seaweeds are commonly available as cultivated species with the production of 3203 metric tonnes per year. Among the total (cultivated and wild) species of seaweeds, India possesses 434 species of red seaweeds, 194 species of brown seaweeds and 216 species of green seaweeds. 

Seaweeds are classified into chlorophyceae (green algae), Phaeophyceae (Brown algae) and Rhodophyceae (Red algae) based on their pigmentation. It has been used as manure, cattle feed, food for human consumption and as a source of phyccolloides such as agaragar and carrageenan which are derived from red seaweeds viz., Gelidiella, Gracilaria, Chondrus, Hypnea etc. and alginate from brown seaweeds like Ascophyllum, Laminaria, Turbinaria, Sargassum etc. These products are difficult to synthesize chemically because of the formidable chemical barriers and hence for these commercially important products we have to depend on seaweed resources. The importance of seaweeds for human consumption is well known since 300 BC in China and Japan. These two countries are the major seaweed cultivators, producers and consumers in the world. In the Indian Ocean region countries like Malaysia, Indonesia, Singapore, Thailand, Korea etc., are used the seaweed in salad, jelly, soup etc. In India, however, seaweed consumption is negligible except in the preparation of porridge from Gracilaria sp. and Acanthophora sp. in coastal states of Kerala and Tamil Nadu.

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Apart from the human and animal consumption, it is being used in various purposes like medicines, cosmetics, fuel, waste water treatment process and manures. In recent times, seaweed extract has assumed as natural organic fertilizer because it contains many growth promoting hormones like auxin, gibberellin and cytokinin, macronutrients (N, Na, K, P, Ca, Mg, S and Chlorine), trace elements (Fe, Cu, Zn, Co, Mo, Mn, Ni, Iodine), vitamins (A, Bl, B12, C, D & E), aminoacids etc (Challen and Hemingway, 1965).

**Soil conditioner**

The use of seaweeds as manure in farming practice was very ancient and common practice among the Romans and also practiced in Britain, France, Spain, Japan and China. The use of marine macro algae as fertilizer in crop production has a long tradition in coastal areas all over the world. Seaweed cast continued to be so valuable to farmers in the early 1900’s. In many countries, seaweed and beach cast are still used in both agriculture and horticulture. Generally, the fertilizing effect of composted seaweeds is dependent on their biochemical composition, mineralization pattern and the synchronization of the mineralization of nutrients with the crops demand. The fresh seaweed contains equal amount of nitrogen, higher amount of potassium and lower level of phosphorus as compared to farm yard manure. Production of 500 million tons of seaweed would absorb 135 million tons of carbon, about 3.2% of the carbon added annually to seawater from greenhouse gas emissions, offering the potential of using carbon credits to improve the profitability of seaweed businesses.

Mostly brown seaweeds species viz., Ascophyllum, Ecklonia and Fucus are readily available in large quantities and sold as soil additives and function as both fertilizer and soil conditioner. The chemical constituent of brown seaweed ie., Alginic acid acts as a soil conditioner that improves water-holding characteristics of the soil and helps formation of crumb structure by developing the high molecular weight polymer due to the cross linkage between alginic acid and metallic radicals of the soil. This in turn leads to better aeration and capillary action, and these stimulate the root systems of plants to further growth, and so stimulate the soil bacteria to greater activity. The substances secreted by soil bacteria in the presence of seaweed include organic chemicals known as polyuronides. Polyuronides are chemically similar to the soil conditioner alginic acid and they have soil-
stabilizing properties. Application of Ascophyllum species are highly preventing the top soil loss and making the steep slopes for cultivation. Maerl is a fertilizer mainly used to neutralize acid soils, as a substitute for agricultural lime which are derived from red seaweeds viz., Phymatolithon calcareum and Lithothamnion corallioides that grow with a crust of calcium carbonate at depths of 1-7 m and mostly found on the coast of France, where the water temperature must be 13°C or higher Agricultural Research Council’s unit of soil metabolism reported in 1947 that 0.1 of a gram of sodium alginate added to 100 grams of soil increased its water-holding power by 11 per cent.

**Plant conditioner**

Seaweed-derived liquid extracts have been extensively used as Seaweed Liquid Fertilizer (SLF) by foliar application for enhancing the nutrient absorption efficiency in plants. The nutrients are not leached down in to the soil but are available to the plant through leaf openings such as lenticels, hydathods and stomata. Leaves absorb nutrients within 10 to 15 minutes of its application. Seaweed extract products are more beneficial for crops because of the presence of macronutrients, trace elements, vitamins, antibiotics, amino acids polysaccharides (fucoidan, alginic acid, carrageenan and agar) and natural plant growth hormones (cytokinin, auxin, gibberellins) as well as other plant biostimulants (betaines, polyamines, oligosaccharides), that improve plant growth and yield, resistance and tolerance to environmental disease and insect stresses. Seaweed, when applied to plants as a foliar spray, can increase the rate of cell division and elongation in those plants or when applied to the soil as a meal or used as a root dip, the hormones plays well to increase the root growth and germination. So, the seaweed liquid fertilizes can be applied either to the soil or leaf or seed to stimulate the germination, improve growth, induce resistance to frost, fungal and insects attack, reduce red spider, aphid and nematode infestation and increase nutrient uptake from soil (Mooney and Van Staden, 1986) thereby increasing the crop yield.

Recent researches proved that seaweed fertilizers are better than other fertilizers since they are very economic and ecofriendly (Gandhiappan and Perumal, 2001). For a year long ago, seaweed extracts available commercially worldwide. Many brands of seaweed liquid fertilizers like Maxicrop (UK), Kelpak 66 (South Africa), Seagrow (New Zealand), Algifert (Norway), Plantozyme, Shaktizyme (India) etc are available in the market.

**References**


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**22. AGRICULTURAL**

**Alternative Splicing (AS) Mechanism**

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Splicing is a post-transcriptional modified mechanism, which generates mature RNA transcripts from intron containing eukaryotic genes in multiple ways. It has evolved from simple self-splicing introns (reminiscent of an early RNA world) to a highly complex ribonucleo protein (RNP) machine, the spliceosome. Most constitutively spliced genes are also alternatively spliced in response to developmental/environmental cues to enhance...
transcriptome and proteome diversity for different functions. In recent years, the availability of high-throughput RNA-Seq datasets coupled with refined bioinformatics predictions have enhanced understanding about its prevalence and importance in animals and plants (Reddy et al., 2012).

Alternative splicing events such as intron retention (IR), exon skipping (ES), alternative splice site recognition (5´ASS and 3´ASS) and mutually exclusive exons (MEE) generate RNA/protein isoforms with altered stability, activity and cellular localization (Reddy et al., 2013). Alternative splicing events are not equally prevalent among organisms; IR events are most predominant in plants, contributing to ~40% of their total (Syed et al., 2012).

IR isoforms are generally subject to nonsense-mediated decay (NMD), a cytosolic decay pathway, but in plants they often escape this fate suggesting a different mode of regulation and function. Such new functional modes of IR transcripts have been reported in gametophyte development (Boothby et al., 2013) and neurogenesis (Yap et al., 2012), and these reports also suggest the importance of IR-type alternative splicing events in development and stress responses.

Alternative splicing is always operative, and produces several RNA/protein isoforms with functional significance in a diverse array of cellular functions. It is affected by developmental and environmental cues, and is important for cellular responses in such conditions. Ling et al. (2018) showed that heat-stress priming enables plants to ‘remember’ constitutive splicing patterns right after relief from second or recurring exposure to heat stress and generate correct transcripts/ proteins that ensure survival.

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23. AGRICULTURAL ECONOMICS

**Green Tax and their Regulation in India**

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**Introduction:** Green Tax is an excise duty on goods that cause environmental pollutants. Green tax is an environmental tax that aims at ensuring that polluters are duly punished for the activities that deter the environment by charging them a penalty for the harm caused to others. According to Economic theory, charging taxes on emissions that cause pollution will lower environmental impairment in a cost-effective manner by encouraging behavioural changes in households and firms that need to decrease their pollution. It is also called as *pollution tax or environmental tax*. Green tax is imposed either in direct or indirect terms.

*Direct green taxes* imposes on perpetrators of GHGs emissions is an economical means to lower their pollution to the extent where further reduction could potentially turn out to be more expensive than paying the tax itself.

*Indirect green taxes* like taxes on alternative policies or related goods such as authorized technology standards have the potential to reduce pollution, but the costs involved may be rather high.

**Why does impose Green Tax:** This tax is in a way levied to discourage people from using old vehicles as they are not as per upgraded pollution standards. The collected tax will be a part of the state’s fund. It will be used for developing infrastructure. They are useful to achieve a range of goals, which can be broken down into three broad categories:

- **Environmental benefits:** Everyone must make a conscious effort to reduce pollution. Servicing your vehicle on a regular basis, carpooling, using public transport, etc. can help towards reducing the impact of your vehicle on the environment.
- **Raising fiscal revenues and increasing fiscal efficiency**
- **Encouraging economic growth, innovation and job creation.**

**Who are Negatively Impacted by an Environmental Tax:** A wide cross-section of the economy is impacted by the imposition of an environment tax. In general, the adverse effects of pollution, such as climate changes, impaired health and noxious odours are forms of an impaired environment borne by everyone whether or not they contribute towards pollution. Governments/Revenue Authorities—may not necessarily benefit from the imposition of such a tax because imposition amount is less than environmental improvement cost.

**United Nations Framework Convention on Climate Change:** In response to the global warming crisis, in Rio de Janeiro of Brazil, the 1992 UN Conference on the Environmental and Development clearly raised the concept of “sustainable development”. Through this conference more
than 150 countries had established “United Nations Framework Convention on Climate Change”. UNFCCC is the first convention to take full control of greenhouse gas emissions including CO₂ discharge, and is an international convention to fight global warming which causing a lot of and adverse effect to the development of society and economy. In December 1997, the third Conference of the Parties (COP) under the UNFCCC held in Kyoto of Japan, which aimed at limiting carbon emissions in developed countries. The conference ended with an agreement of “Kyoto Protocol”. India comes under the third category of signatories to UNFCCC. India signed and ratified the Protocol in August, 2002 and has emerged as a world leader in reduction of greenhouse gases by adopting Clean Development Mechanisms (CDMs) in the past few years. The Protocol took formal effect in February 16th 2005. Presently 192 parties are signed to UNFCCC. “Kyoto Protocol” is internationally binding and enforceable agreements that will encourage countries to reduce greenhouse gas emissions. Kyoto Protocol worked out three mechanisms of the energy conservation and emission reduction:

1. **Clean Development Mechanism (CDM):** Under the UNFCCC, charter any company from the developed world can tie up with a company in the developing country that is a signatory to the Kyoto Protocol. These companies in developing countries must adopt newer technologies, emitting lesser gases, and save energy. Only a portion of the total earnings of carbon credits of the company can be transferred to the company of the developed countries under CDM. There is a fixed quota on buying of credit by companies in Europe.

2. **Joint Implementation (JI):** It allows a country with an emission reduction or limitation commitment under the Kyoto Protocol (Annex B Party) to earn emission reduction units (ERUs) from an emission-reduction or emission removal project in another Annex B Party, each equivalent to one tone of CO₂, which can be counted towards meeting its Kyoto target. Joint implementation offers Parties a flexible and cost-efficient means of fulfilling a part of their Kyoto commitments, while the host Party benefits from foreign investment and technology transfer.

3. **Emissions Trade (ET):** Parties with commitments under the Kyoto Protocol (Annex B Parties) have accepted targets for limiting or reducing emissions. These targets are expressed as levels of allowed emissions, or “assigned amounts,” over the 2008-2012 commitment periods. The allowed emissions are divided into “assigned Amount Units” (AAUs). Emissions trading, as set out in Article 17 of the Kyoto Protocol, allows countries that have emission units to spare – emissions permitted them but not "used" - to sell this excess capacity to countries that are over their targets.

**Green Tax Regulation Acts in India:**
The Department of Environment was established in India in the year 1980 to ensure a healthy environment for the country. Environmental Protection Act, 1986, which came into force soon after Bhopal Gas Tragedy. The Water (Prevention and Control of Pollution Act-1974, the Air (Prevention and Control of Pollution) Act-1981 and the Environment Protection Act-1986 were came into force in the country. In Indian context, various types of taxes are imposing central and state government on environment affecting goods and services.

1. **Carbon Tax:** A carbon tax is a tax levied on the carbon content of fuels. It is a form of carbon pricing. Carbon is present in every hydrocarbon fuel (coal, petroleum, and natural gas) and converted to carbon dioxide (CO₂) and other products when combusted. On July 1, 2010, India introduced a nationwide carbon tax.

2. **Fuel tax:** A fuel tax is an excise tax imposed on the sale of fuel. In most countries the fuel tax is imposed on fuels which are intended for transportation. Fuels used to power agricultural vehicles, and/or home heating oil which is similar to diesel are taxed at a different, usually lower rate. The fuel tax is used as an ecotax, to promote ecological sustainability. In India, the pricing of fuel varies by state, though central taxes still are part of the pump price of fuel.

3. **Pollution Tax:** Ecological taxation or ecotax is a tax levied on activities which are
considered to be harmful to the environment and is intended to promote environmentally friendly activities via economic incentives. Ecotaxes are examples of Pigouvian taxes.

4. **Land/location value tax:** It is an ad-valorem levy on the unimproved value of land. A land value tax is generally favored by economists as (unlike other taxes) it does not cause economic inefficiency, and it tends to reduce inequality. This tax burden falls on titleholders in proportion to the value of locations, the ownership of which is highly correlated with overall wealth and income. Land value taxation is not currently implemented in India.

Green Activism through Green Cess Levied in States of India:

<table>
<thead>
<tr>
<th>State/Union Territory</th>
<th>Green Initiatives</th>
<th>Date of Effective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delhi</td>
<td>Delhi &amp; Green Cess</td>
<td>November 1, 2015</td>
</tr>
<tr>
<td>Gujarat</td>
<td>Go Green Gujarat</td>
<td>2011-12</td>
</tr>
<tr>
<td>Himachal Pradesh</td>
<td>Green Cess levied in Hill Station in HP</td>
<td></td>
</tr>
</tbody>
</table>

Examples of Carbon Trading in India:

1. **Jindal Vijaynagar Steel** is selling $225 million worth of saved carbon. This was made possible since their steel plant uses the Corex furnace technology which prevents 15 million tones of carbon from being discharged into the atmosphere.

2. **Powerguda village in Andhra Pradesh** is selling 147 tonnes equivalent of saved carbon dioxide credits. The company has made a claim of having saved 147 MT of CO₂. This was done by extracting bio-diesel from 4500 Pongamia trees in their village.

3. **Handia Forest in Madhya Pradesh** is estimated that 95 very poor rural villages would jointly earn at least US$300,000 every year from carbon payments by restoring 10,000 hectares of degraded community forests.

**Conclusion**

Green economy is one in which policies and innovations enable society to generate more of value each year, while maintaining the natural systems that sustain us. Clearly, it will require technological innovation. But it requires lots of other changes too to the way we organize businesses; the way that we design cities; the way we move people and goods around; the way we live, essentially. Effecting changes of this sort requires the engagement of all sectors, including policymakers, businesses and individual citizens. And that in turn implies the need for a mass of information to guide and inform decision-making.

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24. **PLANT PHYSIOLOGY**

**Reactive Nitrogen Species (RNS) production in plants and its effects**

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**Introduction**

The term Reactive Nitrogen Species (RNS) includes radicles like nitric oxide (NO⁻) and nitric dioxide (NO₂⁻) and nitrate radicle (NO₃⁻), as well as non-radicals such as nitrous oxide.
(HNO₂), nitrosonium cation (NO⁺), nitroxyl anion (NO⁻), peroxynitrite (ONOO⁻), dinitrogen trioxide (N₂O₃), and dinitrogen tetroxide (N₂O₄). NO⁻ has an important function as a key signalling molecule in plant growth, development, and senescence. RNS like ROS (Reactive Oxygen Species) also play an important role as signalling molecules at low concentration for the regulation of growth and development and for defence in response to environmental (abiotic stress).

Table 1: Main reactive nitrogen species (RNS)

<table>
<thead>
<tr>
<th>Source</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell organelles</td>
<td>Mitochondria NO₂⁻</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td>L-Arg + NOS cofactors</td>
</tr>
<tr>
<td>chloroplasts</td>
<td>NO⁻ and L-Arg</td>
</tr>
</tbody>
</table>


The use of NO by plants observed by Fewson and Nicholas (1960) and the emission of NO by plants has been described by Klepper (1979), in soybean plants treated with herbicides. Delledonne et al. (1998) pointed to a new function of NO in plants which, until then, had not been considered. They postulated the role of nitric oxide as a signal molecule in plant defence (or resistance) to bacterial infection.

In plants, there are several potential sources of NO including enzymatic and non-enzymatic systems. In plants there is little information on sub-cellular sites where NO is produced the presence of NOS activity in peroxisomes was first demonstrated in plant tissues, besides, peroxysomes other cell organelles where the generation of NO has been clearly demonstrated are mitochondria and chloroplast.

Table 2: Main sources of NO in plants

<table>
<thead>
<tr>
<th>Source</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-enzymatic</td>
<td>Acid pH (ASC)</td>
</tr>
<tr>
<td>Enzymatic</td>
<td>NO₂⁻ + NADH</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>L-Arg + NOS cofactors</td>
</tr>
<tr>
<td>NOS-like activity</td>
<td>Hydroxyurea + H₂O₂</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>NO₂⁻ + reduced Cyt-c</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td></td>
</tr>
</tbody>
</table>

(Source: Corpas et al., 2009. New Phytologist. 184: 9-14.)

Nitric oxide has an important function as an inter- and intra-cellular signalling molecule in plant growth and development. Apparently, NO regulates different processes by including gene transcription or by activating secondary messengers. NO has multiple functions in different plant physiological and pathological processes and mainly include seed germination, pollen tube growth, cell wall lignification, root organogenesis, establishment and functioning of legume-Rhizobium symbiosis, flowering, fruit ripening and senescence and biotic and abiotic stresses.

Signalling function of RNS in plants under physiological and environmental stress conditions:

NO is a key mediator, in co-operation with ROS, in the defence response to pathogen attacks in plants. The response of the rapid production of NO and ROS unchain a designated programme cell death (PCD) process. In this process, both NO and ROS plays a key function. PCD is an important mechanism to regulate aspects of growth and development, as well as to eliminate damaged or infected cells during responses to environmental stresses and pathogen attacks (Wang et al., 2013)

NO in presence of O₂ can react with reduced glutathione (GSH), by an s-nitrosylation reaction, to form S-nitrosoglutathione (GSNO) which is an important mobile reservoir of NO bioactivity in plants. The RNS peroxynitrite (ONOO⁻) is a powerful oxidant/nitrating species, formed by the rapid reaction between O₂⁻ and NO (Radi, 2013) and its occurrence in plant organelles like peroxisomes are reported by Corpas and Barroso, 2014.

Presence of NO and GSNO in plant tissues can cause the generation of ONOO⁻, post-translational modifications (PTMs) can also takes place, such as S-nitrosylation and nitration of proteins. S-nitrosylation inturn in peroxisomes inhibit the activity of catalase and glycolate oxidase activity, this could regulate the key
signalling molecules like H₂O₂.

Generation of ONOO⁻ can produce tyrosine nitration of plant proteins and leads to nitrosative damage in plant cells.

**FIGURE 1:** Post-translation modifications (PTMs) mediated by nitric oxide in plant cells.
(Source: Corpas et al., 2015. RNS signalling and communication in plants. Berlin: 267-281.)

**Conclusion**

The subtle balance between the rates of production and scavenging/processing by antioxidant defences/NO metabolism regulates the accumulation of NO in particular cellular location at a given time. The tempo-spatial accumulation of NO is considered as important in conferring specificity to the signals underpinning plant responses to a given stimulus. However, the mechanisms by which cellular stress-perception systems regulate the signalling micro-environments and form discrete niches for specific NO production remain largely unsolved.

**References:**


25. GENETICS AND PLANT BREEDING

Allele Mining for Crop Improvement

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Allele Mining

Searching for different alleles. Allele - alternative forms of a gene found at a given locus on a chromosome. Mining – Searching the
new alleles in the wild germplasm. It is often referred to as 'dissection of naturally occurring variation at candidate genes/loci' or simply 'allele mining'. Identification and access to allelic variation that affects the plant phenotype is of the utmost importance for the utilization of genetic resources, such as in plant variety development. Considering the huge numbers of accessions that are held collectively by gene banks, genetic resources collections are deemed to harbor a wealth of undisclosed allelic variants. The challenge is how to unlock this variation. Allele mining is a research field aimed at identifying allelic variation of relevant traits within genetic resources collections.

**Importance of allele mining**

It helps in tracing the evolution of alleles and identification of new haplotypes for the development of allele-specific markers for use in marker-assisted selection (MAS). This capability will be important for giving breeders direct access to alleles conferring:

- Resistance to biotic stresses
- Tolerance to abiotic stresses
- Greater nutrient use efficiency
- Enhanced yield and improved quality

It can also provide insight into molecular basis of novel trait variations and identify the nucleotide sequence changes associated with superior alleles.
Strategies for allele mining

There are two approaches available for allele mining i.e., modified tilling procedure called ecotilling based allele mining and sequencing based allele mining.

1. Modified Tilling procedures called Eco-Tilling based allele mining

TILLING (Targeting Induced Local Lesions IN Genomes) is a technique that can identify single base-pair allelic variation in target gene (more specifically induced point mutations) while Eco-Tilling technique detects natural mutation. It allows the rapid detection of variation in many individuals and is cost effective because only one individual for each haplotype need to be sequenced.

2. PCR-based or Sequencing-based allele mining

Sequencing based allele mining involves amplification of alleles in several genotypes through PCR followed by identification of nucleotide variation by DNA sequencing techniques. It helps to analyze individual haplotype structure and diversity and such analysis is expected to simplify genetic association studies in plants. This technique does not require much sophisticated equipment or involve tedious steps (Ramkumar et al., 2010).

Different steps involved in two approaches (Eco-Tilling and sequencing) of allele mining

Bioinformatic tools required for allele mining

Allele mining requires various sophisticated bioinformatic tools namely PLACE, plantCARE, TRANSFAC, JASPAR, MEME, Plantprom DB, DCPD, SCPD, BioEdit, ClustalW etc. These tools are useful for sequence alignment in order to compare new genome sequence to reference genome i.e., sequenced genome data (Amaranatha Reddy et al., 2014).

Applications of allele mining

Allele mining can be effectively used for gene prediction, expression study, evolutionary study, identification of new haplotypes, discovery of superior alleles, similarity analysis-inter and intra species and functional molecular marker development for MAS (Sarika et al., 2014).
Status of allele mining in crop plants

<table>
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<th>Trait/name of the protein</th>
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<td>Rye</td>
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<td>Mal d 3</td>
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<td>Apricot</td>
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<td>Blast resistance</td>
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References

26. PLANT BREEDING AND GENETICS

Utilisation of Self - Incompatibility in Plant Breeding

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The exploitation of heterosis in forms of hybrid varieties is one of the major achievement of plant breeding. Fertility regulating mechanisms are the first and foremost requisite for commercial exploitation of heterosis. Control of reproductive behaviour is pivotal in developing new cultivars, seed multiplication and maintenance of genotypic integration as well as varietal purity during course of cultivation. Thus, it is necessary to clearly understand the pollination behaviour of plants and different mechanism for regulation of fertility so that it could be better utilized in plant breeding. The technique of emasculation and bagging emerged as a simple and basic method for fertility regulation but it is time consuming, labour intensive and cost effective. Besides, in plants with small and numerous bisexual flowers, the manual emasculation is even impractical. In this regard, self-incompatibility
qualifies as a better and more systematic alternative for hybrid seed production.

Self-incompatibility (SI) is a widespread mechanism of fertility regulation in flowering plants that prevents autogamy and promotes allogamy thus assisting in achieving hybridisation in otherwise small and bisexual flowers in which manual emasculation is a herculean task. It was first reported in *Verbascum phoeniceum* by Koelreuter in the middle of 18th century. Thisterm was coined by Stout in 1917 and he categorized it in two major categories of heteromorphic and homomorphic self-incompatibility on the basis of flower morphology. The SI event is governed through a chain of bio-chemical reactions and complex cellular interactions between the pollen and stigmatic surface. Heteromorphic system (difference in floral morphology) primarily includes distyly (*Primula* spp.) and tristyly (*Lathyrus salicaria*) while the homomorphic system (physiological or genetic causes of SI rather than differences in floral morphology) again classified into gametophytic system and sporophytic system which are genetically controlled by multi-allelic S-loci. Gametophytic self-incompatibility (GSI) is controlled by the genetic constitution of pollen and have been reported in potato, tomato, red clover, white clover, rye, etc. GSI is governed by either a single gene (monofactorial) or two gene (bifactorial) with multiple alleles that show individual action in the style which inhibit growth of pollen tube in the style or ovary. As against to GSI, sporophytic self-incompatibility (SSI) is governed by the genotype of pollen producing plant i.e. sporophyte. The SSI system was first discovered independently by Hughes and Babcock (1950) in *Crepis foetida* and Gerstel (1950) in *Parthenium argentatum* (Guayule). The sporophytic self-incompatibility system has been reported in radish, cabbage, cauliflower, etc.

**Utilization in Plant Breeding:**

Self incompatibility has great significance in plant breeding. It is used in hybrid seed production while assisting cross pollination. GSI is used for hybrid seed production in *Medicago sativa* (lucerne) and *Trifolium* spp. etc. while SSI is used for hybrid seed production in cabbage, Brussel sprouts and other *Brassica* spp. etc. Two self-incompatible lines are planted in alternate rows for hybrid seed production. The harvest from both lines would be hybrid seed as shown below.

![Diagram of hybrid seed production](image)

**Maintenance and crossing of Self-incompatible lines**

Maintenance of self-compatible lines is achieved by using various techniques that temporarily overcome the barrier of self-incompatibility like bud pollination, shortening the styles exposing either the plants or the styles to high or low temperatures, spray of chemicals etc. These treated plants are allowed to be selfed and thus maintained. These lines are used in various breeding programmes viz. single cross, three-way cross or double cross as and when required.

In horticultural crops it is used in commercial production of seedless pineapple and grapes. In Japan most of the grown cruciferous vegetables are F1 hybrid varieties developed using self-incompatibility. A F1 hybrid variety of Chinese cabbage named “Nagaoka Kohai I Go” was produced by Shojiro Ito. In radish F1 hybrid variety, “Harumaki Minowase”, was produced by a commercial seed company (Agronomist seed, Genetics). In radish, many F1 hybrids are developed using SI namely KGMR-1, H-43, H-44 and H-46. In cauliflower, Pusa Hybrid -2 and Pusa Kartik Sankar were developed by IARI. In *B. napus* ‘271’ and S-1300 can be successfully used for producing three-way hybrids (Ma et al., 1998). The interspecific hybridization between *E. grandis* and *E. urophylla* clones was achieved using self-incompatibility found in
a *Eucalyptus grandis* which yield about 95.9% outcrossed seeds (Junghans *et al.*, 1998). Use of self-incompatibility is a noble technique in plant breeding which allows for imposition of characteristic reproductive system on a plant, thus gaining special recognition in heterotic breeding where it ensures high degree of assisted cross pollination for better yield of outcrossed seeds.

**Sources of information**


Image Source: Google images


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**27. BIOTECHNOLOGY**

**Introduction to polymerase chain reaction (PCR)**

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Amplification of DNA by PCR is even faster than cancer cells, which are known for their high reproductive rates. PCR can make a biological sample with trace amounts of DNA in it. We want to work with the DNA, perhaps characterize it by sequencing, but there isn’t much to work with. This is where PCR comes in. PCR is the amplification of a small amount of DNA into a larger amount. It is quick, easy, and automated. Larger amounts of DNA mean more accurate and reliable results for later techniques.

The technique was developed by Nobel laureate biochemist Kary Mullis in 1984 and is based on the discovery of the biological activity at high temperatures of DNA polymerases found in thermophiles (bacteria that live in hot springs). Most DNA polymerases (enzymes that make new DNA) work only at low temperatures. But at low temperatures, DNA is tightly coiled, so the polymerases don’t stand much of a chance of getting at most parts of the molecules.

But these thermophile DNA polymerases work even at 100 °C, a temperature at which DNA is denatured (in linear form). This thermophilic DNA polymerase is called Taq polymerase, named after *Thermus aquaticus*, the bacteria it is derived from. Taq polymerase, however, has no proofreading ability. Other thermally stable polymerases, such as Vent and Pfu, have been discovered to both work for PCR and to proofread.

Four things to perform PCR on a sample:

1. **The target sample.** This is the biological sample you want to amplify DNA from.
2. **A primer.** Short strands of DNA that adhere to the target segment. They identify the portion of DNA to be multiplied and provide a starting place for replication.
3. **Taq polymerase.** This is the enzyme that is in charge of replicating DNA. This is the polymerase part of the name polymerase chain reaction.
4. **Nucleotides.** You’ll need to add nucleotides (dNTPs) so the DNA polymerase has building blocks to work with.

There are three major steps to PCR and they are repeated over and over again, usually 25 to 75 times. This is where the automation is most appreciated.
1. Denaturation of template DNA:
Target DNA sample is heated. This denatures the DNA, unwinding it and breaking the bonds that hold together the two strands of the DNA molecule, leaving you with single stranded DNA (ssDNA).

2. Annealing of Primer
Temperature is reduced and the primer is added. The primer molecules now have the opportunity to bind (anneal) to the pieces of ssDNA. This labels the portions of DNA to be amplified and provides a starting place for replication.

3. Extension (Synthesis of new strands)
In this step, new pieces of ssDNA are made. Taq polymerase catalyzes the generation of new pieces of ssDNA that are complimentary to the portions marked by the primers. The job of Taq polymerase is to move along the strand of DNA and use it as a template for assembling a new stand that is complimentary to the template. This is the chain reaction in the name polymerase chain reaction. PCR is so efficient because it multiplies the DNA exponentially for each of the 25 to 75 cycles. A cycle takes only a minute or so and each new segment of DNA that is made can serve as a template for new ones.

Perhaps the most important thing to remember is to be very aware of contamination. If, for example, we unknowingly slough off a piece of skin into our sample, then our DNA may be amplified in the PCR reaction. Here are some other factors to optimize results with PCR:

i. Annealing temperature.
Starts at the low end of what we think will work, then move up as necessary. If the temperature is too low, the primers will make more mistakes and we'll get too many bands when we run our sample on a gel. If the temperature is too high it will not give results and gel will be blank. An annealing temperature should be about 3°C to 5°C below the melting temperature (Tm). A rough formula for determining Tm is Tm=4(G+C)+2(A+T).

ii. Magnesium concentration.
You want your Mg²⁺ concentration to be about 1.5mM to 3mM. If you go too high, the polymerase will make more mistakes.

iii. Think carefully about primer design.
Both primers should have approximately the same Tm so they both anneal at the same temperature. Two out of three bases on the 3’ end should be G or C to get good hybridization (G and C have three H-bonds so you get better polymerization). Lastly, avoid primer dimers, which occur when the primers have ends that will anneal to each other. This will produce no product.

iv. More is not necessarily better.
More polymerase produces more nonspecific product, so don't just carelessly dump in a bunch of polymerase. Additionally, PCR reactions don't work if there is too much DNA.
28. PLANT PATHOLOGY

Immunoglobulins- Structure and Function of Various Domains and Hybridoma Technology

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Introduction:
The most important and widely used methods for assay, detection and diagnosis depend on the surface properties of viral proteins. For most plant viruses, this means the protein or proteins that make up the viral coat. Different procedures may use the protein in the intact virus, the protein subunits from disrupted virus or proteins expressed from cloned cDNA or DNA in a system such as Escherichia coli or insect cells. More recently non-structural proteins coded for by a virus have been used in diagnosis.

Serological procedures are based on the interaction between a protein or proteins (termed the antigen) in the pathogen with antibodies raised against them in a vertebrate. The theories of the immune response and the practical applications of serology have been reviewed by Harlow and Lane (1988), Hampton et al. (1990) and van Regenmortel and Dubs (1993).

Immunoglobulins:
The term 'immunoglobulin' is often used interchangeably with 'antibody'. However, strictly an antibody is a molecule that binds to a known antigen, whereas immunoglobulin refers to this group of proteins irrespective or not of whether their binding target is known. Antibodies are secreted by B lymphocytes. They are a large family of glycoproteins that share key structural and functional features. Structurally they are composed of one or more copies of a characteristic unit that can be visualized as forming a Y shape (Fig.1).

Each Y contains four polypeptides:
Two identical copies of the heavy chain.
Two identical copies of the light chain joined by disulphide bonds.

Classes of antibodies:
IgG, IgM, IgA, IgD and IgE

IgG molecules have three protein Domains (Fig.2):
Two of the domains, forming the arms of
the Y, are identical and are termed the Fab domain. They each contain an antigen-binding site at the end, making the IgG molecule bivalent.

The third domain, the Fc domain, forms the stem of the Y.

The three domains may be separated from one another by cleavage with the protease papain. The Fc region binds protein A, a protein obtained from the cell wall of *Staphylococcus aureus*, with very high affinity. This property is used in several serological procedures.

**IgG:**

It is the most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules and exist in monomers.

- 75% of serum Ig is IgG.
- Molecular weight of 1,50,000-1,60,000.
- Contains 2-4% carbohydrates.
- Lowest electrophoretic mobility.
- Major Ig in vascular spaces.
- It is the only class of Ig that crosses the placenta.

**IgM:**

IgM normally exist as pentamer but it can also exist as monomers.

- Most protein and amino acid sequence has not yet been determined.
- 576 aminoacids and has a molecular weight 9,50,000.
- First antibody synthesized by a newborn animal or humanbeing.
- Divided into daughter cells, which produce IgG.
- Promotes phagocytosis of microorganisms by macrophages and polymorphonuclear leukocytes.
- Donot cross placenta.

**IgA:**

Serum IgA is a monomer but IgA found in secretions is a dimer.

- Molecular weight of between 1,80,000 and 4,00,000.
- Higher carbohydrate content of 5-10%.
- Found in higher concentration in bloods.
- Cannot cross the placenta.

**IgD:**

Exist only as monomer.

- No antibody activity is attributed.
- Low levels in serum.
- Found on B cell surfaces where it functions as a receptor for antigen.
- Does not bind complement.

**IgE:**

Exist as monomer.

- Extra domain in the constant region.
- Appears in serum at very low concentrations.
- Molecular weight of 1,90,000.
- Allergic diseases show high IgE concentrations.

**General functions of immunoglobulins:**

**Antigen binding**

Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host. The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

**Effector Functions**

Frequently the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary “effector functions” of antibodies. The immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions. Such effector functions include:

- **Fixation of complement** - This results in lysis of cells and release of biologically active molecules.
- **Binding to various cell types** - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some
function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the fetus and newborn.

Hybridoma Technology:

Hybridoma technology is a technology of forming hybrid cell lines (called Hybridoma) by fusing a specific antibody-producing B cell with a myeloma (B cell cancer) cell that is selected for its ability to grow in tissue culture and for an absence of antibody chain synthesis. The antibodies produced by the hybridoma are all of a single specificity and are therefore monoclonal antibodies (Kohler and Milstein, 1975).

What are monoclonal antibodies?

Monoclonal antibody (MAb) is a single type of antibody that is directed against a specific antigenic determinant (epitope). Monoclonal antibodies are specific to antigen and are homogenous.

Steps involved in production of monoclonal antibodies:

- **Immunization**
- **Cell fusion**
- **Selection of hybridomas**
- **Screening the products**
- **Cloning and propagation**
- **Characterization and storage**

**Immunization:**

Immunize an animal usually mouse by injecting with an appropriate antigen along with Freund's complete or incomplete adjuvant.

Adjuvants are non specific potentiators of specific immune responses.

Injections of antigens at multiple sites are repeated several times for increased stimulation of antibodies.

3 days prior to killing of animal a final dose is given intravenously.

Spleen is aseptically removed and disrupted by mechanical or enzymatic methods to release the cells.

By density gradient centrifugation lymphocytes are separated from rest of the cells.

**Cell fusion:**

Lymphocytes are mixed with HGPRT deficient myeloma cells and are exposed to PEG for a short period.

The mixture is then washed and kept in a fresh medium.

The mixture contains hybridomas, free myeloma cells, and free lymphocytes.

**Selection of hybridomas:**

The above mixture is cultured in HAT medium for 7-10 days.

Due to lack of HGPRT enzyme in myeloma cells, salvage pathway is not operative and aminopterin in HAT medium blocks the de novo synthesis of nucleotides. Hence free myeloma cells are dead.

As the lymphocytes are short lived they also slowly disappear.

Only the hybridoma that receives HGPRT from lymphocytes are survived.

Thus hybridomas are selected by using HAT medium.

Suspension is diluted so that each aliquot contains one cell each. These are cultured in regular culture medium, produced desired antibody.

**Screening the products:**

Screening is done for antibody specificity.

For this we need to test the culture medium from each hybridoma culture for desired antibody specificity.

Common test like ELISA is used for this.

In these tests the antigens are coated to plastic plates.

The antibodies specific to the antigens bind to the plates. The remaining are washed off.

Thus the hybridomas producing desired antibodies are identified.

The antibodies secreted by them are homogenous and specific and are referred as monoclonal antibodies.

**Cloning and propagation:**

The single hybrid cells producing the desired antibody are isolated and cloned.

Usually two techniques are commonly employed for this:

- Limiting dilution method: Suspension
of hybridoma cells is serially diluted so the aliquot of each dilution is having one hybrid cell. This ensures that the antibody produced is monoclonal.

- Soft agar method: In this method the hybridoma cells are grown in soft agar. These form colonies and the colonies are monoclonal in nature.

**Characterization and storage:**

Biochemical and biophysical characterization are made for desired specificity. It is important to note the monoclonal antibody is specific for which antigen. MAbs must be characterized for their ability to withstand freezing and thawing.

**Large scale production:**

Encapsulating the hybridoma cells in alginate gels and using a coating solution containing poly-lysine is employed. These gels allow the nutrients to enter in and antibodies to come out.

Damon biotech and cell-tech companies are using this technique for commercial production of MAb. They employ 100-litres fermenters to yield about 100g of MAb in about 2 weeks period.

**Advantages of monoclonal antibodies:**

They are homogenous in nature.

They are specific to a particular antigen with a particular epitope.

**Limitations**

As they are specific to a particular antigen, they cannot distinguish molecule as a whole.

Mice used in MAb production carry adenovirus, Hepatic virus, Retrovirus, reovirus, cytomegalovirus, thymic virus.

The presence of some of these viruses is detected in hybridomas. This poses a great danger since there is no guarantee for MAb produced is totally virus free.

For this reason US food and drug administration insists that MAb for human use should be totally free from all pathogenic organisms including viruses.

**References:**


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**29. PLANT BREEDING AND GENETICS**

**Bulked Segregant Analysis: An effective approach for QTL mapping**

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**Introduction**

Bulked segregant analysis (BSA) is a technique used to identify genetic markers associated with a mutant phenotype. This allows geneticists to discover genes conferring disease resistance or susceptibility. This technique involves forming two groups that display opposing phenotypes for a trait of interest. For example, the individuals in one group are resistant to a disease, whereas those in the second group are not. Two bulked DNA samples are then created by pooling the DNA of all individuals in each group. These two bulked samples can then be analyzed using techniques such as Restriction
fragment length polymorphism or RAPD to detect similarities and differences in the various loci of the genome. The two groups will have a random distribution of alleles in all loci of the genome except for loci that are associated with the mutation (Phillip, 1992). A consistent difference on a locus between the two bulked samples likely means that the locus is associated with the mutation of interest.

Time and effort saving approach is bulk segregant analysis (BSA) (Michelmoore et al., 1991). In BSA, DNA of progenies corresponding to the phenotypic extremes is extracted and pooled. Therefore, only two pools of extreme lines along with the parents are genotyped for the identification of markers linked with the trait of interest. BSA was first applied to the identification of markers linked with disease resistance. Initially, it was applied to the diseases in which resistance was mostly governed by major genes, usually qualitative in nature (Michelmoore et al., 1991). Recently, BSA has been applied for quantitative traits also such as QTLs for heat tolerance in rice (Zhang et al., 2009).

The first published example of BSA was the identification of downy mildew genes in lettuce: Michelmore et al. (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations (Proc Nat Acad Sci USA 88: 9828-9832).

- **Bulk:** it makes use of bulked samples
  - a) saves time and money
- **Segregant:** it makes use of a segregating population
  - a) but it does not require map information!!
- **Analysis:** it screens the whole genome
- **Typical experiment:** parents and 2 bulks + hundreds to thousands of molecular markers
- **Rapidly identify molecular markers linked to trait of interest**
- **The success** relies on the dramatic reduction in the number of marker assays when compared to building a genetic map for the purpose of identifying markers associated with a phenotype.
- ‘**Collapse**’ the 2D matrix of marker assays (DNA samples × markers) into a 1D vector of genotypic differences between two DNA bulks.
- **Highly multiplexed marker technologies such as SSR, SFP, DArT and SNP can further ‘collapse’ the vector of genotypic differences between bulks into a single whole-genome assay.**

![Diagram](image)

**Figure 1:** The “bulk segregant analysis” method consists of bulking the DNA of extreme phenotypes for a trait. These bulks can be screened with molecular markers to find a DNA fragment that segregates with the trait and is therefore linked to it.
BSA compares 2 pooled DNA samples of individuals from a segregating population originating from a single cross. Within each bulk the individuals are identical for the locus of interest but are arbitrary for all other loci.

Markers polymorphic between the pools are markers putatively linked to the locus involved in the trait of interest.

**Method** involves comparing two pooled DNA samples of individuals from a segregating population originating from a composite population/single cross.

**Theory** - Each pool, or bulk, contains individuals selected to have identical genotypes for a particular genomic region ("target locus or region") but random genotypes at loci unlinked to the selected region (Michelmore et al., 1991). (Grouping of the informative individuals together)

**Bulking** - Two pools contrasting for a trait (e.g., resistant and susceptible to a particular disease) are analyzed to identify markers that distinguish them.

Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait of interest.

**How to set-up an experiment?**

Create a segregating population from a single cross (for example, F1 or BC progeny) Phenotype the progeny and identify individuals with extreme trait-phenotypes. Construct DNA bulks of the individuals displaying the most extreme trait-phenotypes. Genotype the parents and the bulks using hundreds to thousands of DNA-markers. Identify those markers which distinguish the bulks and the parents.

**Two types BSA technique**

Bi-parental population - cross between two parental lines. Composite populations - plants with diverse genetic backgrounds.

In both applications, when using co-dominant markers (e.g. RFLPs or SSR), where several marker alleles may be present, more than 15 individuals would need to bulk to ensure that each allele frequency represented that in the population as a whole.

**Linkage** between a polymorphic marker and the target locus is confirmed and quantified by using the segregating population from which the bulks were generated. Journal of Biotechnology Vol. 11(61), pp. 12436-12442, 31 July, 2012.

The Wmc25 of the tolerant parent was smaller than the sensitive parent. This locus was inherited in a Mendelian co-dominant manner. There was clear co-segregation between the
amplification of the smaller Wmc25 and the F2 plants showing tolerant phenotypes. In the homozygous sensitive F2 plants, only the large Wmc25 was amplified.

**Minimum size of the bulk**

Determined by the frequency with which unlinked loci might be detected as polymorphic between the bulked samples. This in turn will depend on the type of marker being screened (dominant or codominant) and the type of population used to generate the bulks (F2, backcross, full sib, etc.). As smaller bulks are utilized, the frequency of false positives will increase.

**30. HORTICULTURE**

**Vegetables: A Potential Source of Nutraceuticals**

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**INTRODUCTION**

Nutraceutical, a combination of the words “nutrition” and “pharmaceutical”, was coined in 1979 by Dr. Stephen L. DeFelice, founder and chairman of the Foundation of Innovation Medicine (FIM) (Crawford, New Jersey). It refers to any substance that is food or part of food and provides medicinal or health benefits, including prevention and treatment of diseases. Present day, vegetable contribute an essential part in balanced diet. They are termed as protective food because of their richness in vitamin, minerals, carbohydrates, dietary fiber and organosulfur contents. These chemicals have tremendous impact on health care system and provide medical health benefits including the prevention and treatment of diseases and physiological disorders. Therefore the plant substances important to human nutrition must be clearly identified and should be intend to breed cultivars with improved nutritional attributes through conventional and molecular breeding approaches.

**MAJOR GROUP OF NUTRACEUTICALS ARE:**

- **Anthocyanidins:** These are the type of flavonoids also known as flavonals give rise to blue-purple-red colour of pigmentation in plant parts. It provides cross-links that hook up and strengthen the intertwined collagen protein strands found in tissues and act as free radical scavenger in tissue fluids.
- **Carotenoids:** These are lipid soluble yellow-orange-red pigment found in all higher plants. There are more than 600 naturally occurring carotenoids. Among them oxy-carotenoids or xanthophylls such as lutein and zeaxanthin and non-oxy carotenoids (hydrocarbon carotenoids) such as beta carotene and lycopene has been identified.
- **Flavonoids:** It constitutes a sub class of phenols that improve the effect of ascorbate-Vitamin C. They can act as potent antioxidants and metal chelators. They possess anti-inflammatory, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral and anti-carcinogenic properties. These are also helpful in protection of vascular system.
- **Glucosinolates:** These are generally present in cruciferous vegetables. They convert to isothiocyanates (contain sulfur) and indoles (do not contain sulfur) when vegetables containing them are cut. They detoxify enzymes in liver, white blood cells and cytokines ultimately helps in boosting immunity. The isothiocyanates (in radish), dithiolthiones and sulforaphane (present in broccoli) are bio-transformation products of glucosinolates that prevents enzymes that are responsible for tumorous growth in liver, lung, breast and gastrointestinal tracts.
- **Catechins:** Chemically slightly differs from other flavonoids but it has the same chemo protective activity.
- **Phenols:** This group of phyto-chemical protects human body from oxidative damages. It gives blue, blue-red
pigmentation. It blocks specific enzyme that cause inflammation and protects platelets from clumping by modifying enzymatic pathway.

Lipoic acid: These are antioxidants which can effectively quench the hydroxyl radicals and are active on both lipid and tissue fluid. They can protect catalase and glutathione, thus helpful in liver detoxification.

Nasunin: It is the major type of anthocyanin substance that provides the dark pigment in the brinjal. It protects brinjal from environmental damage and has high antioxidant property.

Prebiotics: These are the short chain polysaccharides that have unique chemical structures which are not digested by human generally found in chicory roots, tomato and alliums. This dietary ingredient beneficially affects the host body by altering the metabolism.

Thiols: these are sulphur containing phyto-nutrients. Generally found in onion, garlic and cruciferous vegetables. They have anti-mutagenic and anti-carcinogenic properties. It activates liver detoxification enzymes and effective against tumors, bacteria, fungi, viruses, parasites and cholesterol.

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<td>Red cabbage, brinjal</td>
<td>Prevent urinary tract infections</td>
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<td>Sulphoraphane</td>
<td>Broccoli</td>
<td>Anti-cancerous</td>
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<td>Lignan</td>
<td>Broccoli</td>
<td>Anti-oxidant, anti-ageing</td>
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<td>Ferulic acid</td>
<td>Tumip</td>
<td>Protection against skin</td>
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<td>Luteoline</td>
<td>Cauliflower, celery</td>
<td>Carotenoid with eye benefit</td>
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<td>Betanin</td>
<td>Beet, chard</td>
<td>Prevent the oxidation of Low-density lipoprotein (LDL) oxidation</td>
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<td>Angelicin, Xanthotoxin</td>
<td>Parsnips</td>
<td>Treatment against skin disorders</td>
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