IN THIS JOURNAL

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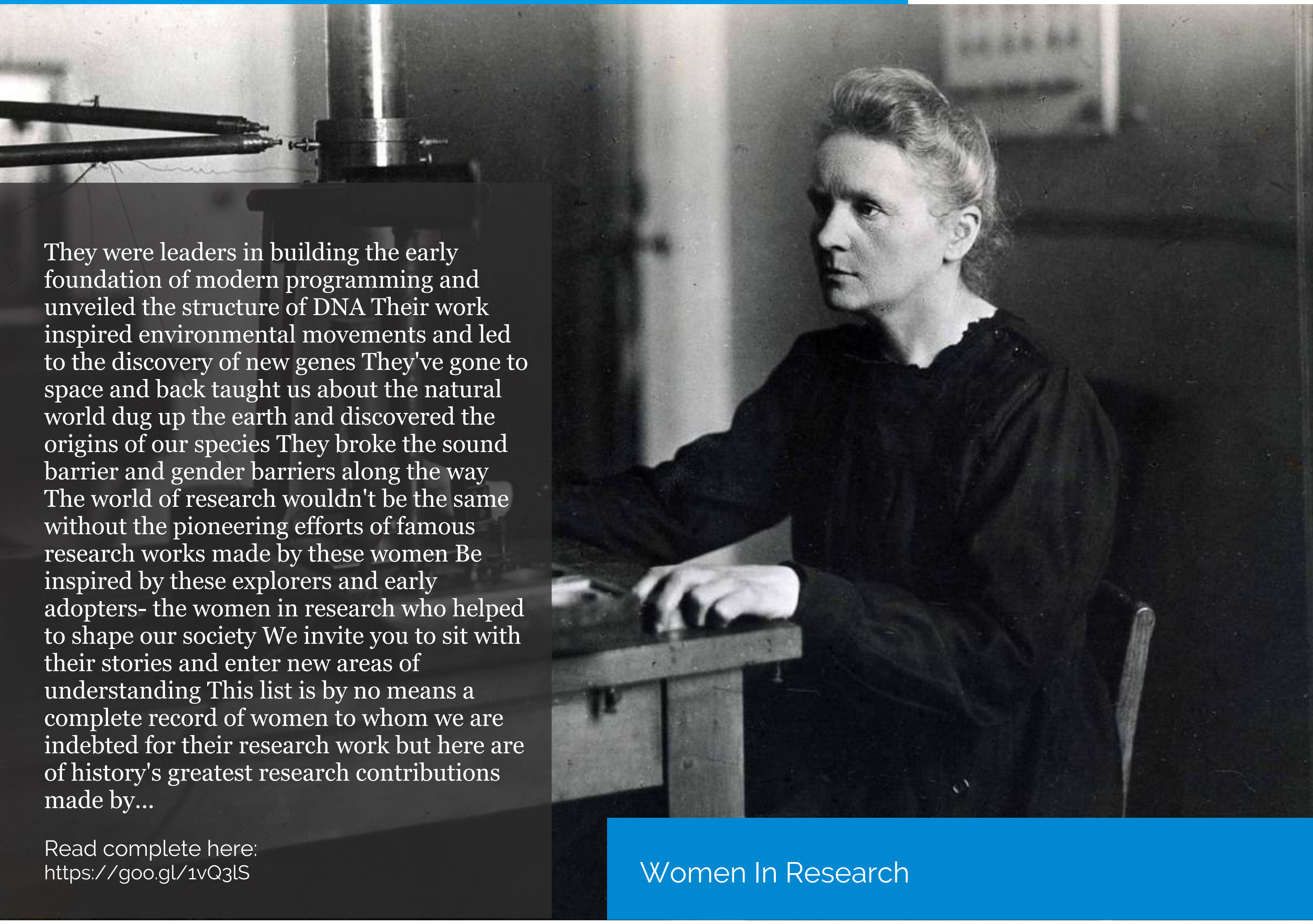

Women In Research

They were leaders in building the early foundation of modern programming and unveiled the structure of DNA Their work inspired environmental movements and led to the discovery of new genes They've gone to space and back taught us about the natural world dug up the earth and discovered the origins of our species They broke the sound barrier and gender barriers along the way The world of research wouldn't be the same without the pioneering efforts of famous research works made by these women Be inspired by these explorers and early adopters- the women in research who helped to shape our society We invite you to sit with their stories and enter new areas of understanding This list is by no means a complete record of women to whom we are indebted for their research work but here are of history's greatest research contributions made by...

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Research Project on Rotational Dynamics Orbiting and Rotation of Celestial Bodies

Gabriel Barceló Rico-Avello

ABSTRACT

We will attempt to summarize in this text the private scientific research that our team has conducted on rotational dynamics over the past forty years. The document analyses the rotation and orbiting of rigid solid bodies, and specifically of celestial bodies, arriving at a Theory of Dynamic Interactions (TDI) as a solution to the problems not resolved by Kepler's and Newton's laws, by taking into account the simultaneous orbital and rotational movements of planets.

The text describes experiments that demonstrate how a body in rotation and translation can alter its trajectory, thus challenging the classical Newtonian model. The TDI explains the behaviours of bodies that simultaneously orbit and rotate, and it can be applied to dynamic phenomena that meet those conditions, such as the case of boomerang flight, gyroscopes, or the anomalies observed in the Pioneer probes, offering a new interpretation of these behaviours.

Keywords: theory of dynamic interactions, tdi, rotational dynamics, dynamic interactions, rotation acceleration, solar system dynamics, dynamic equilibrium, momentum, orbiting, rotation, gyroscopic effects.

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The text describes experiments that demonstrate how a body in rotation and translation can alter its trajectory, thus challenging the classical Newtonian model. The TDI explains the behaviours of bodies that simultaneously orbit and rotate, and it can be applied to dynamic phenomena that meet those conditions, such as the case of boomerang flight,[1] [2] gyroscopes, or the anomalies observed in the Pioneer probes,[3] offering a new interpretation of these behaviours.

The text suggests that this theory provides new insights into understanding rotational dynamics and the harmony of the universe, proposing a defined mathematical model for modern physics and for certain natural phenomena.

Keywords: theory of dynamic interactions, tdi, rotational dynamics, dynamic interactions, rotation acceleration, solar system dynamics, dynamic equilibrium, momentum, orbiting, rotation, gyroscopic effects.

Author: Advanced Dynamics CB, España, Madrid E.T.S.I. Industriales de Madrid, L. C. Physics. U. Complutense, Madrid, (Spain).

I. INTRODUCTION

Driven by our interest in observing the universe—both the dynamics of the cosmos and the interactions of atomic particles—we began an investigation into the dynamic behaviour of rotating rigid bodies. We carried out the necessary experimental tests, subjecting these bodies to new, non-coaxial rotations, ultimately developing a new theory: the so-called *Theory of Dynamic Interactions* (TDI),[4] supported by a specifically conceived mathematical framework.

In the solar system, all planets rotate on their own axes and orbit around the Sun. The first modern scientist to study the motion of the planets was the German astronomer Johannes Kepler in the 17th

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[1] Barceló, Gabriel; *Theory of Dynamic Interactions: The Flight of the Boomerang*, Journal of Applied Mathematics and Physics, 2, 569-580. (2014) doi: 10.4236/jamp.2014.27063.

[2] Barceló, Gabriel*: Theory of Dynamic Interactions: The Flight of the Boomerang II*, Journal of Applied Mathematics and Physics, Vol.3 no.5, Mayo 2015. DOI: 10.4236/jamp.2015.35067, https://www.youtube.com/watch?v=mGfrGW5fhOg&feature=youtu.be

[3] Barceló Rico-Avello, Gabriel: The Pioneer probes. http://dinamicafundacion.com/wp-content/ uploads/2014 /02/ ANOMAL%C3%8DAS-DIN%C3%81MICAS-EN-LAS-SONDAS-PIONEER1.pdf

[4] Alvarez Martínez, Alejandro: *Theory of dynamic interactions: innovations*. World Journal of Mechanics. Special issue: Rotational Dynamics: Theory of Dynamic Interactions. 7, 101-119. Vol.7 No.3, March, 2017. DOI: 10.4236/wjm.2017.73010

century. Kepler formulated three fundamental laws for understanding celestial mechanics, which laid the foundation for the development of orbital physics.

Later, Newton explained these behaviours through his *Law of Universal Gravitation,* which Einstein accepted and incorporated into his *Theory of Relativity*. However, Kepler's laws referred exclusively to the orbiting of planets and did not analyse their axial rotation.

The Theory of Dynamic Interactions (TDI)[5] that we propose, offers an update to this problem, and provides justification for the simultaneous rotation and orbiting of celestial bodies—an aspect not previously considered, as I have already presented in other articles and developed through various cases and assumptions.[6] Therefore, the TDI we present justifies this behaviour, and its application allows us to understand the simultaneous rotational and orbital movement of planets and other celestial bodies.

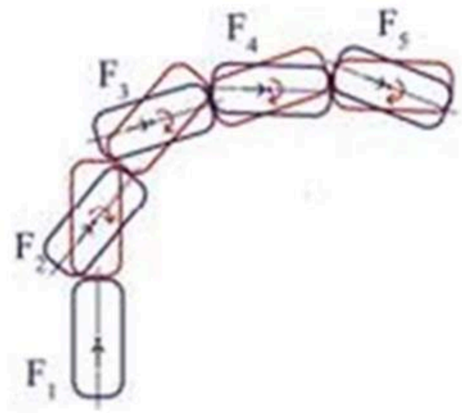

Figure 1. Trajectory of a body with translational velocity and rotation around its main axis, when subjected to a new rotation not coinciding with the existing one.

TDI[7] explains that a body in space, following a rectilinear trajectory and possessing translational and rotational velocity around its main axis, when subjected to an external torque not aligned with its intrinsic rotation, does not generate a new rotation, but rather modifies its trajectory.

In our experimental tests,[8] we concluded that the velocity field generated, for example, by a torque resulting from buoyancy and weight—when not aligned with the intrinsic rotation of the object—causes the object to rotate around a vertical axis perpendicular to the external torque (Figure 1), thereby altering its trajectory.

In that figure, the displaced object is shown in red (with its previous orientation), and in black is the new orientation of the object due to the resulting dynamic coupling. We concluded that under these conditions, a coupling occurs—an interaction between two velocity fields (the translational field already present and the anisotropic one generated by the new torque). As a result, the object's trajectory changes, initiating an orbit similar to that of Earth around the Sun, which would be closed if the external conditions remain constant.

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[5] Gauna, J: Theoria Dynamics Interaction's A New Paradigm in Physics. – Alf Gauna

[6] Barceló, Gabriel: *Analysis of the Orbitation and Rotation of Celestial Bodies*, Journal of Applied Mathematics and Physics (September, 2023).

[7] Sierra Márquez, Jordi: *Teoría de Interacciones Dinámicas* El libre pensador, 06/07/2012, https://ellibrepensador.com /2012/07/06/teoria-de-interacciones-dinamicas-por-gabriel-barcelo/

[8] Merino, Justo: *The works and days of Gabriel Barceló*. WJM. Vol.7 No.3. 3, 2017, DOI: 10.4236/wjm.2017.73006.

With that initial experimental test, we concluded that the accepted model attempting to explain Earth's behaviour around the Sun was incorrect, and that it is not solely the Law of Universal Gravitation that enables the observed orbital trajectory simultaneous with Earth's rotation.

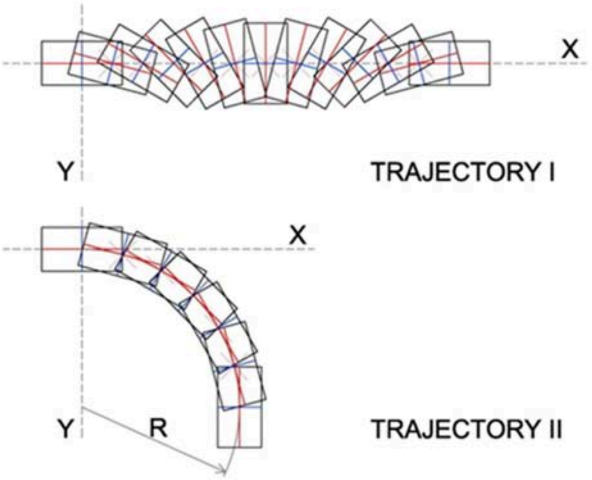

Figure 2: Trajectory I is the one predicted by classical mechanics, while trajectory II is the result of the Theory of Dynamic Interactions that we propose and which has been verified in numerous trials and tests.[9]

II. INTERPRETATIVE ERROR

For me, all of this began many years ago when I wanted to investigate the research of my professor Miguel Catalán.[10] Miguel Catalán[11] was a spectroscopist and, in the 1920s, in the laboratories of Imperial College London, he discovered multiplets in the manganese spectrum — groups of lines with characteristic regularities. Catalán demonstrated that the study of multiplets led to a better understanding of the energy states of atomic electrons and allowed for the definition of the structure of matter. However, the rotational and orbital behaviour of an atom's outer electrons remained a mystery in those years.

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[9] Barceló, Gabriel: *New Paradigm in Physics*. Volume I and II. Amazon. 1917/1918.

[10] Barceló, Gabriel: *Miguel A. Catalán Sanudo: Memoria Viva*. Editorial Arpegio, 2.012. http://advanceddynamics.net/en/memoria-viva/

[11] Barceló, Gabriel: *Miguel A. Catalán's CXXV Anniversary*. December 10, 2019. Advances in Historical Studies Vol.8 No.5 December 10, 2019 , DOI: 10.4236/ahs.2019.85017

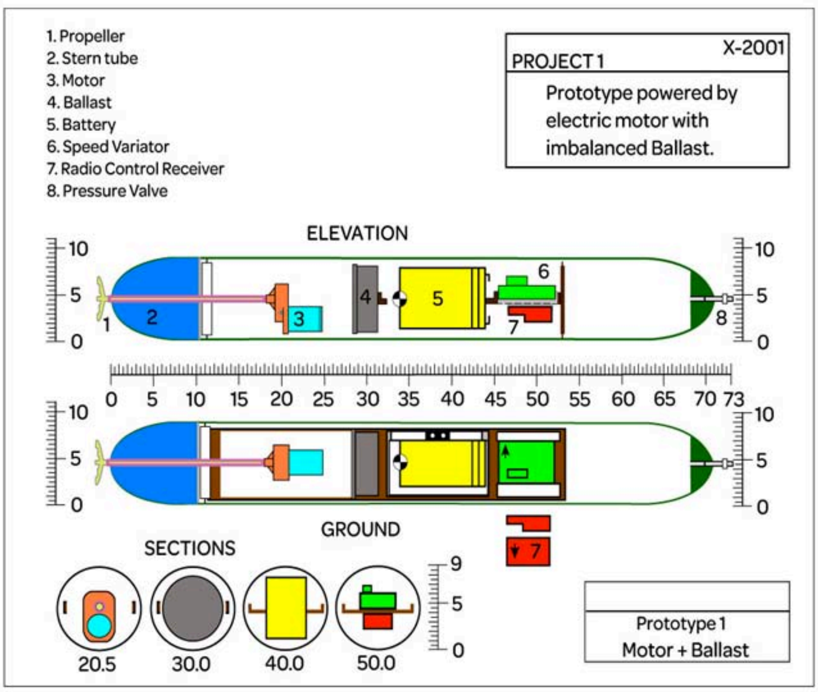

Figure 3: Model of a ship-submarine prototype with water tanks at the stern and bow, which demonstrated its trajectory change without the need for a rudder, as predicted by TDI.[12]

In this context, we concluded that Newton's laws could be valid under assumptions without accelerations, but not when dealing with moving bodies that rotate. To test this, we conducted an experimental trial with a waterborne vehicle, designed according to the scheme shown in Figure 3.

As shown in the video https://advanceddynamics.net/wp-content/uploads/2014/ 02/31Submarino_Prototipo_I.mp4, we can assume the vehicle follows a straight-line trajectory, equipped with translational velocity and intrinsic rotation around its principal axis of inertia, which is subjected to a new torque not coaxial with the intrinsic rotation — for example, the buoyancy/weight pair, contained in the drawing plane, as in our experiments.

In such a case, the anisotropic velocity field generated by this buoyancy/weight pair forces the vehicle to perform a turn around a vertical axis, perpendicular to the external torque acting upon it.

We repeated the experiments with other vehicles (see Figure 5) and came to the conclusion that the model proposed by Newton to justify the Earth's orbit — which was later upheld by Einstein — was, in our opinion, clearly flawed and needed to be replaced by the model conceived by the TID (Theory of Dynamic Interactions).

Moreover, Newton's statement would necessarily produce a variable or undulating orbit for both the Earth and the Moon: their trajectories would be influenced by gravitational forces at each point of their orbits. In the case of the Moon, its orbit would fluctuate depending on the relative positions of the Sun and the Earth with respect to the Moon. The resulting orbit would not be the same if these bodies were in conjunction or in opposition.

On the other hand, the *Theory of Dynamic Interactions* not only justifies the orbits of Earth, the Moon, and celestial bodies, but also provides insight into other natural dynamic phenomena, such as: the

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[12] Barceló, Gabriel: *New Paradigm in Physics*. Volume I and II. Ed. Amazon. 1917/1918.

flight of the boomerang,[13] the spinning top's dance, the gyroscope, the gyroscopic pendulum, epostracism, atmospheric vortex phenomena,[14] dynamic confinement,[15] the dynamic anomalies of the Pioneer probes, dynamic lever mechanics, steering of vehicles without rudders, spinning balls and curveballs, roll coupling in airplanes, bouncing bombs from World War II, the Euler disk, or soda cans that lift off without an upward force — among many other dynamic examples we can observe in bodies with intrinsic rotation.

A multitude of cases whose behaviour, in our opinion, had not been correctly explained until now.

All of these examples, and many more, are described in the second volume of the book:

NEW PARADIGM IN PHYSICS,[16] and can also be explored via the portals:

https://advanceddynamics[17].net/http://www.dinamicafundacion.com/and in various videos.

The experimental tests carried out are easily reproducible according to the scientific method. Advanced Dynamics has announced three successive contests for possible refutation or antithesis of the proposed theory, without receiving any response. The presentation videos of the TID can also be found at:

https://youtu.be/keFgx5hW7igor http://www.youtube.com/watch?v=k177OuTj3Gg&feature=related.

This strange behaviour of rotating bodies is due to the phenomenon of *precession*, which occurs when a rotating body in motion is forced to perform a new rotation around a new axis. Newton's mechanical model of the Universal Law of Gravitation should be considered an approximation — although it has endured for centuries in our scientific paradigm.

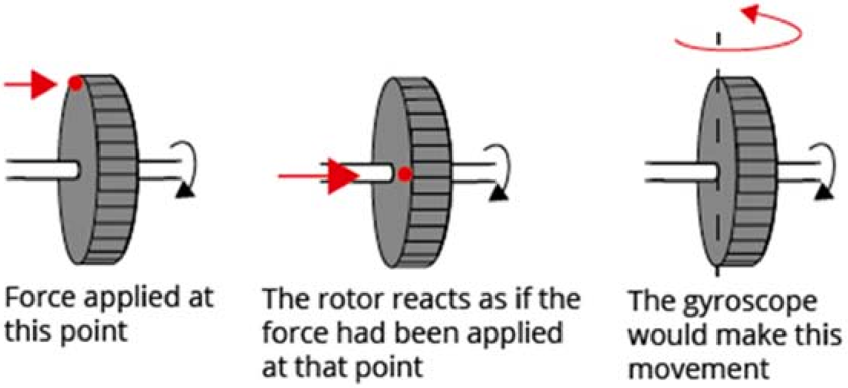

Figure 4: Precession in rotational dynamics is generated when a force is applied to a rotating body, forcing it to perform a new rotation around a new axis. The body's response is not that new rotation, but rather a reaction as if the force had been applied at another point, forcing it into an orbital motion — due to the coupling of the new induced rotation, with the existing translational velocity — thereby generating the orbital trajectory of celestial bodies.

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[13] Martín, Almudena: *The Flight of the Boomerang: Comments*. WJM, Vol.7 No.3, March 30, 2017. DOI: 10.4236/wjm.2017.73007

[14] Barceló, Gabriel: Dynamic Interactions in the Atmosphere, Atmospheric and Climate Sciences, Vol.4 no.5, November 20, 2014, DOI: 10.4236/ACS.2014.45073

[15] Barceló, Gabriel: *Dynamic Interaction: A New Concept of Confinement*, Global Journal of Science Frontier Research: A Physics and Space Science, Vol 16 no.3 , Junio 2016.

[16] Barceló, Gabriel: *New Paradigm in Physics: Assumptions and applications of the theory of dynamic interactions*, Volume II: Theory of Dynamics Interactions, Amazon, 2018. http://advanceddynamics.net/

[17] Barceló, Gabriel*: On Motion, Its Relativity and the Equivalence Principle*. Journal of Modern Physics, Vol.5 no.17, November 14, 2014, DOI: 10.4236/jmp.2014.517180

Our TID hypotheses are based on the coupling of translational velocity fields at each point of the moving object — even if these fields are caused by translational movements or generated by external actions that could create new rotations not aligned with any other preexisting ones in the body.

We believe that our proposals and the results obtained from our experimental tests suggest new horizons for *rotational dynamics* and new keys to understanding the harmony of the universe. The universe is made up not only of forces but also of their effects, as they constantly act on celestial bodies in rotation with constant translational velocity, resulting in a closed orbit. Thus, it is a system in motion, yet also in a constant state of *dynamic equilibrium*. Isn't this very equilibrium we observe in the cosmos the essence of celestial mechanics?

The orbital movements we observe in celestial bodies are the result of dynamic coupling not foreseen in Classical Mechanics, as expressed in the TID, enabling a secular dynamic equilibrium. Consequently, we believe the TID mathematical model we propose is of great conceptual importance.

Furthermore, we think it is not only necessary to understand the dynamics of rotating bodies, but also that of the cosmos — with bodies that orbit and have constantly recurring motions — which make possible systems that have been in dynamic equilibrium for centuries, and are not necessarily in a process of unlimited expansion.

We even believe that this new dynamic theory improves our understanding of the universe and the matter from which it is made.

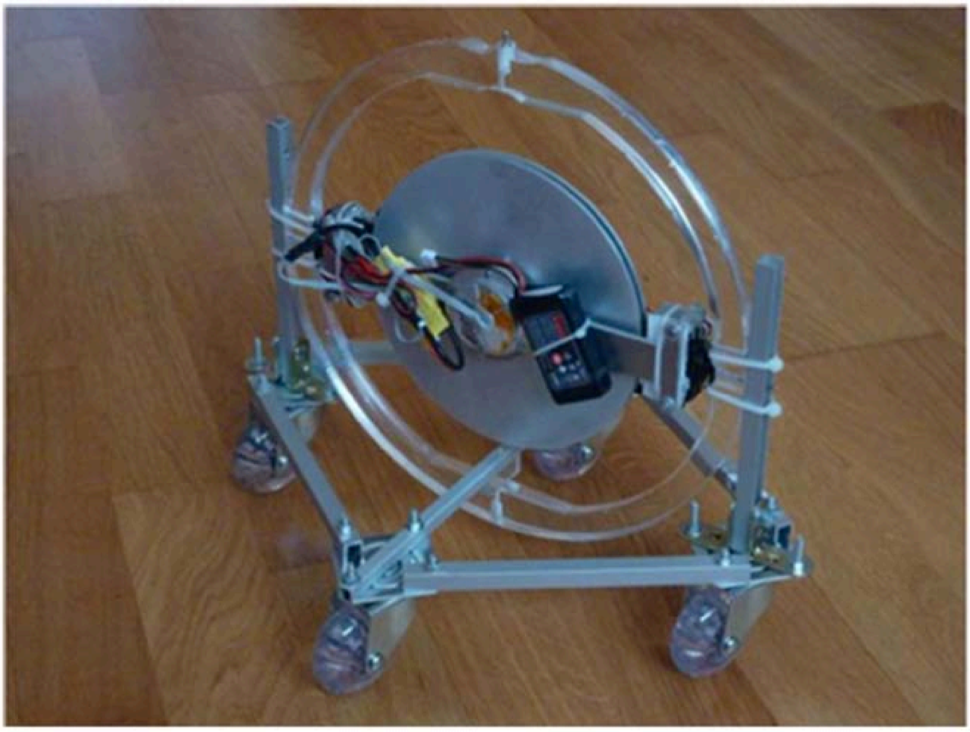

Figure 5: Terrestrial Prototype II with rotating wheels, which allowed directional control, with results similar to those of the submarine.[18]

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[18] Pérez, Luis Alberto: *New evidence on rotational dynamics*. WJM, DOI: 10.4236/wjm.2013.33016

III. MATHEMATICAL FORMULATION

Non-coaxial moments generate an anisotropic distribution of velocities, which, when coupled with the translational velocity field, causes a rotation in the velocity of the center of mass.[19] The variation in the direction of the velocity can be obtained by applying the mathematical formula of our model:

$$\vec{v} = \vec{\vec{\Psi}}\, \vec{V}_0$$

The operator matrix $\vec{\vec{\Psi}}$ will be:

$$\begin{pmatrix} \cos\alpha & -\operatorname{sen}\alpha & 0 \\ \operatorname{sen}\alpha & \cos\alpha & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

So, the velocity vector will rotate by an angle alpha due to this dynamic coupling. The velocity vector will be in the form, in the body frame:

$$\vec{V}_0 = V_{0x}\vec{i}\,'$$

The proposed mathematical formulation has been subsequently complemented and developed by Arturo Rodriguez Palenzuela in his book: *Rotational Field Theory.* [20]

The author proposes a new description of two types of motion already known in physics, namely *rotation* and *precession* of objects, which, according to the theory presented, experience them in a unified manner.

These rotational and precessional movements would be generated, starting from rest, by the action of new macroscopic forces, whose associated fields would be dynamically related by equations similar to Maxwell's equations of Electromagnetism.

In his book, and in his writings,[21] Rodriguez Palenzuela also describes the connection with the *Dynamic Interactions Theory*. As a demonstration of the validity of the *Rotational Field Theory,* he presents the result of a simulation of Chandler's motion developed from the postulated model.

These proposals result from private scientific research carried out by Advanced Dynamics team for over 40 years, seeking nomological relationships of non-inertial systems. The objective of these investigations was to understand the dynamic laws of rotating bodies in space, by analyzing their behavior.

As a result of this research project, dynamic behavior laws have been found in environments where Classical Mechanics laws are not applicable. A new dynamic and mathematical theory for bodies with intrinsic rotation has been proposed. Through repeated experimental tests, this dynamic theory has been confirmed with certainty, revealing how to conceive the true development of scientific knowledge in this area of nature.

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[19] Barceló, Gabriel (2019) *Rotational Mechanics. Generalization of Movement in Space*. International Journal of Innovative Studies in Sciences and Engineering Technology, https://ijisset.org/storage/Volume5/Issue12/IJISSET-051119.pdf

[20] Rodríguez Palenzuela, Arturo: *Teoría de Campos Rotacionales: La Teoría de Interacciones Dinámicas, Campos Rotacionales y el movimiento de Chandler*, Editorial Amazon, 2022.

[21] Palenz, Arthur: *Theory of Gyroscopic Fields. An introduction*. GMAERO 2024, 2Nd, Madrid, 5 august, 2024.

This new paradigm suggests new keys to understanding the cosmos and posits celestial mechanics equilibrium as a logical and rational result of these new dynamic hypotheses, but also allows us to imagine the poetry of our universe, comparing the movement of celestial bodies to the flight of a boomerang.[22]

New Paradigm

In our opinion, this represents a substantial shift in the basic assumptions or models within the dominant theory of dynamic science. We are truly proposing a paradigm shift.

This new physical framework defines a new image of the world and makes it possible to justify behaviours that, until now, were considered chaotic or insufficiently understood. Our objective is to share the surprising results obtained and to attract interest toward the research of this new area of knowledge — rotational dynamics — and its many remarkable scientific and technological applications.

The Theory of Dynamic Interactions (TDI) is a fascinating concept. We believe our proposal offers entirely original content, and that the conclusions we present constitute a new paradigm in physics, one that had not been formulated until now.

IV. SUMMARY OF CONCLUSIONS

As a result of this dynamic research work, we propose the following summary of our conclusions:

1. There is a broad area of research, still insufficiently developed, in rotational dynamics for rigid solid bodies subjected to accelerations caused by simultaneous non-coaxial rotations.
2. This area of knowledge can be analysed under both relativistic and non-relativistic mechanics frameworks.
3. The hypotheses are based on new criteria regarding velocity coupling and rotational inertia.
4. After conducting the non-relativistic experimental tests presented, we concluded that new general behavioural laws can be deduced based on the analysis of the dynamic fields generated.
5. We have also derived an equation of motion for rigid solid bodies undergoing translational movement with intrinsic angular momentum, when subjected to new, non-coaxial torques or moments. This defines their dynamic behaviour and has led to a deep mathematical analysis and development of the behaviour of bodies with intrinsic rotation and orbitation.
6. We found a clear physical and mathematical correlation between rotation and translation. This mathematical connection allows us to identify a physical relationship between the transfer of rotational kinetic energy and translational kinetic energy, and vice versa.
7. This mathematical model implies that bodies with intrinsic rotation and translational movement, when subjected to successive non-coaxial forces, will begin an orbiting motion as a result of the dynamic interactions generated.
8. As long as the initial angular momentum remains constant and a second non-coaxial torque or moment acts, the center of mass of the moving body will follow a closed orbit, without requiring the existence of any centripetal force.
9. There is no need to include unstructured effects, fictitious forces, or supposedly deduced expressions such as dark energy or dark matter in order to conceive a model of the cosmos and a dynamic behaviour of nature that is coherent with observational experience.
10. A revision of the mathematical models derived within the framework of General Relativity is advisable, incorporating the equation of motion we propose and the dynamic criteria of the TDI.

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[22] García Moliner, F. (2017) *Physical-mathematical models in rotational movements*. WJM, 7, 35-38. doi: 10.4236/wjm.2017.73004 .

11. The Theory of Dynamic Interactions also allows us to answer the initial aporia: to become aware of and understand the physical and mathematical correlation between orbitation and intrinsic rotation.

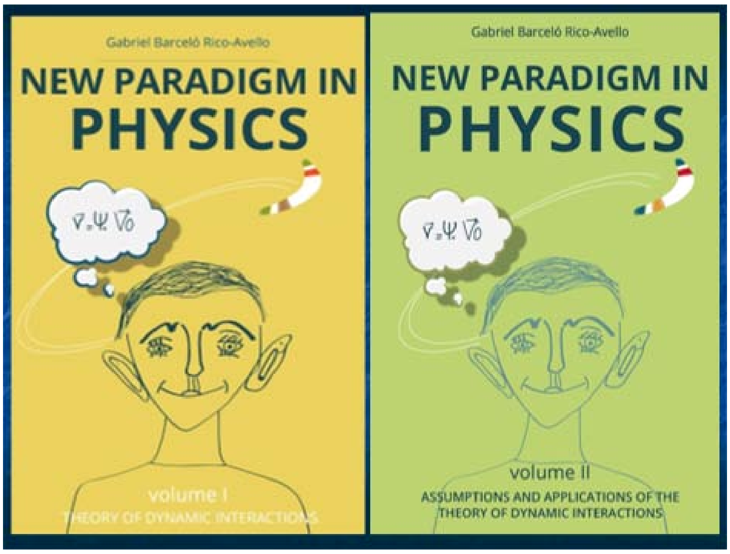

Figure 6: Cover of the Two Volumes of New Paradigm in Physics

Several books have been published on the results obtained, in Spanish or in English, as well as over seventy articles throughout the course of the research.

We recommend reading Section 5.4 of Volume I of *New Paradigm in Physics*, as well as the book *Theory of Rotational Fields* by Arturo Rodríguez Palenzuela (2022), which proposes the mathematical development of the Theory of Dynamic Interactions, rotational fields, and the Chandler motion.

An antithesis prize for the TDI was announced three times, and on all three occasions, it remained unawarded.

To access more complete documentation on the Theory of Dynamic Interactions, please visit:
http://www.advanceddynamics.net/
http://www.dinamicafundacion.com/

V. EXPERIMENTAL TESTS AND VIDEOS

Over recent years, various experimental tests have been conducted with fully satisfactory results. These tests confirm the dynamic hypotheses on which the Theory of Dynamic Interactions is based.
Videos of these experiments can be seen at the following links:

Theory of Dynamic Interactions_1
http://www.youtube.com/watch?v=P9hGgoL5ZGk&feature=related
Theory of Dynamic Interactions_2
http://www.youtube.com/watch?v=XzTrGEtJGXU&feature=related
Theory of Dynamic Interactions_3.avi
http://www.youtube.com/watch?v=dtMqGSU9gV4&feature=related
Theory of Dynamic Interactions_4.avi
http://www.youtube.com/watch?v=qK5mW2j2nzU&feature=related
Barceló, G.: *Theory of Dynamic Interactions.*

Videos, 2002.
http://www.youtube.com/watch?v=P9hGg0L5ZGk&list=PL3E50CF6AEBEED47B
http://www.youtube.com/watch?v=XzTrGEtJGXU&list=PL3E50CF6AEBEED47B
http://www.youtube.com/watch?v=dtMqGSU9gV4&list=PL3E50CF6AEBEED47B
http://www.youtube.com/watch?v=qK5mW2j2nzU&list=PL3E50CF6AEBEED47B
http://www.youtube.com/watch?v=vSUkd4slHGQ
http://www.youtube.com/watch?v=P9hGg0L5ZGk&feature=c4-overview-vl&list=PL3E50CF6AEBEED47B
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http://www.youtube.com/watch?v=vSUkd4slHGQ
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We have tried to summarize in this article, for the first time, a summary of the research work developed in Rotational Dynamics, proposing a new model to understand the behaviours of rigid bodies, planets, or celestial bodies when they have intrinsic rotation and orbit.

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Deciphering Root Dynamics and Functional Architecture in Rice for Strategic Redesign of the Hidden Half to Enhance Water and Nutrient Acquisition

Abdou NjifenjouPrasanta Kumar Majhi, Kishore Chandra Sahoo, Monika Ray, Subhakanta Samantaray, Sukumar Tudu, Sujata Das, Partha Pratim Beherav, Abhishek Nanda, Sarita Pradhan, Gyana Ranjan Mohanty, Sandeep Kumar Mallik & Srimanta Kumar Sahoo

Odisha University of Agriculture and Technology

ABSTRACT

The present scenario of climate change on agriculture in the form of groundwater depletion, flood, salinity, several new borne species of pathogens, and insect-pests, brings serious attention to developing rice varieties with higher resilience. As most of the above-ground plant parts are already explored to combat these situations, now the time came to improve the yield by reshaping the below-ground plant parts. Designing the root system architecture (RSA) in rice is one of the most imperative traits for such conditions, as modification in the root architecture in rice will be the best strategy to improve water uptake and nutrient acquisition. Many quantitative trait loci (QTLs) and genes playing the role for RSA have been recognized to improve the root parameters and the confirmed QTLs can be introgressed through marker-assisted backcross breeding to develop ideal genotypes. The recent advances in molecular plant breeding including genome editing, mutation study and genetic engineering has shown their potency in this direction.

Keywords: climate change, genome-wide association studies, genome editing, rice, root system architecture, QTLs.

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Prasanta Kumar Majhi[α], Kishore Chandra Sahoo[σ], Monika Ray[ρ], Subhakanta Samantaray[Ѡ],
Sukumar Tudu[§], Sujata Das[χ], Partha Pratim Behera[ν], Abhishek Nanda[Θ], Sarita Pradhan[ζ],
Gyana Ranjan Mohanty[£], Sandeep Kumar Mallik[€] & Srimanta Kumar Sahoo[₣]

ABSTRACT

The present scenario of climate change on agriculture in the form of groundwater depletion, flood, salinity, several new borne species of pathogens, and insect-pests, brings serious attention to developing rice varieties with higher resilience. As most of the above-ground plant parts are already explored to combat these situations, now the time came to improve the yield by reshaping the below-ground plant parts. Designing the root system architecture (RSA) in rice is one of the most imperative traits for such conditions, as modification in the root architecture in rice will be the best strategy to improve water uptake and nutrient acquisition. Many quantitative trait loci (QTLs) and genes playing the role for RSA have been recognized to improve the root parameters and the confirmed QTLs can be introgressed through marker-assisted backcross breeding to develop ideal genotypes. The recent advances in molecular plant breeding including genome editing, mutation study and genetic engineering has shown their potency in this direction. Even though most of the root architectural traits in rice are not documented properly, the present review will make comfortable to the future researchers on the aspects of molecular mechanisms involved in root traits development viz., genes functioning for root traits, their development, physiological role for moisture, and mineral-nutrient uptake under stressful environment.

Keywords: climate change, genome-wide association studies, genome editing, rice, root system architecture, QTLs.

Authors α σ ρ Ѡ § χ Θ: Regional Research and Technology Transfer Station (RRTTS), Odisha University of Agriculture and Technology (O.U.A.T.), Keonjhar-758002, Odisha, India.

ν: International Crops Research Institute for Semi-arid Tropics (ICRISAT), Hyderabad-502324, Telangana, India

ζ: College of Agriculture, Odisha University of Agriculture and Technology (O.U.A.T.), Chiplima-768025, Odisha, India.

£: Department of Genetics and Plant Breeding, Palli Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan-731236, West Bengal, India.

€: Department of Seed Science and Technology, Faculty of Agriculture, Uttar Banga Krishi Vishwavidyalaya, Coochbehar-736165, West Bengal, India.

₣: Regional Research and Technology Transfer Station (RRTTS), Odisha University of Agriculture and Technology (O.U.A.T.), Semiliguda-764036, Odisha, India.

I. INTRODUCTION

Rice is considered to be the vital crop for billions and got ranked as the second-best significant cereal food in the world after wheat. This crop is being cultivated under various natural climatic conditions (Dokku *et al.*, 2013; Manna et al., 2025; Rasheed *et al.*, 2020). Conversely, the yield potentiality is hindered by several biotic and abiotic factors, including increasing water scarcity, flood, soil salinity and temperature fluctuation (Navea *et al.*, 2017). Asia is contributing maximum rice (90%) to the World's food security, while water scarcity affects around 50% of rice-cultivable land (Khan *et al.*, 2013; Shukla et al., 2024). The reason behind this is that it takes 3000 litres of water to yield one kilogramme of rice. Drought affects the crop at every stage of growth because of its shallow roots and thin cuticle, making it one of the most vulnerable plants (Figure-1), with a potential yield loss of 100% (Oladosu *et al.*, 2019). In the near future, rice could be subjected to more severe challenges, including drought, soil salinization, and a lack of readily available nutrients due to diminished fertilizer or moisture availability (Poot and Lambers, 2008; Shukla *et al.*, 2024). Roots are very essential for crop production and productivity because of their vital functions to the plant, including water uptake, nutrient gain and anchoring into the soil to withstand lodging (Yang *et al.*, 2019). The root system architecture (RSA) is the vital part towards overcoming the environmental complexity and ensuring an ideal response. They also have an impact on their surroundings by inducing physico-chemical and microbiological processes that affect essential factors such as nutrient bioavailability, organic matter cycling, soil mineral weathering, and water quality (Li *et al.*, 2025; Ma *et al.*, 2025). Most of the researchers are generally focused on the above-ground plant parts like leaf, stem, panicle and grain quality to achieve higher yield under stress. However, the root system scattering under ground is the main role performer to improve the above-ground parts. Therefore, more efforts are required to the ground to study the root architectural traits to enhance its capability to absorb sufficient moisture and mineral elements from the ground to boost the yield under diverse climatic conditions. The three-dimensional location of roots in the soil is referred to as a plant RSA, and this encompasses the morphology, topology, and distribution of roots (Li *et al.*, 2025). As a plant grows, its root system undergoes elongation, branching, and spacing changes, all of which affect RSA's ability to maintain its original shape. Root systems can show a high degree of developmental plasticity because of their ability to integrate environmental information into their growth programme (Novoplansky, 2002). In a dynamic, ever-changing environment, plants with root plasticity can adapt their root system's three-dimensional shape to maximise growth. An important part of root stability, as well as hormone biosynthesis and nutrition and water absorption, is the root system's design (Coudert et al. 2010). Specialized root architectures are effective in such conditions for a specific stress-prone environment (Poot and Lambers, 2008; Panda *et al.*, 2021). However, the molecular mechanisms of genes regulating root development and the physiological role (*e.g.*- nutrient uptake), root plasticity, plant-soil microbial interaction, high-throughput root phenotyping methods are not clearly understood. So it needs further analysis for proper understanding.

Root architecture, lateral root growth, and root symbiosis with host plants are all examples of how water and nutrient uptake rates can be changed to improve survival and production (Figure-1). Mutational analysis is a powerful technique for deciphering these processes and identifying genes that could be used to improve root function. The usage of numerous sorts of mutants will improve genetic studies on the detection of regulatory mechanisms. EST libraries and mutant protein/metabolite profiles, among other genomic and proteomic methods, add a new level to understanding root function (IAEA, 2006). Plant ecophysiology and genetics research should be combined to improve crop tolerance to adverse environments. Designing an optimum root system for a specific edaphic environment through gene mutation is ideal breeding without phenotypic selection (Uga, 2021; Li *et al.*, 2025). Therefore, this is highly essential to identify the unique QTLs or genes families or transcription factors accompanying yield attributing root traits in rice to design high-yielding cultivars. Keeping all the above facts in view, the present review illuminates the understanding of the genetic networks underlying in root-traits development and associated QTLs in rice and breeding innovation in the molecular era to develop an ideal climate resilient genotype thru root architectural trait modification.

II. DEVELOPMENT OF ROOTS IN RICE AND ITS GENETIC BASIS

Remarkable progress has been made to identify the genes or QTLs linked to the root traits in rice with the assistance of advanced molecular biology and biotechnology tools especially DNA sequencing technology. Most of the wild relatives of the cultivated crops have sufficient root systems to fight against drought by capturing water from a deeper layer. Therefore, genetic enhancement in the architecture of roots, regarded as an effective methodology to enhance the yield of crops. But, it is time-consuming and more laborious to select the below-ground parts (traits related to root) than aboveground traits. In rice including the wild species, a diverse variation for root characteristics has been observed. Different types of root traits in rice are illustrated in Figure-2 and listed in Table-1, which helps to withstand the plant under stress. The molecular mechanism involved in root improvement in rice has been discovered mostly based on QTL study and this was described by Champoux *et al.* (1995) for the first time. In rice, a number of QTLs have been found linked with root growth angle, length, thickness and volume, more or less which affect the RSA (Rebouillat *et al.*, 2009). A list of QTLs related to 29 root traits was summarized by Courtois *et al.* (2009) and many genes for root growth have been cloned in mutant genotypes with unusual root phenotypes (Li et al., 2025; Kong et al., 2024; Wu and Cheng, 2014). Yet, the hereditary mechanisms involved in these genes are understood poorly. Therefore, here, in brief we are discussing the genes/QTLs interrelated to quantitative variations of RSA in rice.

2.1. Genes controlling root Length and root number

Cell differentiation, expansion, and elasticity all contribute to root elongation. The investigation of mutant lines revealed the importance of quantitative trait loci (QTLs) underlying cell wall development, growth, and auxin signalling during root cell differentiation and elongation (Wang *et al.*, 2014). Two QTLs, QUICK ROOTING 1 (QRO1) and QRO2, were identified by Kitomi et al. (2018) as determinants of maximum root length. Transgenic rice overexpressing the *OsEXPA8* gene has managed to perform a variety of functions, including increasing crown root, seminal root, and lateral root length. Due to an increase in root and shoot vascular bundles, these also increase plant height, leaf size, and leaf number (Shin *et al.*, 2005). A low auxin concentration is most likely to blame for the short elongation zone. The far more important genomic regions containing QTLs for several traits (root length, root diameter, and root dry weight) were discovered on chromosomes number 1, 4, 9, 11, and 12 (Courtois *et al.*, 2003). Jonathan *et al.* (2015) discovered a QTL for total lateral root number; TLRN (*qTLRN-12*) flanked at 25.5 cM at the seedling stage (hydroponics system). The gene (*qLLRN-12*) which was discovered during the vegetative stage, controls lateral root number in rice.

2.2. Genes directing root growth angles

The environmental factors like temperature, light, water potential, and gravity are combined to control the root growth angle (Uga *et al.* 2015a). In rice, a few QTLs for the root gravitropism reaction have been identified (Manna *et al.*, 2025; Norton and Price, 2009), but the causal genes need to be identified. A significant QTL (DRO1), which plays a role in root development angle and gravitropism, was initially identified (Uga *et al.*, 2013a). Many other researchers have done extensive work on DRO series genes (*DRO2*, *DRO3*, *DRO4*, and *DRO5*) for growth angle root (Uga *et al.*, 2013b; Uga *et al.*, 2015b; Kitomi *et al.*, 2015). A variety "Kinandang Patong" is a prominent donor for this trait which was studied by the earlier researchers. This can be used as a contributing parent to tailor efficient rice genotypes.

2.3. Genes linked with other root related traits

The marker-assisted selection method was employed to fine-map a QTL (STELE TRANSVERSAL AREA 1; STA1) on chromosome 9 that influences root stele transversal area (Uga *et al.*, 2010). Other genes, such as *qRT9* and *STA1*, are close to *DRO1* (16.31 Mb), and the phenotypic roots can be distinguished by the proximity of these associated QTLs. Root plasticity is characterized as the ability of a plant to change the phenotype of its roots in reaction to varying environments (O'Toole and Bland, 1987), and it is critical for plant adaptation. QTLs for soil-surface rooting were reported by Uga *et al.* (2012) from the RIL populations (Gemdjah Beton × Sasanishiki). The female parent has surface rooting, while the male parent is without. The result confirmed that the QTLs located on chromosomes number 3, 4, 6, and 7 (*qSOR*, with major effect).

III. DECIPHERING THE ROLE OF ROOT ASSOCIATED QTLS IN IMPROVED NUTRIENT AND WATER ACQUISITION

Modern agriculture system faced major challenges to improve nutrient acquisition properties of crop plants under dynamic environmental conditions. Thus, RSA is an important trait for genetic improvement of nutrient acquisition from nutrient deficient soils (Kong *et al.,* 2014). The upper and lower crown roots, which emerged from each node's upper and lower sections, respectively (Rebouillat *et al.*, 2009). It's been observed that the crown roots closer to the ground are wider than those closer to the ceiling (Abe and Morita, 1994). The root system's vertical distribution is established by the relative growth inclination of the upper and lower crown roots. Roots are more likely to be dispersed in the topsoil when the root growth angle is shallow, and in the subsoil when the angle is steep. Each crown root can only grow so long, limiting the plant's ability to draw moisture and nutrients from the ground (Liu *et al.*, 2023; Ma *et al.*, 2025). Roots that are too short form compact root systems, whereas those that are too long produce vast root systems that are more resistant to lodging. A wide array of quantitative trait loci (QTLs) have been identified and characterized which significantly contribute to enhanced nutrient and water acquisition, especially under suboptimal or stress-prone environments (Table-2). These QTLs govern key root traits such as root length, root depth, root surface area, root hair density, root angle, and biomass allocation, which collectively determine the plant's efficiency in accessing soil nutrients and water.

3.1. QTLs for Nitrogen uptake

The main form of nitrogen under aerobic condition to make available to plants is Nitrate, and is leached by precipitation into subsoil. Therefore, root system architecture greatly affects the acquisition of water and nutrients from soil (Gewin, 2010; Liu et al., 2023). The rice *QTL DEEPER ROOTING 1* (*DRO1*) has been identified from the RIL population derived from the cross between 'IR 64' (lowland cultivar nonfunctional allele of *DOR*, shallow roots) and 'Kinandang Patong' (upland cultivar with functional allele of *DRO1*); and reported on the chromosome number 9 (Uga *et al.*, 2011) and has been cloned by Uga *et al.* (2013a). The yield performance of 'IR64' and *Dro1-NIL* was compared under upland field conditions with no drought, moderate drought, or severe drought (Uga *et al.*, 2013a). Under moderate drought in comparison with no drought, the grain weight of 'IR64' decreased by nearly half, whereas that of *Dro1-NIL* was almost the same. Under severe drought, the grain weight of 'IR64' was very low, whereas that of *Dro1-NIL* was more than 30% of that with no drought. This study suggests that deep rooting induced by *DRO1* enhances drought avoidance, resulting in higher grain yield (Uga *et al.*, 2013a). The *Dro1-NIL* showed about 10% higher grain yield than did 'IR64' irrespective of nitrogen treatment (Arai-Sanoh *et al.*, 2014). Comparison among cultivars with different root and shoot morphologies has suggested that deep roots increase grain yield in paddy (Kawata *et al.*, 1978; Morita *et al.*, 1988). There was no significant difference between IR64 and *Dro1-NIL* in nitrogen content before heading, but nitrogen uptake was higher after heading in *Dro1-NIL* than in IR64. The results suggest that deep rooting induced by *DRO1* enhances nitrogen uptake from lower soil layers, resulting in better grain filling (Uga *et al.*, 2015a). The *QTL NITRATE TRANSPORTER 1.1* has the ability to transport for the first time a functional link between root development, auxin and nitrate availability in soil (Puiga *et al.*, 2012). Low nitrogen (N) availability, in contrast to P constraint, encouraged the elongation of primary and LRs in particular, whereas LR density remained substantially unaltered (Lopez-Bucio *et al.*, 2003; Liu *et al.*, 2023; Gruber *et al.*, 2013). Such RSA alterations are expected to boost the plant's ability to more efficiently forage the soil in quest of hardly available nutrients, or to collect N before it leaches out of the rooting zone, as part of the 'steep, cheap, and deep' root ideotype advocated for maize (Fu *et al.*, 2023; Lynch, 2013).

3.2. QTLs for Phosphorus uptake

Phosphorus (P) is a key inorganic plant nutrient that is required for cell growth and division in living organisms. P fertilizer application for crop development has expanded dramatically in recent decades, yet P-use efficiency has decreased to a low of 10-20% (Wissuwa *et al.*, 1998). Furthermore, much of the applied P has polluted the environment severely. The development of cultivars that are more resistant to P deprivation is thought to be a viable solution to this problem. Rice growth and development are severely hampered by phosphorus shortage. P deficiency can cause plant growth to be stunted, resulting in dark green leaves, reduced root formation, and reduced tillering (Dobermann and Fairhurst, 2000). Under P stress, high sterility, maturity delaying, and plant height reduction are all common. In P-deficient soils, root extension has been reported in a variety of plant species (He *et al.*, 2003; Shimizu *et al.*, 2004). Three primary ways for plants to adjust to low P-deficiency are root P-interception, P acquisition efficiency, and internal P-use efficiency (Ismail *et al.*, 2007). Under P deficit, changes in root architecture are considered an adaptation that improves phosphorus uptake (Lynch, 1995). Much evidence indicates that root hair development is also stimulated in response to (high/low) phosphate (Desnos *et al.*, 2008). Results found that the effect of low phosphate on RSA is opposite to that of low nitrate. Shallow and shorter root architecture with more branches is an ideal trait for immobile resource acquisition such as phosphorus, potassium, iron, and manganese in topsoil. The optimal RSA is also related to the plant's carbon status, air temperature, and planting density (Postma *et al.*, 2014) because the topsoil tends to hold less water but more immobile nutrients such as phosphorus than does the subsoil (Gewin, 2010). Breeders have focused their efforts on developing rice cultivars that are resistant to P deficiency. In practise, P-deficiency tolerance has been determined by directly measuring dry weight or grain yield in low-phosphorus soils (Fageria *et al.*, 1988), or indirectly evaluating relative tiller number and relative dry weight (Fageria *et al.*, 1988; Chaubey *et al.*, 1994). However, previous efforts to generate tolerant high yield varieties have been impeded by the complexity of the characteristics involved in P-deficiency tolerance and the lack of a screening criterion suitable for use in breeding programmes. Several plant features are complicated quantitative qualities in nature that are impacted by many genes and the environment (Li *et al.*, 2003). QTL analysis is a powerful method for understanding the genetic basis of complicated traits like P-deficiency tolerance. In rice, certain QTLs for characteristics linked to P-deficiency tolerance have been discovered (Wu and Ni, 2000; Shimizu *et al.*, 2004, 2008). Using a recombinant inbred line (RIL) population from the rice cross between IR20 and IR55178-3B-9-3 cultivated in P-deficiency and P-sufficiency nutritional solutions, mapped QTLs for relative tillering ability, relative shoot dry weight, and relative root dry weight. Wissuwa *et al.* (1998) used BC lines cultivated in P-deficient soil to map QTLs for P absorption, internal P-use efficiency, dry weight, and tiller number. On chromosomes 6 and 12, Wissuwa *et al.* (2001, 2002) identified two QTLs for P uptake. In addition, in the Kasalath Gimbozu population, a QTL for P deficiency-induced root elongation was mapped on chromosome 6 and fine mapped (Shimizu *et al.*, 2004; Shimizu *et al.*, 2008). Using molecular markers to discover genetic factors and incorporate them into a high yield variety is one efficient way to improve rice's adaptability to P deficit. Only a few publications have been published on QTLs for P uptake, P use efficiency, and P-related characteristics

(Ismail *et al.*, 2007). The bulk of P-deficiency tolerance genes have yet to be discovered. Furthermore, earlier studies mainly looked at additive QTL, ignoring epistatic effects and QTL-environment (Q × E) interaction effects. As a result, more research is needed to identify genes linked to resistance to P-deficiency and to decipher its complicated genetic architecture. Introgression lines can be used to find QTL, detect hidden genetic variation, evaluate genetic interaction, and provide valuable resources for map-based clone and marker assisted breeding. Under P-deficiency and P-sufficiency circumstances, a report on a set of 271 introgression lines (ILs) was used to assess seedling responses to low P availability and to discover QTLs for root characteristics, biomass, and plant height. P-deficiency inhibited plant height, total dry weight, shoot dry weight, and root number, whereas P-deficiency stress enhanced maximum root length (MRL) and root-shoot ratio (RS). P-deficiency tolerance may be influenced by the two QTL *qRN5* (influence root number) and *qRDW5* (influence root dry weight) which is consistently expressed to promote trait stability (Anis *et al.*, 2018). Twelve intervals were used to cluster QTLs for P-deficiency tolerance, and one QTL (*qRRS8*) showed pleiotropic effects on both P-deficiency and drought tolerance, suggesting that these QTLs could be employed in future marker-assisted breeding programmes (Li *et al.*, 2009). In rice, overexpression of *PHOSPHORUS-STARVATION TOLERANCE 1* (*PSTOL1*) enhanced grain yield and P acquisition increased in P-deficient soil through regulation of RSA (Gamuyao *et al.*, 2012). *Pup1* (*Phosphorous uptake1*) having *PSTOL1* is suitable in improving the phosphate uptake under rainfed/upland conditions in rice (Shin *et al.*, 2020) and *Pup1* was mapped on chromosome 12 of traditional rice variety Kasalath (*aus*-type) (Wissuwa *et al.*, 2002).

3.3. QTLs for heavy metal stress

Cadmium (Cd) is an extremely poisonous heavy metal that can kill living things. Rice grains containing an excessive amount of Cd pose a major concern to persons who eat rice as a staple food. Chronic exposure to Cd may result in a variety of health issues (Bertin and Averbeck, 2006; Clemens *et al*, 2013). It is critical to produce premier rice cultivars with minimal Cd accumulation, particularly indica types, which can accumulate more Cd than japonica varieties. Tang *et al.* (2017) used the CRISPR/Cas9 method to generate a novel indica rice line with minimal Cd accumulation by altering the metal transporter gene *NRAMP5*. Because this transporter is involved in Cd uptake at the root, mutations in this gene result in a significant drop in Cd concentration. The ninth exon of the gene is targeted by two sgRNAs. The root growth angle influences the efficiency of nitrogen, phosphorous absorption; it might also affect the uptake of other minerals such as heavy metals. In Cd-contaminated soil, the grain and straw Cd concentrations were significantly higher in 'IR64' than in *Dro1-NIL* (Uga *et al.*, 2015a). Hence, the plants with shallow rooting will capture Cd from top soil layer and the allele occurring shallow rooting is a potential genetic resource for phytoremediation under high Cd accumulation. From food safety point of view, the allele giving deep rooting could be a useful resource to avoid absorbing the bioavailable Cd from topsoil (Uga *et al.*, 2015a).

3.4. QTLs for water uptake under moisture stress

Approximately half of the world's rice production depends on rain water which is grown in aerobic upland and rainfed lowland systems and plants are frequently exposed to unpredictable stages of drought stress (Singh and Chinnusamy, 2008). The plant's root is the primary site to perceive drought stress and to initiate a signaling cascade at the molecular level. Hence, an ideal root architecture with extensive root system is desirable for water stress (drought) condition as they maximizing water capturing ability and supporting shoot growth under drought conditions (Gowda *et al.*, 2011; Khan *et al.*, 2013; Mai *et al.*, 2014; Agrawal *et al.*, 2016). Plants adopt diverse strategies like enhance their water uptake ability by developing their root system (by increasing root density, deep rooting, and root/shoot ratio); improve their water-storage ability in specific organs; reduce their water loss (by leaf rolling and rapid stomatal closure); and accelerate or decelerate the conversion from vegetative to reproductive stage to avoid complete abortion in severe drought conditions to deal efficiently with water stress. The mechanisms of interactions between root system architecture and drought stress in rice would have a noticeable impact on overcoming drought stress (Gowda *et al*., 2011; Wu and Cheng, 2014). Early seedling vigor can cope with drought stress during the seedling stage, which is mostly determined by the environments of germination, genetic or inheritance pattern, and early seedling vigor (Zhang *et al*., 2004; Qun *et al*., 2007; Yang *et al*., 2015). Sandhu *et al*. (2014) investigated the seedling vigor under drought stress conditions in rice, and a QTL analysis was performed using genotyping-by-sequencing (GBS) technique. A total of 162 recombinant inbred lines (RILs) of rice derived from the cross of two varieties, Milyang23 and Tong88-7, were subjected to seedling vigor evaluations which are grown under water stress condition for two weeks. A total of 6 main-effect QTLs (M-QTLs) and 21 epistatic QTLs (E-QTLs) associated with root morphological traits were identified on all chromosomes and information will be useful for molecular breeding of drought-tolerant rice with higher seedling vigor Sandhu *et al*. (2014). Though enhancement in seedling vigor is a promising strategy to overcome the negative effect on plant growth under drought conditions (Rebolledo *et al*., 2013), the molecular basis for the seedling vigor under drought stress has not been fully explained. Root growth at soil depths below 30 cm may provide access to critical soil water reserves during drought in rainfed lowland rice. The lines evaluated by Henry *et al*. (2011) genotype Dular, which facilitate improvement in drought resistance in rice through dehydration avoidance and showed greater drought resistance associated with deep root growth and highest drought response index (less reduction in yield by drought stress). The QTL *qRFW9* reported by Price *et al*. (2002) for root fresh weight was detected only on chromosome 11 in rice and it might be a novel QTLs to develop molecular markers for breeding drought-tolerant rice varieties. To design new root ideotypes to adapt under diverse environmental stresses, amelioration of ideotype breeding with root trait QTLs through marker-assisted selection is a requirement (Coudert *et al*., 2010). For this, updated understanding of the genetic mechanism associated with root system architecture, information on gene networks involved in root formation has been accumulated (Coudert *et al*., 2010; Rebouillat *et al*., 2009). The identified QTLs advancing the rice breeding technology by understanding molecular biology and with the help of DNA sequencing technology.

IV. MOLECULAR APPROACHES TO REDESIGN THE UNDERGROUND HALF OF RICE

Roots are vital organs that help plants capture water and nutrients from the soil. The extent of the zone of the soil where water and nutrients can be obtained is determined by root system architecture (RSA). Because roots are front-line organs in the response to abiotic stresses such as drought, flooding, and salinity stress, it will be critical to improve belowground plant parts as well as aboveground plant parts as global climate change increases. However, because roots are hidden underground, conventional breeding focused on phenotypic selection makes it difficult to choose breeding lines with potential RSAs for abiotic stress adaptation. Design-oriented breeding of RSA without phenotypic selection is possible thanks to recent advancements in modelling, molecular biology and biotechnology (Holz *et al*., 2024; Uga, 2021). Integrated breeding strategies for developing climate-resilient rice genotypes with improved yield and root traits are highlighted in Figure-3. Improved root systems are critical for greater water and nutrient uptake, especially in systems with limited water supply, such as aerobic farming (Kharb *et al*., 2015; Meister *et al*., 2014). Increased water extractions are linked to the ability of roots to vary their response developmentally and functionally, and it is usually recognised as a key component feature for yield and adaptation during variations in soil moisture (Catolos *et al*., 2017; Phule *et al*., 2019). The findings implies that plants' ability to acquire mineral elements is linked to their root systems' ability to investigate the soil. Mutants with root systems that better utilise the soil, acquire more mineral elements, and produce higher yields on depleted soils can be created (White *et al*., 2009). Most root features for increasing yield in water-stressed situations are complicated in nature, making them difficult to incorporate into traditional breeding strategies (Jalil *et al*., 2018; Sandhu *et al*., 2019). Breeding for aerobic cultivars could be accelerated by finding Quantitative Trait Loci (QTL) related to features for aerobic adaptation using Marker-Assisted Selection (MAS). Therefore an hypothetical ideal root model is illustrated in Figure-4.

4.1. Mutation breeding to identify novel mutants

Using high-throughput genotyping, the populations are screened for mutations in genes of interest, and phenotypic changes are calculated (reverse genetics). Induced mutations for desirable features, such as root properties, provide a quick way to improve elite adapted germplasm for crop improvement. The creation of structured mutant populations for forward and reverse genetics aids mutant exploitation. These take advantage of the growing amount of sequence data available to confirm gene function. For model species like Arabidopsis, Medicago truncatula, *Lotus japonicus*, and rice, mutation grids or 'TILLING' populations have been produced, but agricultural plants like barley and wheat are also being developed (IAEA, 2006). As more sequencing data becomes available, this list will be expanded to include other species. Argentina (wheat), Australia (lupins), Belgium (annual plants), Brazil (wheat), China (soybean), China (wheat), Cuba (wheat), Germany (maize), Israel (annual food plants), Poland (barley), South Africa (*Vigna unguiculata* and *Vigna subterranean*), Turkey (barley and chickpea), United Kingdom (barley) are among the countries working on specific crops (IAEA, 2006). In comparison to control plants, overexpression of O. sativa *ROOT ARCHITECTURE ASSOCIATED 1* (*OsRAA1*) increases the quantity of crown and lateral roots (Ge *et al*. 2004). *OsRAA1* is an anaphase-promoting complex/cyclosome (APC/C)-targeted protein that stops the cell cycle from entering anaphase (Han *et al*. 2008). Although crown root initiation proceeds normally, a mutation in *rice CULLIN-ASSOCIATED AND NEDDYLATIONDISSOCIATED 1* (*OsCAND1*) causes a deficiency in the development of crown root primordia (Wang *et al*. 2011). In Arabidopsis thaliana, *CAND1* is a *SCFTIR1 E3* ubiquitin ligase involved in the degradation of *Aux/IAA* proteins in response to auxin (Chuang *et al*. 2004; Feng *et al*. 2004). *OsCAND1* is required for auxin signalling in the crown root meristem to maintain the G2/M cell cycle transition and, as a result, crown root emergence (Wang *et al*. 2011). The rice gene *AUXIN RESISTANT 1* (*OsAUX1*), which is evolutionarily related to the auxin influx carrier gene family *AUX1/LIKE AUX 1* (*LAX*), has T-DNA insertion mutations that lower the number of lateral roots (Zhao *et al*. 2015). The nal2 and nal3 double mutant (nal2/3) produces fewer lateral roots than the wild type due to mutations in two identical *OsWOX3A/OsNARROW SHEATH* (*OsNS*) genes on chromosomes 11 and 12, respectively (Cho *et al*. 2013). The decreased number of lateral root initiation in *nal2/3* appears to be due to a problem with endogenous IAA distribution mediated by changes in *OsPIN1* and *OsPIN2* expression (Cho *et al*. 2013).

4.2. Genetic engineering of root traits

Despite substantial limitations in our understanding of the genetic mechanisms that affect root system architecture in agricultural plants, multiple research have reported success in changing crop RSA features utilising various phenotyping methodologies and promoter and reporter expression. The bulk of these root-related genetic loci have not been addressed for transgenic manipulation thus far, and in a few cases, the impacts on RSA were accompanied by pleiotropic effects (Bian *et al*. 2012). Overexpression of transcription factors *OsNAC5/9* and *OsMYB2*, the receptor kinase *PSTOL1*, the G-protein coding Root Architecture Associated (*OsRAA1*), a cell wall extension gene *OsEXPA8*, and the identification of the *DRO1* allele for the deep root system have all been made in rice. There are several reports on RSA changes that boost nitrogen, phosphorus, and water utilisation efficiency, resulting in increased grain yield. Root characteristics are critical for enhancing grain yield, according to the findings. Deeper rooting QTL; *DRO1* (Uga *et al*. 2013b) and phosphorous uptake QTL; *PSTOL1* are two of the most extensively researched examples of where genes have been identified that bestow variations in root architecture (Gamuyao *et al*. 2012). Kinandang Patongan, a highland rice variety, was used to find the *DRO1* QTL. When roots develop longer and deeper, they are able to absorb more water from the deeper soil profile. The QTL, *DRO1* is a well-explained example of genetic alteration of root characteristics in rice, where root angle and rooting depth are targeted via breeding or transgenic techniques to achieve the steep-deep ideotype, as described by Uga *et al*. (2013b). The use of traditional breeding to introduce the *DRO1* allele and transgenic techniques to express the *DRO1* genomic fragment in IR64 gives evidence that steep-deep root architecture boosts yield under drought circumstances Uga *et al* (2013b). *PSTOL1* encodes a receptor-like kinase that has been demonstrated to increase root biomass and maps to a significant QTL for phosphorus shortage tolerance in rice (Gamuyao, *et al*. 2012). The finding of 23 differently regulated genes functionally related to root growth and stress responses came from transcriptional analysis of *PSTOL1* allele overexpression lines. The discovery of these QTLs emphasises the importance of root development and angle as features that influence drought tolerance and phosphorus shortage at the same time. Seo *et al*. (2008) found that *OsPT1* produced by a constitutive promoter in rice enhanced phosphorus concentrations in tissues over wild type, but the plants were shorter and had more tillers. Another phosphate transporter allele, *OsPT1.8*, was overexpressed in rice, and tissue phosphorus concentrations increased when plants were cultivated on high phosphorus levels, but not when levels were below 40 mg inorganic phosphorus/kg soil (Jia *et al*., 2011).

4.3. Mapping of major effect QTLs for root architectural traits

Roots are important for crop yield maintenance, which is critical when plants are grown in soils with insufficient water or nutrients (Bengough *et al*., 2011), and roots are one of the primary sites for stress signal perception, which triggers a cascade of gene expression responses in response to water deficit (Rabello *et al*., 2008). Previous research has shown that plant growth is mostly dependent on the severity of the stress; a slight water deficit inhibits the growth of leaves and stems, but roots can continue to extend (Peng *et al*., 2006). Furthermore, root architecture is an important characteristic for separating genotypic variants in rice responses to water shortages (Henry *et al*., 2011). To increase rice varieties' adaptability to aerobic conditions, a better understanding of the underlying physiological and molecular mechanisms is required. Upland rice's long and deep root system, the ratio of root weight to shoot weight, and root penetration ability contribute considerably to drought tolerance, according to Price and Tomos (1997). Several of the QTL discovered for root length are consistent among mapping populations (Courtois *et al*., 2009), and shared genomic areas for root thickness, root penetration, and stomatal behaviour have been identified across populations and even species (Zhang *et al*., 2001). In lowland and irrigated rice, progress has been made in finding large effect QTL conferring drought tolerance (Price and Tomos, 1997; Serraj *et al*., 2011). For both upland and lowland rice, many QTL for grain yield under drought stress have been identified (Bernier *et al*., 2007; Venuprasad *et al*., 2009). Sandhu *et al*. (2013) found 35 QTL related with 14 traits on chromosomes 1, 2, 5, 6, 8, 9, and 11 in the MASARB25 Pusa Basmati 1460 population and 14 QTL associated with nine traits on chromosomes 1, 2, 8, 9, 10, 11, and 12 in the HKR47 MAS26-derived population in their study. Three large-effect stable QTL for improved yield under aerobic settings, as well as QTL for various root-related features that are likely to boost water and nutrient intake under aerobic conditions, were discovered. Coexisting QTL for root and yield-attributing traits reveal a mechanism associated with better yield of promising lines under dry direct-seeded circumstances, indicating resource movement during grain filling (Sandhu *et al*., 2013).

4.4. Speeding up QTL introgression thru marker-assisted backcross (MABC) breeding

Four QTLs for root characteristics were introduced into an upland rice cultivar using marker-assisted backcross breeding. The QTLs have previously been discovered in a different genetic background under experimental conditions. The introgressed lines and the recurrent parent were grown for 6 years in highland areas in Eastern India by resource-poor farmers, and yields were reported. Under generally favourable field conditions, the QTLs improved yield by 1 $t.ha^{-1}$ when combined. Due to increased variation in soil-water availability in very low yielding conditions and resulting yield variability, the QTL effects were not identified in less favourable trials (Steele *et al.*, 2013). Introgressing QTL clusters into advanced backcross-derived lines and testcrosses resulted in an increase in grain yield, providing direct proof for the feasibility of boosting grain output by modifying root systems (Li *et al.*, 2015). *Deep Rooting 1* (*DRO1*), a significant locus influencing root development angle, was found in a bi-parental population of two rice lines with different drought tolerance by Uga *et al.* (2011). When *DRO1* was cloned and characterised, it was found to play a role in producing a steep root angle and, as a result, boosting drought tolerance and grain yield when introduced into the susceptible parent's genetic background (Uga *et al.*, 2013). Furthermore, using a specific allele of the *PHOSPHORUS STARVATION TOLERANCE 1* (*PSTOL1*) locus in rice or sorghum, a clear genetic relationship between root length and P acquisition as well as yield performance in phosphorus-deficient soil has been shown (Gamuyao *et al.*, 2012; Hufnagel *et al.*, 2014). Developing novel crop cultivars with improved root systems has the potential to improve resource use efficiency and plant adaptation to unstable climates when taken together. In the experimental population IR64 × Kinandang Patong, the locus *Deep Rooting 1* (*DRO1*) has been demonstrated to be a major factor for root angle (Uga *et al.*, 2013). Its orthologous gene in barley (MLOC 3895.5, 48.38 cM) was expected to be on Chr 5H (MLOC 3895.5, 48.38 cM) and mapped near an RSA QTL (*qRSA13*, Chr 5H, BOPA2 12 10899, 43.76 cM). *SCARECROW* (*SCR*) is a transcription factor that is expressed in the stele but moves out to govern endodermal development in Arabidopsis. It is activated by the transcription factor *SHORTROOT* (*SHR*), which is expressed in the stele but moves out to regulate endodermal differentiation (Cui *et al.*, 2007). Short roots are caused by mutations in either gene. *OsSCR1* and *OsSHR1* in rice have the same functional purpose as in Arabidopsis (Kamiya *et al.*, 2003; Cui *et al.*, 2007; Mai *et al.*, 2014).

4.5. Genome-wide association study (GWAS) and Identification of Candidate Genes

The development of emerging high-throughput genomic technology will allow harnessing the genetic diversity (Varshney *et al.*, 2014) for several agro-morphological traits to improve the yield under dry-DSR through genomics-assisted breeding (Kang *et al.*, 2016). As the whole-genome sequence is available, high-density SNP arrays allow for the identification of genetic markers, quantitative trait loci, and significant relations between marker traits *via* GWAS (Zhu *et al.*, 2008). A GWAS study designed for a total of 39 traits was conducted on a complex mapping population. A total of ten substantial marker-trait associations (MTAs) were discovered, as well as 25 QTLs associated with 25 traits (Sandhu *et al.*, 2019). Subedi *et al.* (2019) discovered 15 MTAs that are associated with root hair, root length density, nodal root, and culm diameter. A positive association of grain yield was noticed, related to root-morphological traits, seedling establishment traits, grain yield attributing traits, and nutrient uptake traits, with phenotypic variance ranging from 8% to 84%. According to the findings of this study, several root phenotypical traits are related to grain yield under dry-DSR conditions were reported (Sandhu *et al.*, 2019). Liu *et al.* (2020) conducted a GWAS study with 208 numbers of accessions in rice to identify polymorphic markers (SNPs), that are expressively allied with mesocotyl length. Ten of the sixteen distinct loci corresponded to known quantitative trait loci (QTL) or genes, while the remaining six were potentially novel loci (Liu *et al.*, 2020).

4.6. Genome Editing: to identify the allelic variation

To meet food supply demands and support sustainable development, it is critical to reduce genetic erosion and improve the yield of modern rice cultivars. Hu *et al.* (2019) used the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome editing technique to edit *Semi-Dwarf1* (*SD1*) and *Photosensitivity5* (*SE5*) in the elite landraces Kasalath and TTP (TeTePu), which contain many desirable agronomic traits such as low phosphorous tolerance (Gamuyao *et al* (Singh *et al.*, 2012). The results demonstrated that specific targeting of SD1 for gene editing in Kasalath or TTP produced in new lines with a semi-dwarf plant architecture, which is desirable in modern rice varieties, while retaining most of its progenitors' desired agronomic features (Hu *et al.*, 2019). Using gene editing on existing landraces, researchers can quickly boost genetic variety and create new kinds that meet current production needs. Plant development is negatively impacted by phosphorus deficiency. Phosphatic fertilisers could help to alleviate phosphorus deficiency, however rice varieties' low use efficiency of available phosphorus is a bottleneck that could have negative environmental repercussions. When breeding new rice varieties, it is critical to use landraces that possess genes for high phosphorus usage efficiency. Pup1 (also known as Pstol1), a key quantitative trait locus for phosphorus deficit tolerance, was discovered in Kasalath (Gamuyao *et al.*, 2012). Nipponbare does not have this gene. We conducted phenotypic analyses of Kasalath, sd1-3Kas, and sd1-5Kas in nutrient solution with low P (0.5 mg/L), CK (10 mg/L), and high P (25 mg/L) hydroponics solution over 18 days to see if the sd1 mutation in Kasalath affects phosphorus consumption efficiency. In comparison to Kasalath, the root lengths and surface area of sd1-3Kas and sd1-5Kas rose under low P, but decreased under CK and high-P concentration. These findings showed that in Kasalath mutants with low P, the sd1 mutation has no effect on PSTOL1 function (Hu *et al.*, 2019). *Semi-Dwarf1* (*SD1*) in the elite landraces Kasalath and TeTePu (TTP), which contain many desired agronomic traits such as tolerance to low phosphorous and broad-spectrum resistance to several diseases and insects, was edited using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein9 (Cas9) genome editing system. SD1 mutations result in shorter plants with improved lodging resistance (Hu *et al.*, 2019).

V. CONCLUDING REMARKS AND FUTURE OUTLOOK

Roots have been a neglected topic of research since they are difficult to examine because they are underground. Functional genomics and proteomics are providing powerful tools to help the scientific community examine root function and structure, ushering in a new age in root research. Roots information is directly relevant to agricultural/environmental issues including crop production with little environmental effect and crop production in low-input and stressed conditions. The Crop Root Research Consortium (CRRC) was formed in response to the recent spike in interest in roots. The CRRC's purpose is to coordinate the research activities of an ever-increasing number of research groups working on root screening methodologies, molecular marker creation, root architecture modelling, genetic potential evaluation, environmental effect, and plant breeding. The CRRC platform aims to expand and facilitate information and material exchange, promote concerted development of new resources (populations/libraries), and foster collaboration through voluntary networking within thematic sub-consortia, as well as joint research proposal submission under bi-lateral or multilateral cooperation. Screening for features and finding genes/QTLs to improve the acquisition of N, P, Zn, and Mn, enhancing the uptake and efficient use of water, and minimising the entry of harmful components into the food chain are among the current root-related research efforts on rice. Knowledge of the genes that influence mineral acquisition can be used to develop genotypes of other common crops that can be used in extreme environments to improve their ability to grow on resource-poor soils, increase mineral accumulation for animal nutrition, and reduce toxic element accumulation. These results should improve population health by boosting nutritious content and minimising harmful substances in food ingested, as well as increasing the sustainability of agriculture on both a subsistence and industrial level. However, understanding and being able to manipulate components of signalling networks and transduction pathways that respond to translocated signals, both of which occur before physiological adjustment, would allow for intervention at a far earlier and more essential stage of the response. Such knowledge, as well as the genes that control signal flow, would be new and strong weapons in the arsenal of plant breeders. Recent advances in forward and reverse genetic approaches including; Next-generation mapping (NGM), MutMap, Targeting Targeting Induced Local Lesions in Genome (TILLING), Eco-TILLING, and Clustered Regularly Interspaced Short Palindromic Repeats mediated genome editing (CRISPR/Cas), have enabled successful demonstration and isolation of casual mutation sites, genes, or QTLs accompanying with the specific trait of importance. Improvement in the high-throughput root phenotypic screening method coupled with automated data analysis can resolve some major issues which are faced during manual root screening. The characterization of root traits and identification of QTL for rot related traits are not much successful with the biparental mapping populations (e.g. RILs) which show segregation in subsequent generations.

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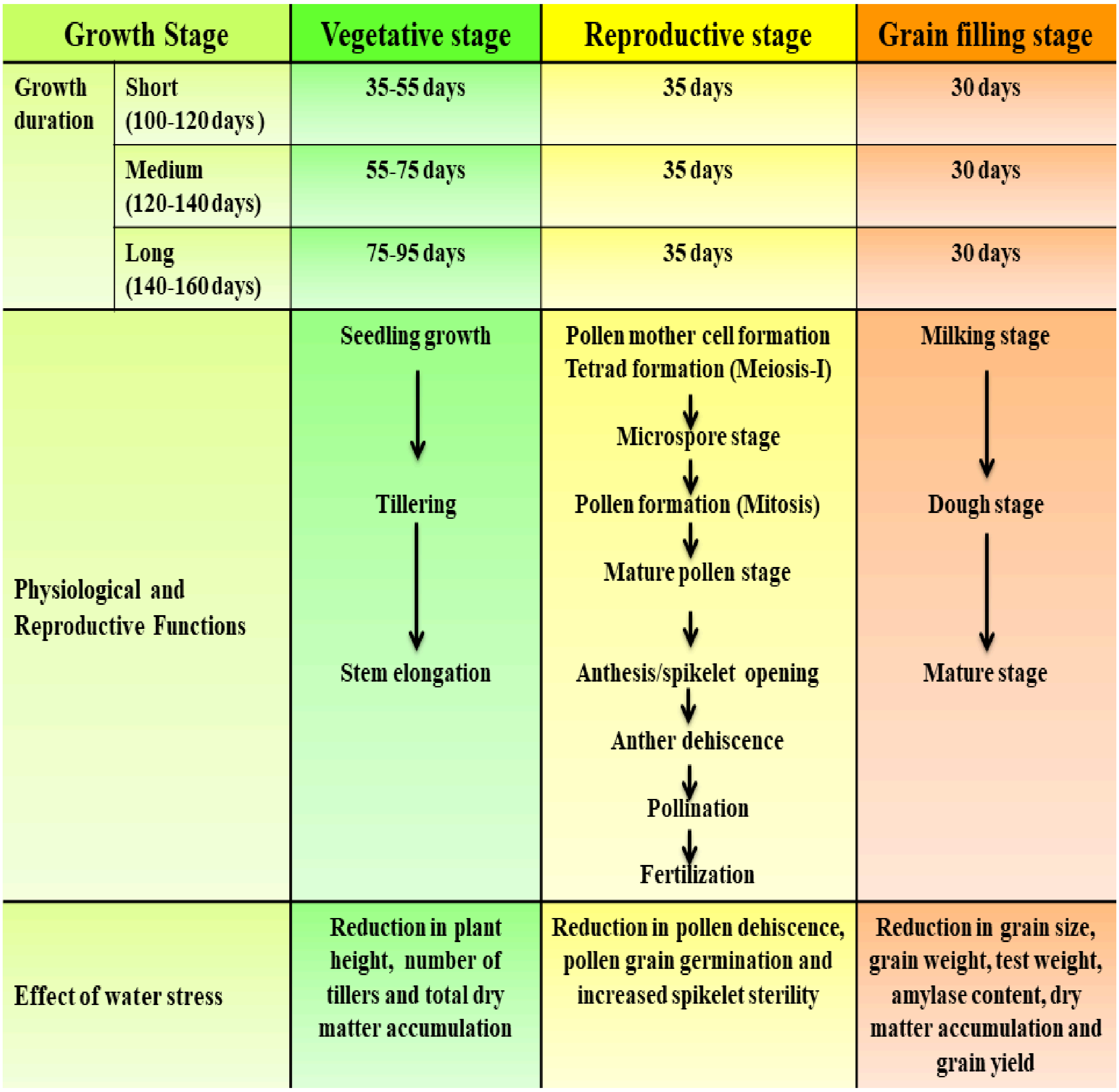
Table-1: Genes/QTLs controlling root traits development in Rice

Sl. No.	Root Traits	Genes/ QTLs	Position on Chromosome	Reference
1.	Root Length	*QRO1*	1	Kitomi *et al.* (2018)
		QRO2	2	
		OsEXPA8	8	Shin *et al.* (2005)
		$qRL_{8.1}$	8	Qu *et al.* (2008)
		$qRL_{8.2}$	8	Qu *et al.* (2008) Sandhu *et al.* (2013)
		$qRL_{9.1}$	9	Qu *et al.* (2008)
2.	Root number	*qTLRN-12*	12	Jonathan *et al.* (2015)
		qLLRN-12	12	

		qRN5	5	Anis *et al.* (2018)
3.	Root growth angles	*DRO1*	9	Uga *et al.* (2013a)
		DRO2	4	Uga *et al.* (2013b); Uga *et al.* (2015); Kitomi *et al.* (2015)
		DRO3	7	
		DRO4	2	
		DRO5	5	
4.	Root hair density	*qRHD*$_{1.1}$	1	Sandhu *et al.* (2015)
		qRHD$_{5.1}$	5	
5.	Root hair length	*qRHL*$_{1.1}$	1	Sandhu *et al.* (2015)
6.	Root volume	*qRV*$_{2.1}$	2	Bernier *et al.* (2007)
7.	Root lodging resistance	*SCM3*		Ma and Yamaji, (2006)
8.	Root stele	*STA1*	10	Uga *et al.* (2010)

Table-2: QTLs linked with improved Nutrient and Water Acquisition

Sl. No.	Nutrient and Water Acquisition	Genes/QTLs	Position on Chromosome	Reference
1.	Nitrogen uptake	*DRO1*	9	Uga *et al.* (2011); Uga *et al.* (2013a); Arai-Sanoh *et al.* (2014)
		NRT $_{1.1}$	1	Puiga *et al.* (2012)
2.	Phosphorous uptake	*qRN5*	5	Anis *et al.* (2018)
		qRDW5	5	
		qRRS8	5	Li *et al.* (2009)
		PSTOL1	12	Gamuyao *et al.* (2012) Shin *et al.* (2020)
		Pup1	12	Shin *et al.* (2020) Wissuwa *et al.* (2002)
3.	Heavy metal tolerance	*NRAMP5, OsLCD1*	7	Tang *et al.* (2017)
		Dro1-NIL	-	Uga *et al.* (2015a)
4.	Water uptake	*qRFW9*	11	Price *et al.* (2002)
		DRO1	9	Uga *et al.* (2013a)



Growth Stage		Vegetative stage	Reproductive stage	Grain filling stage
Growth duration	Short (100-120 days)	35-55 days	35 days	30 days
	Medium (120-140 days)	55-75 days	35 days	30 days
	Long (140-160 days)	75-95 days	35 days	30 days
Physiological and Reproductive Functions		Seedling growth ↓ Tillering ↓ Stem elongation	Pollen mother cell formation Tetrad formation (Meiosis-I) ↓ Microspore stage ↓ Pollen formation (Mitosis) ↓ Mature pollen stage ↓ Anthesis/spikelet opening ↓ Anther dehiscence ↓ Pollination ↓ Fertilization	Milking stage ↓ Dough stage ↓ Mature stage
Effect of water stress		Reduction in plant height, number of tillers and total dry matter accumulation	Reduction in pollen dehiscence, pollen grain germination and increased spikelet sterility	Reduction in grain size, grain weight, test weight, amylase content, dry matter accumulation and grain yield

Figure-1: An illustration for effect of water stress on different growth stages of rice.

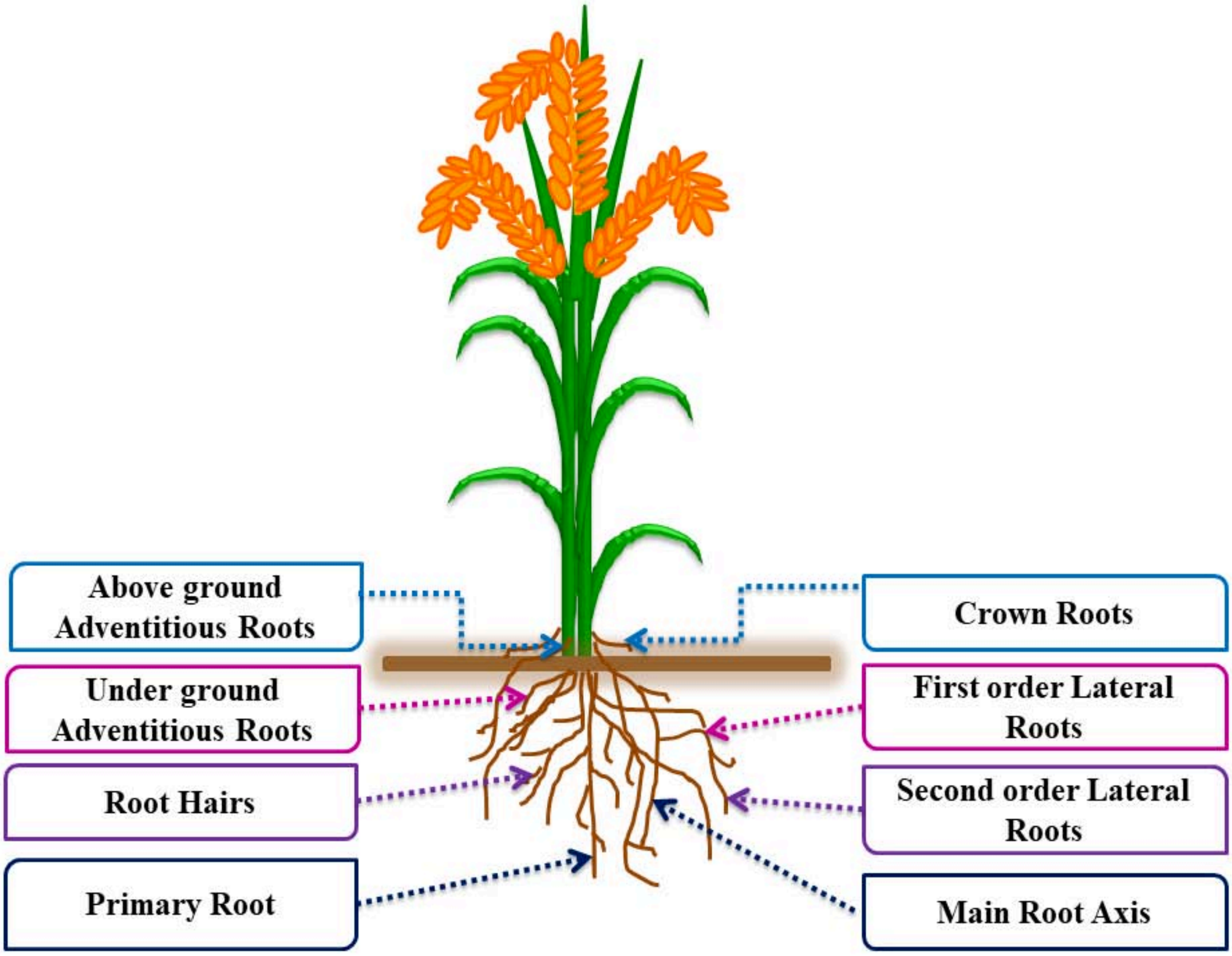


Figure-2: Structural Organization of the Rice Root System Showing Key Root Types and Their Hierarchies

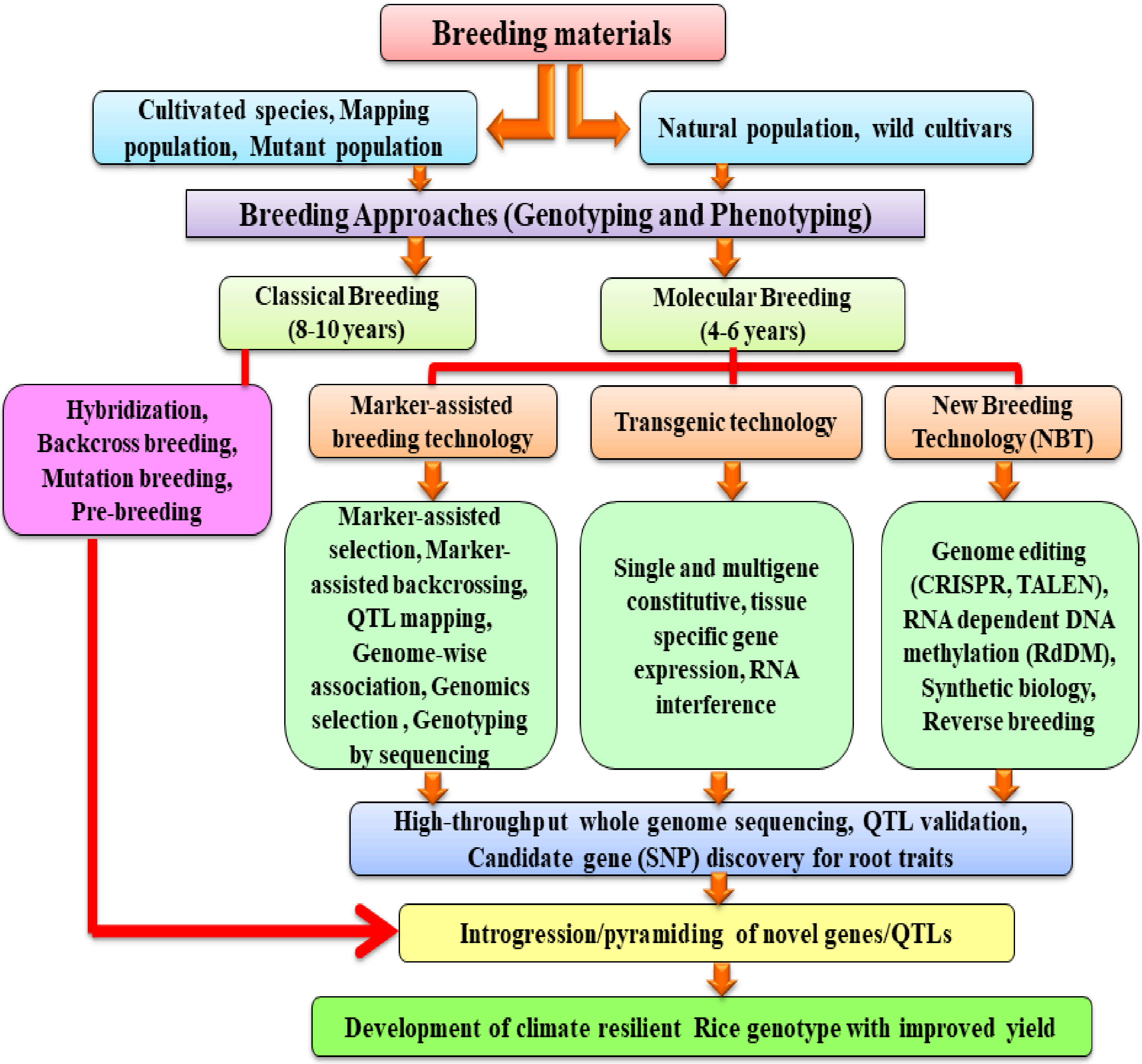


Figure-3: Integrated Breeding Strategies for Developing Climate-Resilient Rice Genotypes with Improved Yield and Root Traits

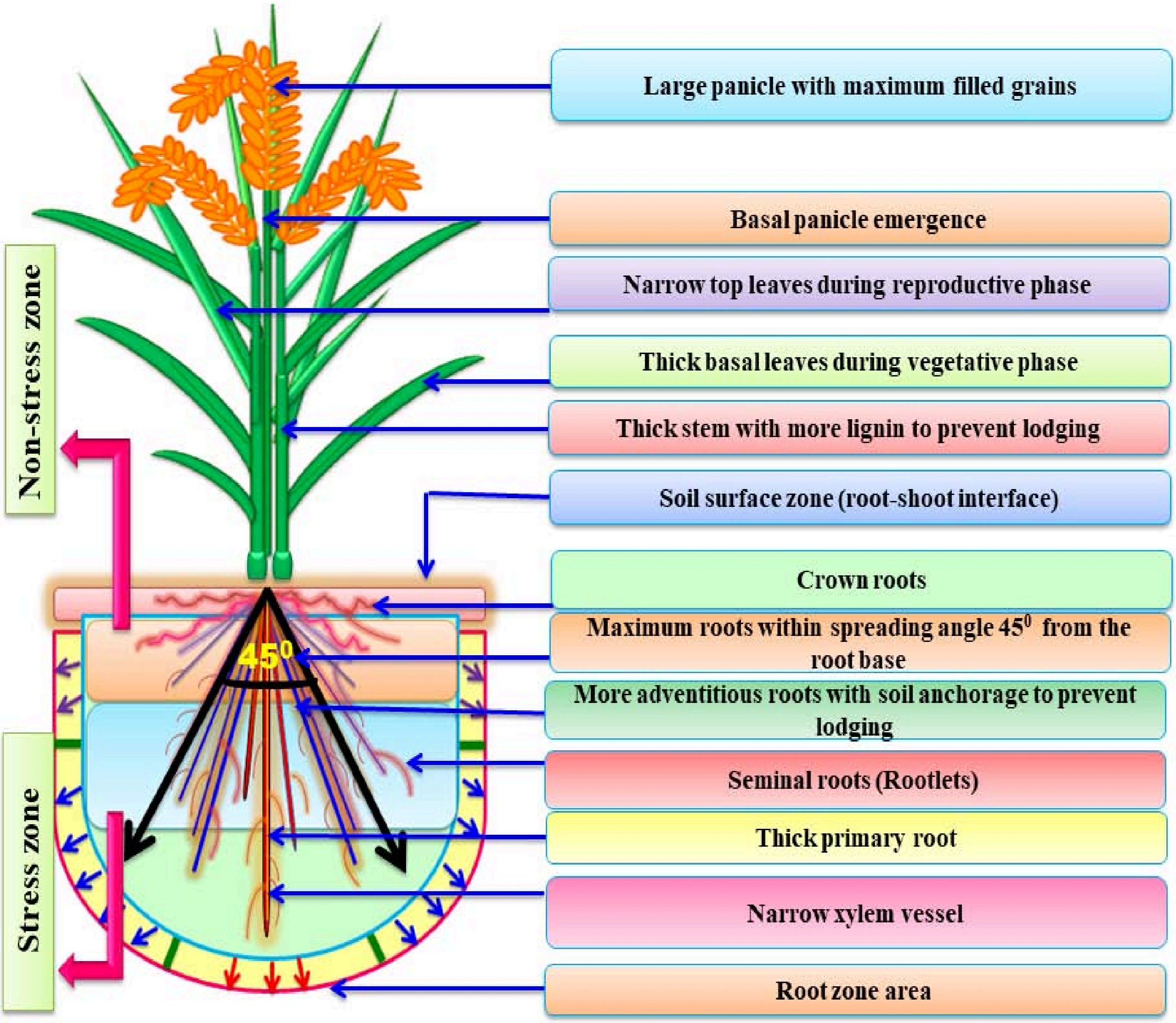


Figure-4: A hypothetical root ideotype for rice with improved dimorphic vigorous root system to optimize soil anchorage, water absorption and nutrient acquisition.

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Biodiversity and Conservation of Fruit Crops with their Wild Relatives: A Review

Ashok Kumar, Jagdish Grover, S.R. Singh, M.C. Yadav, Vijay Kumar Yadav, Arvind Kumar & Pooja Yadav

Guru Kashi University

ABSTRACT

The conservation of biodiversity within fruit crops is essential for sustaining both ecological and agricultural systems. Fruit crops, as integral components of nature's orchard, play a pivotal role in global food security and ecosystem health. India is one of the 12 mega biodiversity centres with 2 biodiversity hotspots which are the reservoirs of plant genetic resources. India stands at 7th place in the global agricultural biodiversity status. Among fruit and nut crops, there are about 117 cultivated species with 175 wild relatives of which only 25 species have been domesticated. Genetic resources conservation of fruit trees is intricate and complex as they are belonging to various genera and species which require specific climate. Hence, in situ and ex situ conservation can go simultaneously. The western ghat and North eastern India are centres of diversity for several important native fruits including Mango, Jackfruit and Citrus. Apart from the major fruit crops, India is home to several underutilized fruit crops. However, due to increased pressure on land use several of the wild types, which are a great source of genes governing useful traits, are disappearing. Thus, there is an urgent need to conserve them in both in situ and ex situ conditions. The genetic diversity and modes of conservation of tropical fruits are discussed in this paper.

Keywords: conservation, ex-situ, fruits, gis, germplasm, in- situ, tropical, varieties and wild species.

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Ashok Kumar[α], Jagdish Grover[σ], S.R. Singh[ρ], M.C. Yadav[Ѡ], Vijay Kumar Yadav[§],
Arvind Kumar[χ] & Pooja Yadav[v]

ABSTRACTS

The conservation of biodiversity within fruit crops is essential for sustaining both ecological and agricultural systems. Fruit crops, as integral components of nature's orchard, play a pivotal role in global food security and ecosystem health. India is one of the 12 mega biodiversity centres with 2 biodiversity hotspots which are the reservoirs of plant genetic resources. India stands at 7th place in the global agricultural biodiversity status. Among fruit and nut crops, there are about 117 cultivated species with 175 wild relatives of which only 25 species have been domesticated. Genetic resources conservation of fruit trees is intricate and complex as they are belonging to various genera and species which require specific climate. Hence, in situ and ex situ conservation can go simultaneously. The western ghat and North eastern India are centres of diversity for several important native fruits including Mango, Jackfruit and Citrus. Apart from the major fruit crops, India is home to several underutilized fruit crops. However, due to increased pressure on land use several of the wild types, which are a great source of genes governing useful traits, are disappearing. Thus, there is an urgent need to conserve them in both in situ and ex situ conditions. The genetic diversity and modes of conservation of tropical fruits are discussed in this paper.

Keywords: conservation, ex-situ, fruits, gis, germplasm, in- situ, tropical, varieties and wild species.

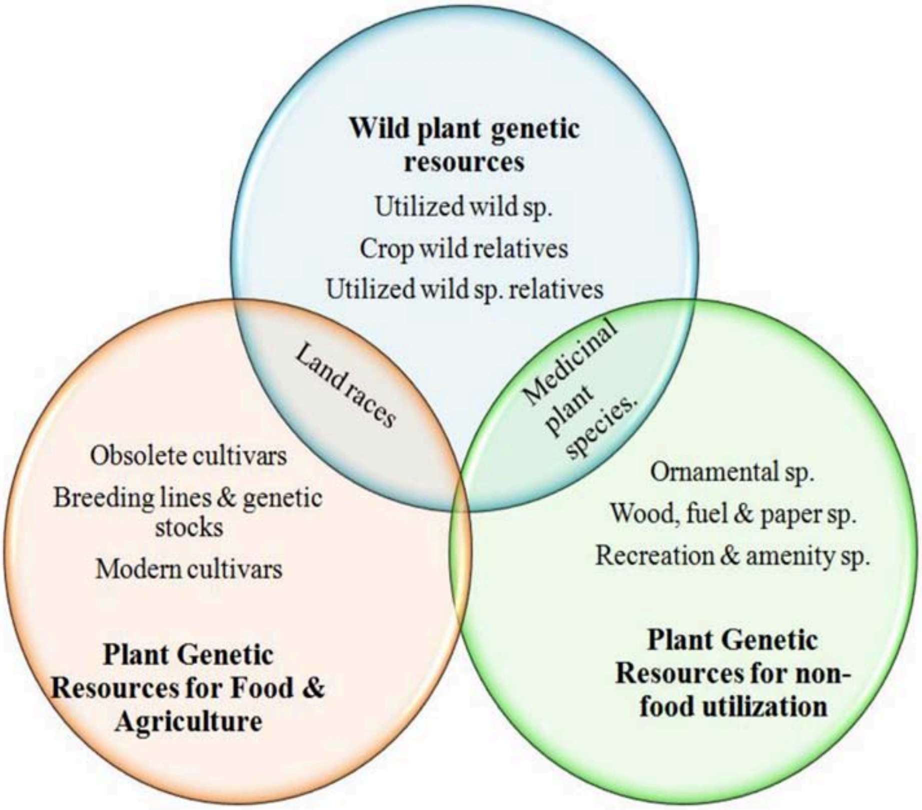
Author α σ: Professor Horticulture-Fruit Science, Guru Kashi University, Bhatinda-161302, Punjab, India.
ρ: Principal Scientist, Indian Institute of Sugarcane Research (ICAR), Lucknow, U.P., India.
Ѡ: Principal Scientist, NBPGR (ICAR), Pusa Campus, New Delhi-110012, India.
§: Professor Genetics & Plant Breeding, CSAUA&T- Kanpur, U.P., India.
χ: Principal Scientist, Indian Council of Agricultural Research, Head Quarters, Pusa Campus, New Delhi-110012.
v: Assistant Professor, Agrometeorology, Guru Kashi University, Bhatinda-161302, Punjab, India.

I. INTRODUCTION

India is one of the reservoirs of plant genetic resources which stand at 7th place in the globe in terms of richness of agricultural biodiversity. There are about 117 cultivated species of fruits and nuts with 175 wild relatives of which only 25 species have been domesticated for the use. Genetic resources conservation of fruit trees is intricate and complex in view of vast diversity of tropical, subtropical and temperate fruits germplasm belonging to various genera and species available in the country and consequent requirement of specific and complimentary conservation approaches encompassing both in situ and ex situ conservation. Plant genetic resources are of great importance as they form the basic raw materials to meet the current and future needs of crop improvement programmes. A wider genetic base, thus, assumes priority in plant breeding research aimed at developing new varieties for increased crop production. This diversity comprises of native landraces, local selections, elite cultivars and wild relatives of crop plants. The collection and conservation of this diversity in a systematic manner is the primary responsibility of all plant genetic resources institutes/ centres. The mention of use and cultivation of fruits can be seen in epics like 'Ramayana'. Plant genetic resources are thus our heritage, which need conservation for posterity. During the long period of domestication, utilization and cultivation, a wide array of fruit crop variability got generated by natural means and through both conscious and unconscious selection. Huge wealth of variability also got generated/adapted and diversified by crop introductions in the exotic environment or through migration of human population. Although, humankind has used only about 5,000 plant species worldwide to meet food and other needs, this number is just a fraction of the total world flora. With population growth, we are increasingly dependent on most productive plants. Today, only about 150 plant species are important in meeting the food (calories)needs of humans worldwide. Hence, there is a greater dependence on fewer plant species; 20 to 30species? in global context (Harlan, 1975). This gradually, has resulted in the loss of native genetic resources, which are otherwise essential as building blocks of genetic diversity. It is estimated that there are about 500 species of tropical fruit trees in Asia Pacific Oceania region, which include 30 families and 59 genera (Arora, 1998). In Southeast Asia alone, there are 120 major fruit species and 275 minor fruit species (Verheij and Coronel 1992). In Asia, 50-60species belong to the most important indigenous fruits (Arora and Rao 1996). Citrus, mango, banana, rambutan, jackfruit, litchi and durian occupy 80% of Total fruit production in the region.

II. WILD SPECIES AND DIVERSITY

The role of wild species in the fruit improvement programme is increasingly becoming important as the donor source for many of the disease and pest resistance. However, in most of the perennial trees, wild species or the indigenous germplasm has not been evaluated extensively either by morphological or by molecular means. Some geographical? areas may be richer in biodiversity than other areas, and some species may also have more variation than others in a particular area. Conservation of germplasm is very important, because many species are becoming extinct and many others are threatened and endangered. The diversity of some fruits is well documented, while for others relatively little work has been done (Arora, 1994). Gaps in collections are found both between species and between regions. This is especially true for both underutilized species and wild crop relatives, where big gaps are noted. Kostermans and Bompard (1993) indicate that *Mangifera blommesteinii, M. leschenaultii, M. superba* and .. *paludosa* are in real danger of extinction. High genetic erosion has been noticed for jackfruit, Citrus spp. and Litchi chinensis in a survey carried out by the International Centre for Underutilized Crops (ICUC) and IPGRI (Haq, 1994). It is to be mentioned here that collection and utilization of wild species is not an easy task, as they require specific climate and do not so easily get acclimatized to the ex- situ conditions on introduction.



Source: Priyanka et al. 2021. .

Fig. 1: Understanding the Impact of Climate Change on Biodiversity,

III. BIODIVERSITY OF FRUITS

The concept of origin of cultivated plants was first put forth by A de Candolle and the geographic centres of variability were described by Vavilov. He identified Asia as a major centre with "Indian centre" of North East region as primary or secondary centre of origin for many crop plants. This region is centre of diversity for several important native fruits including mango, jackfruit and citrus. The plant genetic resources represent a sum of the diversity that come from wild species and primitive forms, accumulated through evolution and natural selection, plant introduction, migration and domestication and the material developed by artificial selection and breeding. The North Eastern region had remained isolated for a long time even today the accessibility is poor to many parts of this region.

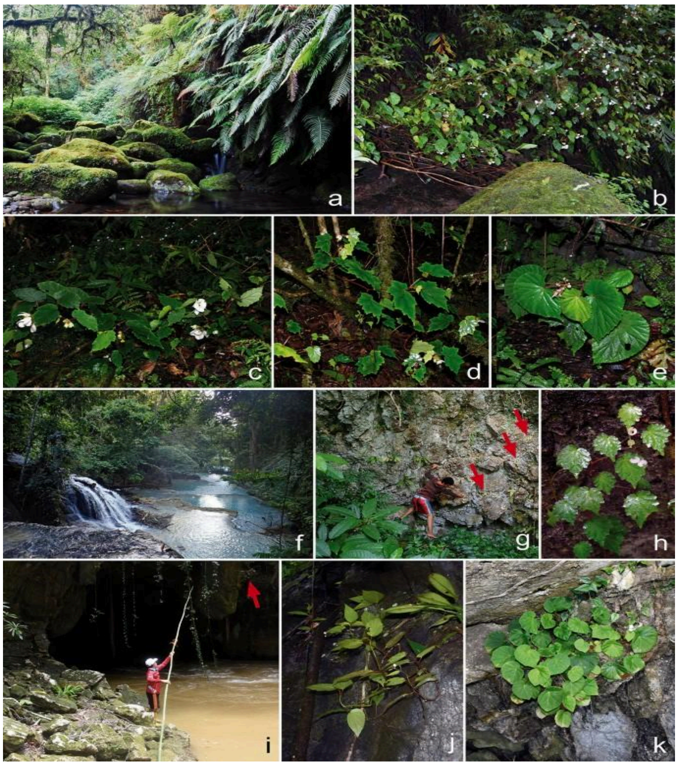


Fig. 2: Typical habitats and growth habits of Sulawesi *Begonia* species. (a) Stream in primary montane forest on Gunung Bawakaraeng, SW Sulawesi. (b) *Begonia bonthainensis* growing terrestrially on the stream bank (wider habitat shown in a). (c) *Begonia sanguineopilosa* growing terrestrially on the forest floor. (d) *Begonia rantemarioensis* growing terrestrially on the forest floor, on a steep slope. (e) *Begonia ozotothrix* growing terrestrially on the forest floor, at the base of a limestone boulder. (f) Stream on limestone bedrock in lowland forest close to Luwuk, eastern Central Sulawesi. (g) Vertical limestone wall at side of stream (wider habitat shown in f), red arrows indicate *Begonia willemii* individuals. (h) *Begonia willemii* growing lithophytically on a limestone wall (wider habitat shown in g). (i) Limestone karst landscape with river and cave in Matarombeo, SE Sulawesi; the red arrow indicates *Begonia watuwilensis* growing lithophytically on a stalactite. (j) *Begonia watuwilensis* growing lithophytically on limestone. (k) *Begonia matarom beoensis* growing lithophytically on a limestone cliff. *Photo credit: a, b, f–k, Wisnu H. Ardi; c–e, Daniel C. Thomas.*

The wet tropics with rain forests, undisturbed environmental conditions and variable altitudes are some of the major reasons for genetic diversity North-eastern Himalayas-wild, semi-wild cultivated species North-west-Semi-wild and cultivated types South-centre mostly cultivated types “Vast diversity in tropical and temperate Thuots cultivated and wild -109 species several wild, endangered and endemic species” Biodiversity can be located both in the wild or in the backyard. Regarding many of the tropical fruit species, the variability can be traced in wild, wherein many species grow naturally even today viz., the occurrence of Mangifera sylvatica in the North-eastern parts of India or M. antimanic and M. Nicobaric in Andaman group of islands. In the wild diversity was generated over a period mainly because of spontaneous mutants and the dispersal of seeds and seedling population. Seedling populations have been the source of diversity in the backyard as noticed in the case of fruits like mango and jackfruit. Diversity due to natural means has come about due to the seed dispersal as in pickling types of mango viz., Appemidi types in Uttara Kannada district of Karnataka or varietal wealth found in the Western Ghat regions.

IV. CHARACTERISTIC FEATURES OF TROPICAL FRUIT TREE DIVERSITY

The main causes for the tropical fruit diversity in India be it mango or an underutilized fruit like jamun, whether in the wild or in the cultivated types have been;

- the presence of high heterozygosity
- cross pollination
- seed propagation
- absence of vegetative propagation in the earlier day's indiscriminate multiplication.

Unlike other crops, where there is a need to create variability, in tropical fruit species, it is the management of diversity, which is the more challenging task. In fact, in crops like mango, the varietal diversity itself is considered as a hindrance to the improvement (Naik et al., 1958).

Sources: Sreekumar, V.B., Sreejith, K.A., Hareesh, V.S. et al.,2020.

Fig. 3: Fruits of wild edible plants grown in the eastern Himalayas, India.

V. BIODIVERSITY AND CONSERVATION OF TROPICAL FRUIT TREES

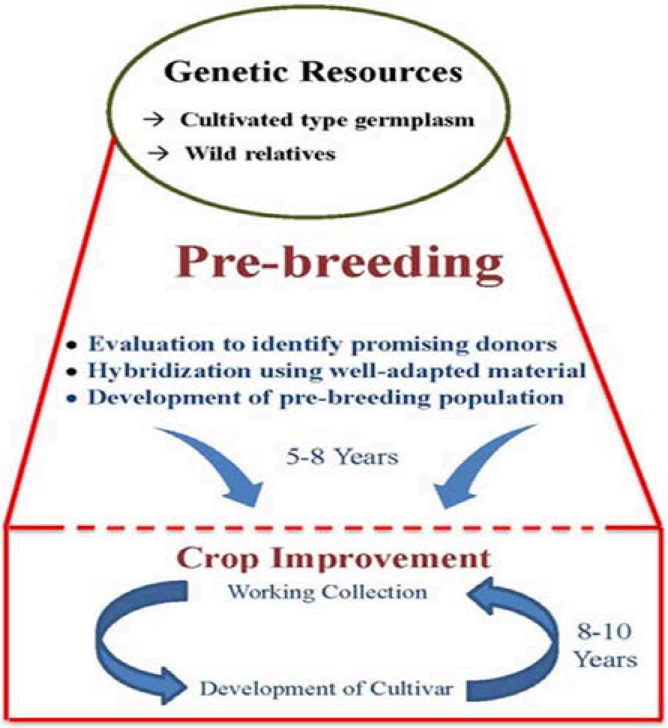
Conservation of plant genetic resources is undertaken at genotype, gene pool, species and ecosystem level using diverse approaches. Plant genetic resources conservation is possible using in situ and ex situ approaches wherein each approach extends further options depending on the biological status, propagation method and population size of the species. Vast genetic diversity of underutilized fruits represents varied germplasm of wild, semiwild species, genetic stocks, cultivars, farmers selections etc. requiring application of more than one method of conservation. It is, therefore, emphasized that a complementary conservation strategy (Rao, 1998; Rao and Sthapit, 2013), involving the use of more than one relevant approach (in situ and ex situ) would be the best option for achieving safe conservation of these underutilized fruit species facing severe threat of extinction. There is big challenge to protect and conserve wild and semi-wild species of several major and minor fruits. Most of the wild species of these fruits occur in the protected areas and buffer zones of forest reserves and National parks. More over regeneration capacity and population size of some of the species is highly inadequate which is a matter of further concern and there is a probability of these being pushed to rare and endangered category (Malik *et al.,* 2006). Due to various developmental projects and changing climate these areas have become highly vulnerable and there is an urgent need to protect and collect the existing important plant diversity for safe exsitu conservation. Coordination with forest department and joint programmes with Ministry of Environment, Forest and Climate Change is imminent to collect the germplasm and to ensure suitable in situ conservation measures.

In situ conservation of tropical fruit tree species is one of the most important aspects in the overall conservation of fruit diversity (Dinesh, 2001). It is well known that many of the species of mango, when introduced to other areas do not perform well or die. It is observed that the Mangifera andamanica, M. camptosperma and M. griffithi when introduced to mild tropics could not survive (Prakash, 2001). It is to be mentioned here that inspite of innumerable problems that are faced in the ex situ conservation, it is still advantageous to maintain them in the field gene bank, as it keeps the biodiversity of a particular species safe when plants are destroyed in the wild. Hence, to rationalize the concept of core collection was introduced, which in a limited set represents the genetic spectrum in the whole collections (Brown, 1989). It is proposed that landraces should be preserved for future generations as they harbour a diversity of interesting traits for future breeding work, for developing new farming systems and moreover, reflect the cultural identity of certain groups of people (Altieri and Merrick, 1987).

Until recently, germplasm conservation of crop landraces, as well as of their wild relatives, relied on ex situ methods (i.e., the conservation of biological material outside its natural habitat, UNCED 1992), mostly in germplasm banks. More recently in situ (on-farm) conservation (i.e. the conservation of biological diversity in its natural habitat) has been proposed as a conservation strategy which allows evolutionary processes to continue rather than being halted as occurs in ex situ conservation (Frankel *et al.,* 1995; Maxted *et al.,* 1997). Fragaria (strawberries), Rubus (raspberries, blackberries) Vaccinium (blueberries, cranberries, lingonberries) and Ribes (currants and gooseberries) are important small fruit crop plant genera in temperate climates. They are predominantly woody perennial dicotyledonous angiosperms (Galletta and Himelrick, 1990). Small fruits are generally heterozygous and do not reproduce true-to-type from seeds. The fruits are fleshy, more-or-less edible with very high levels of vitamin C, cellulose, pectin and anthocyanins possession antitumor, antiulcer, antioxidant and anti-inflammatory activities (Wang et al., 1999). They are highly prized for their varying shapes, textures, Flavours and colours. Wide genetic diversity within a species is required for its survival and adaptation to changing environments. As in other crops, domestication in small fruit crops has limited the genetic diversity to useful genotypes, adapted to the needs of humans as well as local growing conditions. It is necessary to develop reliable methods for characterisation of small fruit germplasm and to assess their genetic diversity/relatedness for practical breeding purposes and proprietary-rights protection. Molecular markers such as DNA-based markers, which allow direct comparison of different genetic material independent of environmental influences, are increasingly used in breeding programs of many horticultural crops.

5.1 Mango

Mango is native to India. Mukherjee (1949,1985) opined that this genus might have originated in the region covering Burma, Siam, Indo-China and Malayan peninsula. The genetic diversity of mango available in India is very rich and at present more than one thousand vegetatively propagated varieties exists in the country. Clonal selection, selections from chance seedlings and breeding efforts have resulted in identification of many elite improved varieties of mango for commercial cultivation in the country. All varieties in mango belong to one species Mangifera indica. Apart from indica, India is also reported to be the home of four other species viz., *andamanica, M. khasiana, M. sylvatica and M. camptosperma* (Mukherjee et al., 1985). The species of Mangifera occur mainly as complex biotic community in tropical humid forests, sub-tropical rain forests and tropical dry forests/woodlands of Indo-Malayan biogeographic realm (Mukherjee, 1985). The Mangifera germplasm can be classified under two categories; 1. Seedling races: This group includes both wild and cultivated types. Under this category the cultivated ones come under the monoembryonic types. The polyembryonic types are seen generally in the Western Ghats of Peninsular India.



Sources: Dr., V. Ramanath Rao

Fig. 4: Collaboration is the key to effective biodiversity conservation

Horticultural races: They include varieties, which when grown under different agro-climatic conditions and propagated vegetatively from the parent material have given rise to clonal variation Varieties like Alphonso, Dashehari and Langra are noticed to have clones resembling them in some of the morphological characters. Yadav and Singh (1985) opined that mango varieties of Northern and Southern regions belong to two different eco-geographic regions. Centres of mango diversity and distribution in India In India, seven centres of mango diversity have been recognized (Yadav and Rajan, 1993). These are the places where maximum diversity has been noticed for species as well as varietal diversity. They are

- Humid Tropical region-Manipur, Tripura, Mizoram and S. Assam
- Chota Nagpur Plateau-Trijunction of Orissa, Bihar and Madhya Pradesh
- Santal Paraganas in Bihar
- South Madhya Pradesh adjoining Orissa and Andhra Pradesh Dhar Plateau of Madhya Pradesh adjoining Gujarat and Maharashtra
- Humid Tropical South Peninsular India
- Andaman and Nicobar Islands

Varietal diversity

In India about thousand varieties of mango are grown. Most of these varieties have arisen as chance seedlings. Each mango-growing region in India grows a different variety. Although, there are more than thousand varieties have been documented in India of which only twenty f ive varieties are cultivated on a commercial scale in different states. Most of the commercial varieties have arisen as a result of selection from seedling types for different fruit characteristics like colour, taste, flavour, size and bearing habit. Although, growth in mango is genetically controlled, the environmental interaction has brought about the change in growth pattern under different agro climatic conditions, which also has contributed for its biodiversity. In India, three main centres contributed to of the diversity of mango i.e., Lucknow -Saharanpur belt of Uttar Pradesh, Murshidabad area of West Bengal and Hyderabad area of Andhra Pradesh. Most of the varieties in these areas have specific fruit? characteristics, require specific climate for optimum performance and have strong regional consumer preference.

5.2. Citrus

The North East hilly region is rich in fruits, vegetables and flowers, especially orchids. It is considered as a centre of origin of Mandarins and few other citrus fruits. Sixteen species of Citrus, 52 varieties, and seven natural hybrids of Assam were described by Bhattacharya and Dutta as early as 1956. They also reported two species of sub genus *Eucitrus* viz., *C. indica* and *C. assamensis* and three species of sub genus of Papeda viz., *C. ichangensis, C. latipes and C. microptera* which grow at high altitudes. *C. indica* is considered to be the most primitive species of citrus and probable progenitor of cultivated species. Diverse forms of Pummelo, Sour Orange, Rough Lemon, Sour Pummelo, Adajamir Sweet Lime etc. are found in this region.

Varietal diversity

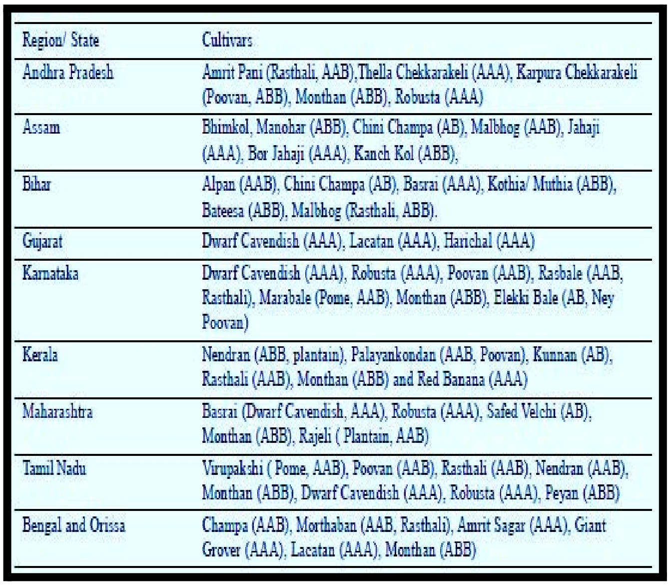
Mandarin orange is concentrated in Maharasthra (Nagpur, Amaravathi, Wardha and Yavatmal), North East region of India (Assam, Arunachal Pradesh and Meghalaya), limited area of Karnataka (Kodagu), Tamil Nadu (Nilgris, Palani and Shevroy hills) and Kerala (Wynad). Sathpura hills of Madhya Pradesh adjoining Vidharbha region of Maharasthra also grow good quality mandarins. Kinnow Orange, a hybrid of King X Willow Leaf Mandarin has recently spread in North West India, especially in Punjab, parts of Himachal Pradesh, Uttar Pradesh and Rajasthan. Cultivation of introduced varieties / hybrids also add to the varietal diversity by throwing spontaneous mutants over a period. Sweet oranges are adapted well to arid tropics and sub tropics. They are commercially grown in Andhra Pradesh, Maharasthra, Punjab and parts of Tamil Nadu, Rajasthan and Utter Pradesh. in Andhra Pradesh Sweet orange cultivar Sathgudi is grown, whereas in Western and Central India sweet orange cultivar Mosambi is popular. In North Western India the cultivars Malta, Jaffa and Valencia are popular. Acid lime is grown on a commercial scale in Tamil Nadu, Andhra Pradesh, Maharashtra and Karnataka states. Lemons are grown commercially only on a limited scale. Eureka lemon in some regions and Assam lemon in North Eastern India are popular varieties under cultivation.

5.3. Banana

Bananas are one of the ancient fruits cultivated by man. It could be assumed that the fruit has evolved with the civilization (Krishnamurthi and Seshadri 1958) and found in Indus valley as early as in 327 B. C. Apart from its mention in Valmiki's Ramayana, it also finds a mention in Kautilya's Arthshastra and ancient Tamil classic Silappadikaram. These evidences suggested the early existence of banana in India. The wild Musa acuminata occurs in Assam, Burma, Siam, Indo-China, the Malayan peninsula and Archipelago and the Philippines The northeastern region of India - including the states of Assam, Arunchal Pradesh, Tripura and Mizoram, lie at a point where Musa balbisiana from the Indian subcontinent meets Musa acuminata from Southeast Asia.

Fig. 5: Diversity of Fruits and their wild relatives

Table 1: Distribution of banana cultivars in India



Region/ State	Cultivars
Andhra Pradesh	Amrit Pani (Rasthali, AAB),Thella Chekkarakeli (AAA), Karpura Chekkarakeli (Poovan, ABB), Monthan (ABB), Robusta (AAA)
Assam	Bhimkol, Manohar (ABB), Chini Champa (AB), Malbhog (AAB), Jahaji (AAA), Bor Jahaji (AAA), Kanch Kol (ABB),
Bihar	Alpan (AAB), Chini Champa (AB), Basrai (AAA), Kothia/ Muthia (ABB), Bateesa (ABB), Malbhog (Rasthali, ABB).
Gujarat	Dwarf Cavendish (AAA), Lacatan (AAA), Harichal (AAA)
Karnataka	Dwarf Cavendish (AAA), Robusta (AAA), Poovan (AAB), Rasbale (AAB, Rasthali), Marabale (Pome, AAB), Monthan (ABB), Elekki Bale (AB, Ney Poovan)
Kerala	Nendran (ABB, plantain), Palayankondan (AAB, Poovan), Kunnan (AB), Rasthali (AAB), Monthan (ABB) and Red Banana (AAA)
Maharashtra	Basrai (Dwarf Cavendish, AAA), Robusta (AAA), Safed Velchi (AB), Monthan (ABB), Rajeli (Plantain, AAB)
Tamil Nadu	Virupakshi (Pome, AAB), Poovan (AAB), Rasthali (AAB), Nendran (AAB), Monthan (ABB), Dwarf Cavendish (AAA), Robusta (AAA), Peyan (ABB)
Bengal and Orissa	Champa (AAB), Morthaban (AAB, Rasthali), Amrit Sagar (AAA), Giant Grover (AAA), Lacatan (AAA), Monthan (ABB)

These two species, as well as other wild relatives have mingled to form a distinctive concentration of genetic diversity, occurring in the semi-evergreen, sub-tropical forests of the hill slopes. Further sources of diversity occur in the valleys and plains where bananas are common as a backyard crop. The habitat of wild bananas is shared with tribal groups who practice a form of shithing cultivation. Unfortunately, a growing number of sites where wild bananas once grew are now denuded. Wild species found in North eastern part of India include Musa acuminata, Musa balbisiana, several species from the section Rhodochlamys as well as Ensete glaucum. The diploid and triploid acuminatacultivars were taken by man to the native areas of balbisiana, which resulted in natural hybridization and formation of hybrid progeny with the genomes: AA, AAB, and ABB. It is thought that subsequent dispersal of edible bananas out of Asia was brought about by man. Secondary diversification with-in the groups of cultivated bananas are the result of somatic mutations. Eumusa and Rhodochlamys are found in Assam region of India and Thailand whereas Callimusa and Rhodochlamys in Borneo, its surrounding the species V. vinifera, has not originated in India.

5.4. GRAPE (Vitis spp.)

European grape V. vinifera is considered to have originated primarily between Caspian and Black Sea, and considered a hybrid between two American spp. V. vulpine and V. labrusca. It also resembles V. parviflora and V. lanata which are found in Himalayan region. This region may be considered as a secondary centre of origin. Native spp. resembling *Vitis lanata* and palmata grows wild in the northwestern Himalayan foothills. Indigenous varieties known as 'Rangspay', 'Shonltu White' and 'Shonltu Red' are grown in Himachal Pradesh even today. Famous Indian medicine scholars, Sushruta and Charaka in their medical treatises entitled 'Sushruta Samhita' and 'Charaka Samhita', respectively, written during 1356-1220 BC, mentioned the medicinal properties of grapes. Kautilya in his 'Arthashastra' written in the fourth century BC, mentioned the type of land suitable for grape cultivation. Cultivated grapes are believed to have been introduced into the north of India by the Persian invaders in 1300 AD, from where they were introduced into the southern parts of India (Daulatabad in Aurangabad district of Maharashtra) during the historic event of changing the capital from Delhi to Daulatabad by King Mohammed-bin-Tughlak. Ibn Batuta, a Moorish traveller who visited Daulatabad in 1430 AD, reported to have seen flourishing vineyards in south India.

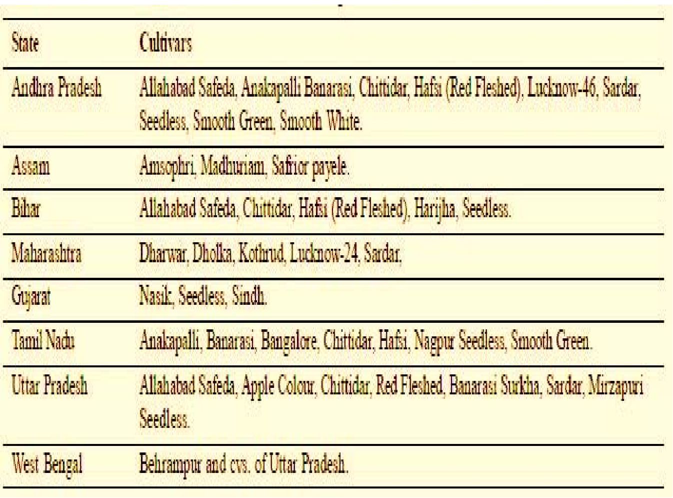
Table 2: Distribution of Vitis species in India

Species	Region	Salient Characters
V. riparia	North Westeran Himalayan region	Small berries, purple black in colour, cold hardy, early flowering
V. lanata	Himalayan region	Purple black berries known for crack resistance, and plant resistant to diseases
V. barbata	Parts of Assam, Khasi hills and Bengal	–
V. parviflora	North West Himalayas from Kashmir to Nepal	Small berries, delicately flavoured
V. tomentosa	Greater part of Deccan peninsula	–

5.5. Guava

Guava is an important fruit crop of India. It is said to have originated from tropical America. It is widely distributed all over the equatorial regions of tropical and sub-tropical climate. Guava is reported to have been introduced during Seventeenth century into India. It has gained considerable prominence on account of its high nutritive value, availability at moderate prices, pleasant aroma and good flavour. It is one of the commonest fruits liked by the rich and the poor alike and is popularly known as the 'apple of tropics. At present, it is grown throughout the length and breadth of the country from sea level to 1300 m altitude and is so much acclimatized that it appears to be native to India. The most important guava growing states are Uttar Pradesh, Bihar, Madhya Pradesh and Maharashtra. The genus Psidium of Myrtacease family comprises of about 150 species of small trees and shrubs. About 20 species have edible fruits, of which the most cultivated is the common guava i.e., Psidium guajava L. It has been reported that the value of the wild Psidium species mainly lies in their utility as rootstocks for regulation of vigour, fruit quality and resistance to pests and diseases. Varietal diversity Guava is mainly a self-pollinated crop but crosspollination is also common. This has resulted in large variability in the seedling population from which promising genotypes have been selected in different agro climatic regions of the country. In India different workers in different regions have described guava varieties. The main centre of variability in guava has been the Allahabad area in Uttar Pradesh. The promising cultivars of different states are given as follows;

Table 3: Distribution of guava varieties in India



State	Cultivars
Andhra Pradesh	Allahabad Safeda, Anakapalli Banarasi, Chittidar, Hafsi (Red Fleshed), Lucknow-46, Sardar, Seedless, Smooth Green, Smooth White.
Assam	Amsophri, Madhuriam, Safrior payele.
Bihar	Allahabad Safeda, Chittidar, Hafsi (Red Fleshed), Harijha, Seedless.
Maharashtra	Dharwar, Dholka, Kothrud, Lucknow-24, Sardar.
Gujarat	Nasik, Seedless, Sindh.
Tamil Nadu	Anakapalli, Banarasi, Bangalore, Chittidar, Hafsi, Nagpur Seedless, Smooth Green.
Uttar Pradesh	Allahabad Safeda, Apple Colour, Chittidar, Red Fleshed, Banarasi Surkha, Sardar, Mirzapuri Seedless.
West Bengal	Behrampur and cvs. of Uttar Pradesh.

I In India, due to seed propagation, varietal diversity is seen for guava, but species diversity is not observed.

5.6. PAPAYA

The papaya (Carica papaya L.) is one of the most important fruit crops valued for its rich nutrient content. It is a rich source of Vitamin A (2020 I.U), Vitamin B1 (40 mg), Vitamin C (46mg), protein (0.5%) and mineral matters (0.4%). Papaya is native to tropical America; its place of origin is said to be in southern Mexico and Costa Rica. It was taken to Manila by Spanish in the mid-16th century, reached Malacca shortly the rewards. It was introduced into India during 16th century. It is grown both in tropical and sub-tropical parts of the world. Wild diversity is not reported in India for papaya.

Table 4: Distribution of papaya varieties in India

State	Cultivars
Andhra Pradesh	CO 2, CO 5, Sunrise Solo, Taiwanese lines
Bihar	Pusa Dwarf, Pusa Majesty, Pusa Nanha, Pusa Giant, Pusa Delicious and Ranchi
Karnataka	Coorg Honey Dew, Washington, Sunrise Solo, CO2, Surya and Taiwanese lines
Maharashtra	Washington, CO2, Pusa Delicious, Pusa Majesty, Ranchi and Taiwanese lines
Orissa	CO2, Coorg Honey Dew, Washington, Ranchi, Pusa Dwarf and Pusa Delicious
Tamil Nadu	Co2, Co3, CO4, CO5, CO6, CO7 and Coorg Honey Dew
Uttar Pradesh	Coorg Honey Dew, Pusa Dwarf, Pusa Delicious, CO3 and Barwani Red

Varietal diversity

In India, varietal diversity is seen for papaya. The variability seen is more because of the open pollination and wide spread multiplication using these seeds. In papaya there are two basic types of varieties. Those varieties, which are dioecious, produce only female and male plants, and 'gynodioecious' that produce both female and hermaphrodite plants. Some of the varieties that are grown in different states are as follows islands, and Indonesia. Australimusa is largely found in Malayan islands, and Indonesia. It is also found in Assam, Indo-China, Malayan and Papua New Guinea, which is a primary centre of cultivated AA types. M. balbisiana occurred in Ceylon, India, Burma, Siam and Malaya where the A X B hybrid have evolved. 4. GRAPE (Vitis spp.) European grape V. vinifera is considered to have originated primarily between Caspian and Black Sea, and considered a hybrid between two American spp. V. vulpine and V. labrusca. It also resembles V. parviflora and V. lanata which are found in Himalayan region. This region may be considered as a secondary centre of origin. Native spp. resembling Vitis lanata and palmata grows wild in the northwestern Himalayan foothills. Indigenous varieties known as 'Rangspay', 'Shonltu White' and 'Shonltu Red' are grown in Himachal Pradesh even today. Famous Indian medicine scholars, Sushruta and Charaka in their medical treatises entitled 'Sushruta Samhita' and 'Charaka Samhita', respectively, written during 1356-1220 BC, mentioned the medicinal properties of grapes. Kautilya in his 'Arthashastra' written in the fourth century BC, mentioned the type of land suitable for grape cultivation. Cultivated grapes are believed to have been introduced into the north of India by the Persian invaders in 1300 AD, from where they were introduced into the southern parts of India (Daulatabad in Aurangabad district of Maharashtra) during the historic event of changing the capital from Delhi to Daulatabad by King Mohammed-bin-Tughluk. Ibn Battuta, a Moorish traveller who visited Daulatabad in 1430 AD, reported to have seen flourishing vineyards in south India.

5.7. Sapota

Sapota (Achras zapota L.) is a popular dessert fruit belonging to the family *Sapotaceae*. It is believed to have originated in tropical America, taken to Philippines by the Spanish and from there has spread to other countries (Purse glove, 1968). In India it is grown in the states of Andhra Pradesh, Gujarat, Karnataka and Orissa. About 30 varieties are reported in India at various places. Several locally grown genotypes identified include Bhuri patti, Morabba, Kali patti, Turi patti, Gole patti, Singapuri, Khabari and Chhumukia type. Among these, a genotype identified in Navsari, in Gujarat locally known as 'Morabba' bears fruits of bigger size and superior quality in comparison to Kalipatti, a local genotype grown in about 80% area of Gujarat. It is a selection from graphed plants collected from nursery located in Golwal. It may have originated as bud mutant and is now being propagated vegetatively. Another somatic mutation having desirable characteristics of the fruit was identified in Paria (Rai, 1995). Wild diversity is not observed for sapota, as it has been grown over the years by using graphs. Biodiversity of underutilized units. In India various native fruits, such as aonla (*Emblica officinalis*), bael fruit (*Aegle marmelos*), jackfruit (Artocarpus heterophyllus), jamun (*Syzygium cuminii*), karonda (*Carissa congesta*), Kokum (Garcinia indica) and phalsa (*Grewia subinaequalis*) with lot of diversity in a wide range of agro ecological situations throughout the tropics, subtropics and temperate regions, which could be grouped as underutilized. Some of these fruits yield juice with excellent flavour, which can be converted into blended beverages and these could play an important role in meeting the demand for nutritious, pleasantly flavoured and attractive natural food of high therapeutic value. Encouraging local people to produce these fruits can help in uncontrolled harvesting from the wild under check and conservation of various species in their native habitats where they perform best.

5.8. Jackfruit

Artocarpus is a genus of small to large evergreen trees, distributed from Sri Lanka and India to South China and through Malaysia to Solomon Islands. Nine species are recorded in India. The spp, A. heterophyllus Lam. is grown for their edible fruits, and *A. chaplasha* Roxb., *A. hirsutus* Lam. and *A. lakoocha* Roxb., are important timber trees. *A. chaplasha* Roxb is distributed in the moist deciduous and evergreen forests of the sub-Himalayan tracts from Nepal eastwards to West Bengal, Assam and Tripura. In West Bengal and Assam, it occurs in moist types of mixed deciduous and evergreen forests. In Andaman and Nicobar Islands it is an important constituent of evergreen and deciduous forests *A. cummunis* J.R. & G. Frost, commonly known as bread fruit is found mainly in Westcoast and Western Ghats, Wynad, in the Nilgris, Lower Plains, the Court Allam hills and the Annamalai's. there are two distinct varieties in this species. One is a seeded type and the other entirely seedless. The seeded types are found in a wild state in its native and are of little economic value. It is not useful in culinary preparations but the seeds, which resemble chestnut, are relished when roasted or boiled. A. heterophyllus Lam. commonly called as jackfruit is one of the most popular fruits of South India. The tree is indigenous to the evergreen forests of the Western Ghatsat altitudes of 450-1200m, but seen growing throughout other hotter parts of India too. Because of seed propagation, the existing population of jack comprises innumerable trees differing from each other in fruit characters of shape, size and quality. A. hirsutum Lam is commonly found in the evergreen forests of Western Ghats from Konkan southwards, is common in North Kanara and Kodagu in Karnataka to Kerala where it is an important tree. It requires heavy rain fall, not less than 174 cm annually and thrives well on lateritic soils at the foot of the Ghats. the tree can stand shade, but thrives best with a fair amount of light. It does equally well in the open and withstands exposure to sun anther the first few years. *A lakoocha* Roxb, is commonly known as monkey jack. In its wild state it is chiefly found in the moist or deciduous forests along the banks of streams and along the site of moist ravines. It thrives best in deep laterite soils and generally comes to bear anther about eight years. It is commonly cultivated throughout the greater part of India as a shade or ornamental tree. It is perhaps one of the foremost among neglected but useful trees. It is distributed in evergreen, semievergreen and moist deciduous forests upto an altitude of 1800 m in eastern and northern India. On the west coast it is found from Konkan southwards to Kerala, and in Tamil Nadu. It is also found growing in many localities in Andaman Islands.

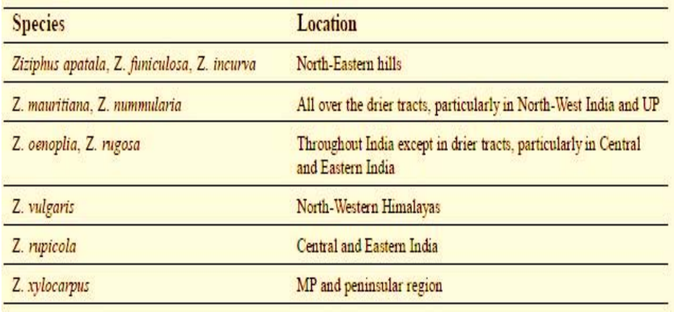
5.9. Aonla

Aonla or Indian gooseberry (Emblica officinalis Gaertn.) is considered as a wonder fruit for health-conscious population. It is being grown in India for more than 3500 years. Sushruta, the father of ancient medicine (during 1500 BC-1300 BC), has mentioned about its usefulness in 'Ayurveda' in detail. It belongs to family Euphorbiaceae and is one of the important indigenous fruits of Indian subcontinent. In different parts of India, it is known by different vernacular names such as Amla or Aonla in Hindi (Pathak, 2003). The plant and fruit of aonla are regarded as sacred by 'Hindus' and have great mythological significance. The aonla tree is native to tropical Southeast Asia, particularly central or southern India, Pakistan, Bangladesh, Sri Lanka, Malaya, Southern China and to Mascarene Islands. Seedling trees are of common occurrence in the mixed deciduous dry forests of India, ascending from sea level (western and Eastern Ghats, Aravali and Vindhya hills) to 1300 m above sea level, from northwest Himalayas (Jammu & Kashmir, Himachal Pradesh, Uttranchal) to eastern Himalayas in Assam, Meghalaya, Mizoram, Manipur and Tripura (Pathak, 2003). The natural distribution of wild aonla is found on the Himalayas, Chota Nagpur, Bihar, Orissa, West Bengal, North Circars, Deccan, Karnataka and in Western Ghats (Rawat and Uniyal 2003). In India, the homeland of aonla, domestication first began in Varanasi (earlier known as Benaras) district of Uttar Pradesh with the initiative of Maharaja of Kashi. Banarasi, a superior genotype was selected from the wild aonla trees available in large number in the nearby Vindhyan hills. Authentic information regarding its cultivation dates to 1881-82 in the Pratapgarh district of Uttar Pradesh. The wild aonla germplasm is mostly confined in the mixed forests with sloppy topography and sometimes even difficult to approach. A rich genetic diversity of aonla exists in northeastern region of India, particularly in lower Assam, Maghalaya, Mizoram and Tripura (Yadav et al., 2001). Aonla grows abundantly in the forest of Khasi and Garo hills of Meghalaya and locally known as “Sohmylleng” (Pandey et al., 1993). The natural population of aonla in west Khasi hills (Nongkhyllum, Rajaju, Khonjoy area) of Meghalaya warrants in situ conservation, which may even be declared as gene sanctuary for this species (Hore, 1998). Mizoram is homeland of wild aonla and star gooseberry (Phyllanthus acidus), which has potential as dwarfing rootstock for aonla. Wild Star gooseberry trees are found in forests of Kolasib,thingdawl and Champhai in Mizoram. Madhya Pradesh forests have rich diversity of aonla. Jharkhand and adjoining areas of Chhatisgarh have rich diversity of aonla in the native forest. the important sites in Jharkhand are Lali Forest near Ranchi, Dalma range of Jamshedpur, Theo Ghat Forest of West Singhbhoom, Tiamara valley area between Ranchi and Jamshedpur, Ramgarh area of 11. BER Out of the 50 reported species nearly 18 to 20 are native to India. A resume of species Hazaribagh, Parasnath hills of Girideeh, Kodemera and Jaomi areas of Bihar border, Simdega and Netarhat forest areas of Gumla, Belta forest of Daltonganj, Palamu and Garhwa of Jharkhand and adjoining areas of Sarguja and Ambikapur districts of Chha tisgarh and Sahdol district of Madhya Pradesh. The Belta forest (Daltonganj), Netarhat range in Gumla and Dalma range of Jamshedpur has comparatively high plant population of aonla in the natural habitat. In western and eastern ghats, three species of Phyllanthus emblica, Phyllanthus indofisheri and Phyllanthus acidus are of common occurrence. A wild strain of aonla grows in the Himalayas up to an altitude of 1600 m asl. The fruits of wild aonla are relatively smaller. In the mid Himalayas wild aonla is distributed right from western to eastern Himalayas including Nepal (Pathak, 2003).

5.10. Bael

Bael (Aegle marmelos) is native to India and cultivated throughout the South East Asia and East Indian Archipelago. the genus consists of 2 to 3 species. It is found in UP, Bihar and West Bengal. Some important types selected in different regions are UP: NB 1, NB5, NB6: Bihar: Etawah Kagzi, Sewan Large, Mirzapuri and Deoria. availability in different locations in India is given below:

Table 6: Distribution of Bael (*Aegle marmelos*) varieties in India



Species	Location
Ziziphus apatala, Z. funiculosa, Z. incurva	North-Eastern hills
Z. mauritiana, Z. nummularia	All over the drier tracts, particularly in North-West India and UP
Z. oenoplia, Z. rugosa	Throughout India except in drier tracts, particularly in Central and Eastern India
Z. vulgaris	North-Western Himalayas
Z. rupicola	Central and Eastern India
Z. xylocarpus	MP and peninsular region

There are more than 100 named varieties in ber and areas rich in variability have been identified in several places in UP, Rajasthan, Haryana, Gujarat, MP,

5.11. Custard Apple

The genus Annona contains some 120 species originating from warm countries but few important Maharashtra, AP, Karnataka and Tamil Nadu. However, some popular cultivars are Umran, Gola

Reshmi and Illaichi. species became integral part of the Indian culture bearing the names of great heroes of the epic Ramayana. Some important species and their natural distribution are given below:

Table 7: Distribution of Custard Apple varieties in India

Species	Common name	Varieties	Location
A. squamosa	Sweet sop, Sharifa, Sitaphal	a) green types: Balanagar, Mammoth, British Guinea, Washington-95, Barbadose seedling, Arka Sahan, (An F1 hybrid) (b) Red types: Red Sitaphal	Low and medium elevations throughout tropics
A. cherimola	Lakshmanphal	Cherimoyar	Cooler places in India
A. atemoya		Pinks mammoth, Bradley, Keller, Page, African Pride, Island Gem	Adopted to colder climate and alkali soils
A. reticulata	Ramphal or Bullock's Heart	Used mostly as a root stock	
A. glabra	Pond apple	Root stock	Flooded areas
A. muricata	Sour soup, Hanuman Phal	Root stock	Mountain regions of India
A. mantna	Mountain soursop	Used in breeding programme for quality	
A. purpurea	soncoya	Used as resistant source for fruit cracking	
A. scleroderma	Eoshto	Used in breeding programmes for thick hard shell	

5.12. Fig

The original home of origin of fig (*Ficus carica)* is South Arabia. There are four horticultural types in this crop viz., Smyrna, Capri, Sanpedro and Adriatic. This crop has very narrow range of diversity in India and there is a scope for introducing exotic germplasm. However, there are wild relatives found in India and some of them are given as under:

Table 8: Distribution of Fig (*Ficus carica)* varieties in India

Species	Common name	Distribution
F. auriculata	Timla	Bihar,Orissa, Khashi hills, Manipur
F. benghalensis	Banyan	All over Inedia
F. benjamina		
F. carica	Fig	Uttar Pradesh, Rajasthan, Andhra Pradesh, Maharashtra, Karnataka
F. elastica	Indian Rubber tree	Assam and Khasi hills
F. glomerata	Cluster fig	
F. hispida		Throughout India
F. krishnae	Krishna's fig	

F. lucescens		North India, MP and W peninsula
F. palmata		N.W India and Rajasthan
F. religiosa	Peepal tree	All over India
F. rumphii		Punjab, MP, Assam
F. samicordata		Punjab, Assam, Bengal, Khasi hills and Manipur

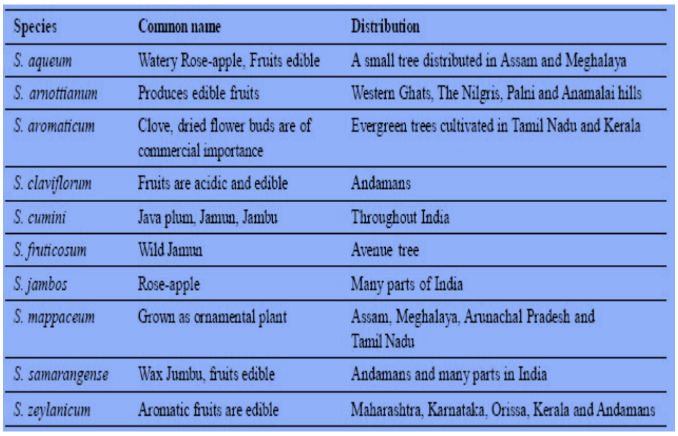
5.13. Syzygium

This genus *Syzygium* comprises about 1000 species of evergreen trees and shrubs; most of them are tropical in origin. Jamun is found in Western Ghats and very extensively in the tropical region. The diversity found is due to the high heterozygosity and seed multiplication. Some of the species are described below:

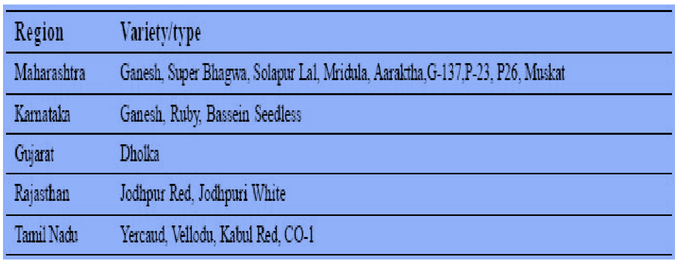
5.14. Pomegranate

Pomegranate (*Punica granatum*) is an ancient fruit, which originated in Persia, Afghanistan and Baluchistan naturalized in Western India very early. Its wild forms are found in lower hills of Himachal Pradesh. Most of the pomegranate types cultivated in India are of seedling origin and thus providing a wide range of variability with respect to fruit shape, size, and mellowness of seed, aril colour, rind colour, sweetness and acidity of juice. Some popular varieties in different regions are furnished below:

Table 9: Distribution of Pomegranate (*Punica granatum*) varieties in India



Species	Common name	Distribution
S. aqueum	Watery Rose-apple, Fruits edible	A small tree distributed in Assam and Meghalaya
S. arnottianum	Produces edible fruits	Western Ghats, The Nilgris, Palni and Anamalai hills
S. aromaticum	Clove, dried flower buds are of commercial importance	Evergreen trees cultivated in Tamil Nadu and Kerala
S. claviflorum	Fruits are acidic and edible	Andamans
S. cumini	Java plum, Jamun, Jambu	Throughout India
S. fruticosum	Wild Jamun	Avenue tree
S. jambos	Rose-apple	Many parts of India
S. mappaceum	Grown as ornamental plant	Assam, Meghalaya, Arunachal Pradesh and Tamil Nadu
S. samarangense	Wax Jumbu, fruits edible	Andamans and many parts in India
S. zeylanicum	Aromatic fruits are edible	Maharashtra, Karnataka, Orissa, Kerala and Andamans



Region	Variety/type
Maharashtra	Ganesh, Super Bhagwa, Solapur Lal, Mridula, Aaraktha,G-137,P-23, P26, Muskat
Karnataka	Ganesh, Ruby, Bassein Seedless
Gujarat	Dholka
Rajasthan	Jodhpur Red, Jodhpuri White
Tamil Nadu	Yercaud, Vellodu, Kabul Red, CO-1

Apart from the above-mentioned fruit species, there are several other species of fruits for which considerable diversity exists in the wild and conservation of such fruits needs to be carried out both *in situ* as well as in *ex situ*. There is also a need to work out the diversity using molecular means, so that the concept of 'core collection' can be practiced effectively.

VI. GENETIC MARKERS AND DIVERSITY ANALYSIS

Different marker systems have been established at morphological, physiological and DNA levels for the assessment of diversity in small fruit plant populations. Various markers including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple (short) sequence repeat (SSR), sequence characterized amplified region (SCAR), sequence-tagged sites (STS), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR) and single nucleotide polymorphisms have been used for diversity analysis in crop plants. Reviews of these techniques are available in literature (Mohammadi and Prasanna, 2003; Debnath, 2008). For an accurate and unbiased estimates of genetic diversity and relatedness, factors including (i) sampling strategies, (ii) utilization of various data sets on the basis of the understanding of their strengths and constraints, (iii) choice of genetic similarity estimates or distance measures, clustering procedures and other multivariate methods in analyses of data and (iv) objective determination of genetic relationships are very important (Mohammadi and Prasanna, 2003). A combination of techniques can be used to gather the useful information as it may be difficult to choose the most appropriate technique. This review deals with diversity analysis in small fruit crops using molecular markers.

VII. DIVERSITY ANALYSIS IN SMALL FRUITS USING MOLECULAR MARKERS

To date, RFLP, RAPD, AFLP, ISSR, SSR, expressed sequence tag (EST)-PCR and cleaved amplified polymorphic sequences (CAPS) derived from EST-PCR markers have been used for diversity analysis in small fruit crops (Debnath et al., 2012). While RFLPs and SSRs are codominant markers, AFLPs, RAPDs and ISSRs are only dominant. Although RFLP has been used for diversity analysis in some small fruit crops, they are labour intensive, time consuming and costly (Kesseli et al., 1994), they are more robust than RAPDs. RAPD markers have been extensively used for estimation of relatedness and diversity in strawberry (Harrison et al., 2000; Degani et al., 2001), blueberry (Albert et al., 2005a, b; Debnath, 2005), cranberry (Polashock and Vorsa, 2002; Debnath, 2005; 2007a), lingonberry (Persson and Gustavsson, 2001) and in Rubus species (Graham et al., 1997; Badjakov et al., 2006). Degani et al. (1998) identified 10 RAPD markers that could distinguish 41 strawberry cultivars grown in the United States and Canada. However, RAPDs were unable to discriminate among the four subspecies of F. virginiana L. (Harrison et al., 2000). Working with five cranberry cultivars and 43 wild clones collected from four Canadian provinces, Debnath (2007a) reported a high proportion of genetic variation (90%) revealed by the analysis of molecular variance (AMOVA). A very high proportion of genetic variation (RAPD-based AMOVA) that can be invaluable in a breeding program, was also observed within V. uliginosum L. (96%, Albert et al. 2005a), *V. mytrillus* L. (86%, Albert et al. 2005b), and V. vitisidaea L. (89%, Persson and Gustavsson, 2001) populations. Debnath (2007a) identified that 10% of total variation was due to geographical distribution in cranberry wild clones. Reasons for the lack of a geographical differentiation might be due to the result of aglacial bottleneck and rapid colonization coupled with autogamous breeding habit of cranberries (Stewart and Nilsen, 1995). RAPD analysis showed positive correlations between geographic and genetic distances for a set lingonberry of populations (Garkava-Gustavsson et al., 2005). RAPDs have also been used to distinguish among Rubus species (Graham et al., 1997). Badjakov et al. (2006) analyzed 28 raspberry genotypes from the Bulgarian germplasm collection including 18 Bulgarian cultivars and breeding lines, eight accessions from outside Bulgaria and two wild species accessions, R. occidentalis and R. adiene. They created a genetic similarity tree with two clusters that corresponded to two pedigree groups among the Bulgarian genotypes. Strawberries have been analyzed for relationship and diversity analysis using AFLP (Degani et al., 2001; Tyrka et al., 2002), ISSR (Arnau et al., 2002; Debnath et al., 2008) and SSR markers (Cho et al., 2007). AFLP was used by Degani et al. (2001) to study genetic relationships among 19 strawberry cultivars from the United States and

Canada. Tyrka et al. (2002) distinguished six strawberry cultivars and 13 salinity tolerant clones using a simplified AFLP assay based on a single cutting enzyme PstI – PstIAFLP. Using AFLPs, Yang et al. (2008) determined sources of the newly founded population and characterized genetic variation of V. membranaceum (black huckleberry). AFLP has also been studied in Rubus species to study genetic diversity among blackberry cultivars and their relationship with boysenberry (Ipek et al., 2009). ISSR markers were used to assess the genetic diversity in 216 accessions of *F. chiloensis*, which represented the two botanical forms present in Chile (F. chiloensis ssp. chiloensis f. chiloensis and F. chiloensis ssp. chiloensis *f. patagonica* (L.) Duch.) (Carrasco et al., 2007). High genetic diversity at the species level (polymorphic ISSR loci [P] = 89.6%, gene diversity [h] = 0.24 ± 0.17, Shannon's index [S] = 0.37 ± 0.24) and a lower genetic diversity in f. chiloensis than *f. patagonica* were observed. The AMOVA showed a moderate genetic differentiation among accessions (fst = 14.9%). No geographic patterns for ISSR diversity were observed. AMOVA, structure, and discriminant analysis indicated that accessions tend to group by botanical form (Carrasco et al., 2007). Arnau et al. (2002) used five ISSR markers to characterize 30 strawberry varieties. With ISSR markers, Carrasco et al. (2007) reported a high genetic diversity at the species level and a lower genetic diversity in F. chiloensis than *F. patagonica*. Using 17 ISSR primers, Debnath et al. (2008) reported a narrow genetic base among 16 strawberry cultivars and 11 breeding lines developed in Canada, ranging from 63% to 77%. Debnath and Ricard (2009) also reported a high degree of genetic similarity among 10 strawberry cultivars and nine breeding lines ranging from 45% to 73% although a wide genetic diversity was observed among the strawberry genotypes for anthocyanin contents and antioxidant activities. Ge et al. (2013) conducted cluster analysis of 16 strawberry cultivars using the 116 SNPs and concluded that the genetic variation between the cultivars was not much as expected. Gil-Ariza et al. (2009) studied the similarity relationships and structure of 92 selected strawberry cultivars with widely diverse origins using EST-SSR markers. As was reported by Debnath et al. (2008) with ISSR analysis, a limited differentiation of modern cultivars, most probably as a consequence of the methodology of strawberry breeding, was noticed. ISSR markers have been used successfully for diversity analysis of Vaccinium (Debnath, 2007b, 2009; Debnath and Sion, 2009) and Rubus species (Debnath, 2007c, d). Using six ISSR markers, Garriga et al. (2013) reported high levels of polymorphism among ten highbush blueberry (V. corymbosum L.) and three rabbiteye blueberry (V. ashei Reade) cultivars. An ISSR analysis with natural populations of Korean black raspberry (R. coreanus) indicated the association of relationships among populations with geographic location (Hong et al., 2003). In a study with nine ISSR primers, a substantial degree of genetic diversity was found among 48 wild cloudberry clones, collected from four Canadian Provinces, indicating a possibility of use of wild germplasm for cloudberry improvement (Debnath, 2007c). There was no pattern of geographical differentiation among the wild clones which could be due a "glacial bottleneck" and rapid colonisation of cloudberries. A study with SSR markers revealed a high genetic diversity in the outcrossing diploid species, F. nipponica compared to self-pollinating F. iinumae as seen from the heterozygosity values (Ho) (0.4071 vs. 0.1336, respectively) and the number of alleles/locus (10.6 vs. 7.3, respectively) (Njuguna et al. (2011). Using 18 SSR markers, Yoon et al. (2012) identified 101 alleles with an average of 5.6 per locus and 21 specific alleles in 59 accessions of cultivated strawberries from Korea, Germany, United States, United Kingdom, and Japan. Despite its economic value, the polyploid constitution of the strawberry has been a major barrier to the genetic characterization of the cultivated species and limited information on the genome structure has been published. Horvath et al. (2011) studied the genetic structure in strawberry cultivars using 23 SSR markers. The important loss of diversity observed in the modern European cultivars and a trend towards using mainly American cultivars for breeding programmes led to the progressive abandonment of old European germplasm. Badjakov et al. (2006) analyzed the 28 red raspberry accessions with four SSR loci and demonstrated high levels of diversity within the collection Bulgarian red raspberries. Polashock and Vorsa (2002) developed SCAR method for cranberry germplasm analysis. Although

SCAR markers can be employed for identifying closely related genotypes, the inferences of more distant genetic relationships are less certain. ESTs are short DNA molecules (300 - 500 bp) reverse-transcribed from a cellular mRNA population (MacIntosh et al., 2001). Rowland et al. (2003) used EST-PCR and EST-PCR-derived CAPSs markers to differentiate blueberry genotypes. A fair correlation between similarity coefficients calculated from marker data and coefficients of co-ancestry was found. Using EST-PCR and EST-SSR markers, Debnath (2014) investigated the genetic structure and diversity in 36 blueberry genotypes. Wide genetic diversity was evident from high values of expected heterozygosities, Shannon's index and polymorphism information content and from AMOVA. Structure analysis grouped the half-high and highbush blueberries into one cluster which was in agreement with the neighbour-joining clustering and principal coordinate analysis. In a previous study of EST-PCR, Bell et al. (2008) detected variation among 25 genotypes of lowbush blueberries from four commercial fields in Maine, USA. Working with highbush blueberries, Boches et al. (2006) found extremely variable EST-SSR loci are in blueberries. Blueberry population structure using SSR markers has also been studied by Bian et al. (2014) where cluster analysis grouped the accessions in a manner consistent with known information regarding species, ploidy levels and pedigree.

Here are some wild edible fruits you can forage:

1. Wild Grapes (Vitis sp.): Commonly found in many regions, these grapes can be eaten raw or made into jelly.
2. American Persimmons (Diospyros virginiana): Sweet and flavorful when ripe, these fruits are great for eating fresh or in desserts.
3. Crabapple (Malus sp.): While tart, crabapples can be used in jellies and preserves.
4. Prickly Pear (Opuntia sp.): The pads and fruits of this cactus are edible and can be used in various dishes.
5. Black Cherry (Prunus serotina): These cherries are sweet and can be eaten raw or used in cooking.
6. Pawpaw (Asimina triloba): Known for its custard-like texture, pawpaw is a delicious fruit that can be eaten fresh.
7. Passion Fruit (Passiflora incarnata): This fruit has a unique flavour and can be eaten raw or used in drinks.

Additionally, you can explore 50+ edible wild berries and fruits and 40+ edible wild fruits for more options. Always ensure proper identification before consuming any wild fruit.

Table 10: Main centres of diversity for fruits in India

Region	Species
Western Himalayas	*Elaeagnus hortensis*, *Ficus palmata*, *Fragaria indica*, *Moms spp.*, *Prunus acuminata*, *P. cerasiodes*, *P. cornuta*, *P. napaulensis*, *P. prostrata*, *P. tomentosa*, *Pyrus baccata*, *P. communis*, *P. kumaoni*, *P. pashia*, *Ribes graciale*, *R. nigrum*, *Rubus ellipticus*, *R. moluccanus*, *R. fruticosus*, *R. lasiocarpus*, *R. lanatus*, *R. niveus*, *R. reticulatus*, Zizyphus *vulgaris*.
Eastern Himalayas	*Fragaria indica*, *Morus* spp., *Myrica esculenta*, *Prunus acuminata*, *P. cerasiodes*, *P. cornuta*, *P. jenkinsii*, *P. napaulensis*, *Pyrus pashia*, *Ribes graciale*, *Rubus lineatus*, *R. ellipticus*, *R. lasiocarpus*, *R. moluccanus*, *R. reticulatus*.

North-eastern region	*Citrus assamensis*, *C. ichangensis*, *C. indica*, *C. jambiri*, *C. latipes*, *C. macroptera*, *C. media*, *C. aurantium*, *Docynia indica*, *D. hookeriana*, *Eriobotrya angustifolia*, *Mangifera sylvatica*, *Musa accuminata/M. balbisiana* complex, *M. manii*, *M. nagensium*, *M. sikkimensis*, *M. superba*, *M. velutina*, *Pyrus pyrifolia*, *P. pashia*, *Prunus cerasiodes*, *P. cornuta*, *P. jenkinsii*, *Ribes graciale*, *Rubus ellipticus*, *R. moluccanus*, *R. reticulatus*, *R. lasiocarpus*, *Myrica esculenta*.
Gangetic plains	*Aegle marmelos*, *Cordia myxa*, *C. rothii*, *Emblica officinalis*, *Grewia asiatica*, *Morus* spp.; *Phoenix* spp.; *Syzygium* spp.; *Zizyphus nummularia* and other species and *Manilkara hexandra* (more in north-western plains).
Indus plains	Meagre occurrence of *Syzygium*, rich variation in *Carissa congesta*.
Western peninsular tract	*Artocarpus heterophyllus*, *A. lakoocha*, *Garcinia indica*, *Diospyros* spp., *Ensete superba*, Mangifera *indica*, *Mimosops elengii*, *Spondias pinnata*, *Vitis* spp., *Zizyphus oenoplia*, *Z. rugosa*, *Rubus ellipticus*, *R. lasiocarpus*, *R. moluccanus*.

Sources: Sankaran M, et al., 2020

Table 11: Wild relatives of some of the fruit crops

S. No.	Family	Species	Remarks
1	Anacardiaceae	*Mangifera andamanica* *Mangifera camptosperma* *Mangifera griffithi* *Mangifera nicobarica* *Mangifera sylvatica* *Semicarpus kurzii* *Spondias pinnata* *S. cytherea* *Bouea oppositifolia* *Dracontomelon dao* *Buchnania splendons*	Possess tolerance to biotic and abiotic stress
2	Annonaceae	*Annona muricata* L. (soursop) *Annona reticulata* L. (bullock's heart) and *Annona glabra* L	*A. glabra* is tolerant to salinity and could be suitably employed as a rootstock for other species of this group
3	Arecaceae	*Areca triandra* *Phoenix andamanensis* *P. sylvestris* (L.) Roxb. *P. rupicola5.*	*P. paludosa* Roxb. All these five species are habitat of seashores
4	Clusiaceae	*Garcinia cowa* Roxb *Garcinia xanthochymus Hook.f* *Garcinia microstigma* *Garcinia speciosa* *Garcinia dhanikhariensis* S.K.Srivast.	About 36 species of *Garcinia* are reported be available in India of which 18 *Garcinia* species are found to exist in Andaman & Nicobar Islands. 6

		Garcinia hombroniana Pierre. *Garcinia lancaefolia* Roxb. *Garcinia andamanica*King. *Garcinia brevirostris* Scheff. *Garcinia cadelliana* King. *Garcinia calycina* Kurz *Garcinia cornea* Linn. *Garcinia dulcis* (Roxb.) Kurz. *Garcinia jelinekii* Kurz. *Garcinia Kingii* Pierre ex Vesque *Garcinia Kurzii* Pierre *Garcinia lanessanii* Pierre. *Garcinia mangostana* Linn.	species which are endemic to Andaman & Nicobar Islands ?? viz. *Garcinia andamanica* King. var. *andamanica, G. cadeliana, G. dhanikhariensis, G. kingii* Pierre ex. Vesque, *G. kurzii* Pierre. and *G. microstigma* Kurz.
5	Dilleniaceae	*Dillenia andamanica* C. E. Parkinson *D. indica* L *D. pentagyna* Roxb	Edible fruits are produced in all the three species.
6.	Ebenaceae	*Diospyrous blancoi* (velvet apple) *D. andamanica*	Fruit of *Diospyrus blancoi* has velvety surface and fragrant, cream-white flesh.
7	Euphorbiaceae	*Baccaurea sapida* (sapida) and *B. ramiflora (khatta phal)*	*Fruits of B. ramiflora are rich in vitamin C.*
8	Moraceae	*1. Ficus carica L.* *2. Ficus racemosa L.* *3. Ficus hispida* *4. Artocarpus heterophyllus (jackfruit)* *5. A. altilis (breadfruit)* *6. A. lakoocha Buch. -Ham. (monkey jack)* *7. A. chaplasha Roxb. (cham pedak)*	*Artocarpus heterophyllus has 10 diversity centres in India. This is found in all states and it has multiple uses*
9	Musaceae	*1. Musa balbisiana var. andamanica* *2. Musa paradisiaca* *3. Musa indandamanensis L. J. Singh* *4. Musa textilis* *5. Musa sabuana*	*Wild species of banana are rich in carotenoid content however the presence of seeds prevents the wider acceptability of the fruits.*
10	Myrsinaceae	*1. Ardisia solanacea Roxb. (Khaariphal)* *2. A. andamanica Kurz.*	*These species are tolerant to salinity*
11	Pandanaceae	*1. Pandanus andamanensium Kurz* *2. Pandanus tectorius Soland. Ex Parkinson* *3. Pandanus lerum Jones ex Fontane var. lerum* *4. Pandanus lerum var. andamanensium (Kurz.) D.C. Stone*	*Nicobari tribes extract the flour from the fruits and cake is prepared out of the flour.* *Pandanus lerum Jones ex Fontane var. lerum, and Pandanus lerum var. andamanensium (Kurz.) D.C. Stone are distributed in the swampy areas and Pandanus tectorius distributed in seashore.*
12	Rhamnaceae	*1. Ziziphus glabrata Heyne* *2. Ziziphus oenoplia (L.) Mill var Oenoplia* *3. Ziziphus oenoplia var pallens*	-

		Bhandari & Bhansali	
13	Myrtaceae	*1. Syzygium andamanicum* *2. Syzygium hookeri* *3. Syzygium kurzii* *4. Syzygium sanjappaina* *5. Syzygium manii* *6. Syzygium claviflorum (wild jamun)* *7. Syzygium aqueum (watery rose apple)* *8. Syzygium samarnagense* *9. Syzygium jambos* *10. Syzygium malaccensis*	-
14	Myristicaceae	*1. Myristica andamanica Hook.f.* *2. Myristica glabra Blume* *3. Myristica glaucescens Hook.f.* *4. Myristica irya Gaertn.* *5. Myristica prainii King* *6. M. elliptica Wall ex. Hook. f. et Thoms.* *7. Knema andamanica (Warb.) de Wilde ssp.* *Andamanica*	*Knema andamanica (Warb.) de Wilde ssp. Andamanica, K. andamanica (Warb.) de Wilde ssp. nicobarica (Warb.) and Myristica andamanica Hook.f are endemic to the*
		8. K. andamanica (Warb.) de Wilde ssp. *nicobarica (Warb.)* *9. K. andamanica (Warb.) W. J. de Wilde subsp. peninsularis*	*Andaman Islands.*
15	Sapotaceae	*1. Manilkara littoralis -* *Hindi - Sea Mohwa*	*Potential rootstock for Sapota*
16	Menispermiaceae	*1. Haematocarpus validus*	*Recorded from North Andaman. This crop has already been domesticated by a farmer in Diglipur area, North Andaman. The farmer has been identified as the custodian farmer*
17	Vitaceae	*1. Vitis parviflora* *2. Ampelocissus barbata (Wall.) Planchon* *3. A. helferi (Lows) Planchon* *4. A. polystachya (Wall) Planchon*	*Vitis parviflora is being used in grapes may be used in grape breeding programme as it has got reflexed stamen.* *Whereas the Ampelocissus barbata is used as a medicinal plant by the tribes of the Island.*

Sources: Sankaran M, et al., 2020

Sources: Romesh Kumar Salgotra and Bhagirath Singh Chauhan, 2021.

Fig. 6: Different sources of genetic diversity and their potential utilization in the development of new crop varieties.

VIII. BIODIVERSITY REFERS TO THE VARIETY AND VARIABILITY AMONG ALL GROUPS OF LIVING ORGANISMS AND THE ECOSYSTEM COMPLEXES IN WHICH THEY OCCUR

From the driest deserts to the dense tropical rainforests and from the high snow-clad mountain peaks to the deepest of ocean trenches, life occurs in a marvellous spectrum of forms, size, colour and shape, each with unique ecological inter-relationships. Just imagine how monotonous and dull the world would have been had there been only a few species of living organisms that could be counted on fingertips! *In the Convention of Biological diversity (1992) biodiversity has been defined as the variability among living organisms from all sources including inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are a part.*

IX. LEVELS OF BIODIVERSITY

Units of biodiversity may range from the genetic level within a species to the biota in a specific region and may extend up to the great diversity found in different biomes.

9.1. Genetic Diversity

It is the basic source of biodiversity. The genes found in organisms can form enormous number of combinations each of which gives rise to some variability. Genes are the basic units of hereditary information transmitted from one generation to other. When the genes within the same species show different versions due to new combinations, it is called genetic variability. For example, all rice varieties belong to the species Oryza sativa, but there are thousands of wild and cultivated varieties of rice which show variations at the genetic level and differ in their colour, size, shape, aroma and nutrient content of the grain. This is the genetic diversity of rice.

9.2. Species Diversity

This is the variability found within the population of a species or between different species of a community. It represents broadly the species richness and their abundance in a community. There are two popular indices of measuring species diversity known as Shannon-Wiener index and Simpson index. What is the number of species on this biosphere? The estimates of actual number vary widely due to incomplete and indirect data. The current estimates given by Wilson in 1992 put the total number of liv ing species in a range of 10 million to 50 million. Till now only about 1.5 million living and 300,000 fossil species have been actually described and given scientific names. It is quite likely that a large fraction of these species may become extinct even before they are discovered and enlisted.

9.3. Ecosystem Diversity

This is the diversity of ecological complexity showing variations in ecological niches, trophic structure, food-webs, nutrient cycling etc. The ecosystems also show variations with respect to physical parameters like moisture, temperature, altitude, precipitation etc. Thus, there occurs tremendous diversity within the ecosystems, along these gradients. We may consider diversity in forest ecosystem, which is supposed to have mainly a dominance of trees. But, while considering a tropical rainforest, a tropical deciduous forest, a temperate deciduous forest and a boreal forest, the variations observed are just too many and they are mainly due to variations in the above-mentioned physical factors. The ecosystem diversity is of great value that must be kept intact. This diversity has developed over millions of years of evolution. If we destroy this diversity, it would disrupt the ecological balance. We cannot even replace the diversity of one ecosystem by that of another. Coniferous trees of boreal forests cannot take up the function of the trees of tropical deciduous forest lands and vice versa, because ecosystem diversity has evolved with respect to the prevailing environmental conditions with well-regulated ecological balance.

X. BIOGEOGRAPHICAL CLASSIFICATION OF INDIA

India's have a different type of climates and topography in different parts of the country and these variations have induced enormous variability in flora and fauna. India has a rich heritage of biological diversity and occupies the tenth position among the plant rich nations of the world. It is very important to study the distribution, evolution, dispersal and environmental relationship of plants and animals in time and space. Biogeography comprising of phytogeography and zoogeography deals with these aspects of plants and animals. In order to gain insight about the distribution and environmental interactions of flora and fauna of our country, it has been classified into ten biogeographic zones (Table 4.1). Each of these zones has its own characteristic climate, soil, and topography and biodiversity.

XI. VALUE OF BIODIVERSITY

The value of biodiversity in terms of its commercial utility, ecological services, social and aesthetic value is enormous. We get benefits from other organisms in innumerable ways. Sometimes we realize and appreciate the value of the organism only after it is lost from this earth. Very small, insignificant, useless looking organism may play a crucial role in the ecological balance of the ecosystem or may be a potential source of some invaluable drug for dreaded diseases like cancer or AIDS. The multiple uses of biodiversity or biodiversity value has been classified by McNeely et al in 1990 as follows: (i) Consumptive use value: These are direct use values where the biodiversity product can be harvested and consumed directly e.g. fuel, food, drugs, fibre etc. Food: A large number of wild plants are consumed by human beings as food. About 80,000 edible plant species have been reported from wild. About 90% of present-day food crops have been domestic cited from wild tropical plants. Even now our agricultural scientists make use of the existing wild species of plants that are closely related to our crop plants for developing new hardy strains. Wild relatives usually possess better tolerance and hardiness. A large number of wild animals are also our sources of food. Drugs and medicines: About 75% of the world's population depends upon plants or plant extracts for medicines. The wonder drug Penicillin used as an antibiotic is derived from a fungus called Penicillium. Likewise, we get Tetracycline from a bacterium. Quinine, the cure for malaria is obtained from the bark of Cinchona tree, while Digitalin is obtained from foxglove (Digitalis) which is an effective cure for heart ailments. Recently vinblastine and vincristine, two anticancer drugs, have been obtained from Periwinkle (Catharanthus) plant, which possesses anticancer alkaloids. A large number of marine animals are supposed to possess anti-cancer properties which are yet to be explored systematically. Fuel: Our forests have been used since ages for fuel wood. The fossil fuels coal, petroleum and natural gas are also products of fossilized biodiversity. Firewood collected by individuals are not normally marketed, but are directly consumed by tribals and local villagers, hence falls under consumptive value. (ii) Productive use values: These are the commercially usable values where the product is marketed and sold. It may include lumber or wild gene resources that can be traded for use by scientists for introducing desirable traits in the crops and domesticated animals. These may include the animal products like tusks of elephants, musk from musk deer, silk from silk-worm, wool from sheep, fir of many animals, lac from lac insects etc, all of which are traded in the market. Many industries are dependent upon the productive use values of biodiversity e.g.- the paper and pulp industry, Plywood industry, Railway sleeper industry, Silk industry, textile industry, ivory-works, leather industry, pearl industry etc. Despite international ban on trade in products from endangered species, smuggling of fur, hide, horns, tusks, live specimen etc. worth millions of dollars are being sold every year. Developing countries in Asia, Africa and Latin America are the richest biodiversity centres and wild life products are smuggled and marketed in large quantities to some rich western countries and also to China and Hong Kong where export of cat skins and snake skins fetches a booming business. (iii) Social Value: These are the values associated with the social life, customs, religion and psycho-spiritual aspects of the people. Many of the plants are considered holy and sacred in our country like Tulsi (holy basil), Peepal, Mango, Lotus, Bael etc. The leaves, fruits or flowers of these plants are used in worship or the plant itself is worshipped. The tribal people are very closely linked with the wild life in the forests. Their social life, songs, dances and customs are closely woven around the wildlife. Many animals like Cow, Snake, Bull, Peacock, Owl etc. also have significant place in our psycho-spiritual arena and thus hold special social importance. Thus, biodiversity has distinct social value, attached with different societies. (iv) Ethical value: It is also sometimes known as existence value. It involves ethical issues like "all life must be preserved". It is based on the concept of "Live and Let Live". If we want our human race to survive, then we must protect all biodiversity, because biodiversity is valuable. The ethical value means that we may or may not use a species, but knowing the very fact that this species exists in nature gives us pleasure. We all feel sorry when we learn that "passenger pegion" or "dodo" is no more on this earth. We are not deriving anything direct from Kangaroo, Zebra or Giraffe, but we all strongly feel that these species should exist in nature. This means, there is an ethical value or existence value attached to each species. (v) Aesthetic value: Great aesthetic value is attached to biodiversity. No one of us would like to visit vast stretches of barren lands with no signs of visible life. People from far and wide spend a lot of time and money to visit wilderness areas where they can enjoy the aesthetic value of biodiversity and this type of tourism is now known as eco-tourism. The "Willingness to pay" concept on such eco-tourism gives us even a monetary estimate for aesthetic value of biodiversity. Ecotourism is estimated to generate about 12 billion dollars of revenue annually, that roughly gives the aesthetic value of biodiversity. (vi) Option values: These values include the potentials of biodiversity that are presently unknown and need to be explored. There is a possibility that we may have some potential cure for AIDS or can cer existing within the depths of a marine ecosystem, or a tropical rain forest. Thus, option value is the value of knowing that there are biological resources existing on this biosphere that may one day prove to be an effective option for something important in the future. Thus, the option value of biodiversity suggests that any species may prove to be a miracle species someday. The biodiversity is like precious gifts of nature presented to us. We should not commit the folly of losing these gifts even before unwrapping them. The option value also includes the values, in terms of the option to visit areas where a variety of flora and fauna, or specifically some endemic, rare or endangered species exist. (vii) Ecosystem service value: Recently, a non-consumptive use value related to self-maintenance of the ecosystem and various important ecosystem services has been recognized. It refers to the services provided by ecosystems like prevention of soil erosion, prevention of floods, maintenance of soil fertility, cycling of nutrients, fixation of nitrogen, cycling of water, their role as carbon sinks, pollutant absorption and reduction of the threat of global warming etc. Different categories of biodiversity value clearly indicate that ecosystem, species and genetic diversity all have enormous potential and a decline in biodiversity will lead to huge economic, ecological and socio-cultural losses

XII. GLOBAL BIODIVERSITY

Following the 1992 *"Earth Summit" at Rio de Janeiro,* it became Evi dent that there is a growing need to know and scientifically name, the huge number of species which are still unknown on this earth. Roughly 1.5 million species are known till date which is perhaps 15% or may be just 2% of the actual number. Tropical deforestation alone is reducing the biodiversity by half a percent every year. Mapping the biodiversity has therefore, been rightly recognized as an emergency task in order to plan its conservation and practical utilization in a judicious manner. Terrestrial biodiversity of the earth is best described as biomes, which are the largest ecological units present in different geographic areas and are named after the dominant vegetation e.g. the tropical rainforests, tall grass prairies, savannas, desert, tundra etc. The tropical rainforests are inhabited by teeming millions of species of plants, birds, amphibians, insects as well as mammals. They are the earth's largest storehouse of biodiversity. Many of these species have developed over the time in highly specialized niches and that makes them more vulnerable to extinction when their natural home or niche is destroyed. About 50 to 80% of global biodiversity lies in these rainforests. More than one-fourth of the world's prescription drugs are extracted from plants growing in tropical forests. Out of the 3000 plants identified by National Cancer Research Institute as sources of cancer fighting chemicals, 70% come from tropical rain forests. Very recently, extract from one of the creeping vines in the rainforests at Cameroon has proved effective in the inhibition of replication of AIDS virus. It is interesting to note that the common Neem tree, so popular in tropical India, known for its medicinal properties has now come into lime light even in the western temperate countries. There is an estimated 1,25,000 flowering plant species in tropical forests. However, till now we know only 1-3% of these species. Needless to say, we must try in every way to protect our tropical rainforests. The Silent Valley in Kerala is the only place in India where tropical rain forests occur. You may recall the case of Silent Valley Hydroelectric Project, which was abandoned mainly because it had put to risk our only tropical rain forest biodiversity. Temperate forests have much less biodiversity, but there is much better documentation of the species. Globally, we have roughly 1,70,000 flowering plants, 30,000 vertebrates and about 2,50,000 other groups of species that have been described. There is a stupendous task of describing the remaining species which may range anywhere from 8 million to 100 million.

It is interesting to know that marine diversity is even much higher than terrestrial biodiversity and ironically, they are still less known and described. Estuaries, coastal waters and oceans are biologically diverse and the diversity is just dazzling. Sea is the cradle of every known animal phylum. Out of the 35 existing phyla of multicellular animals, 34 are marine and 16 of these are exclusively marine.

XIII. BIOLOGICAL DIVERSITY AT NATIONAL LEVEL

(Indian Biodiversity): Every country is characterized by its own biodiversity depending mainly on its climate. India has a rich biological diversity of flora and fauna. Overall six percent of the global species are found in India. It is estimated that India ranks 10th among the plant rich countries of the world, 11th in terms of number of endemic species of higher vertebrates and 6th among the centres of diversity and origin of agricultural crops. The total number of living species identified in our country is 150,000. Out of a total of 25 biodiversity hot-spots in the world, India possesses two, one in the north-east region and one in the western ghats. Indian is also one of the 12 mega-biodiversity countries in the world, which will be discussed later.

XIV. REGIONAL OR LOCAL BIODIVERSITY

Biodiversity at regional level is better understood by categorizing species richness into four types, based upon their spatial distribution as discussed below (i) Point richness refers to the number of species that can be found at a single point in a given space. (ii) Alpha (α-) richness refers to the number of species found in a small homogeneous area (iii) Beta (β-) richness refers to the rate of change in species com position across different habitats. (iv) Gamma (γ-) richness refers to the rate of change across large landscape gradients. α-richness is strongly correlated with physical environmental variables. For example, there are 100 species of tunicates in arctic waters, 400 species in temperate waters and 600 in tropical seas. Thus, temperature seems to be the most important factor affecting α-richness of tunicates β-richness means that the cumulative number of species increases as more heterogeneous habitats are taken into consideration. For example, the ant species found in local regions of north pole is merely 10. As we keep on moving towards the equator and thus add more and more habitats, the number of species of ants reaches as high as 2000 on the equatorial region.

XV. INDIA AS A MEGA-DIVERSITY NATION

India is one of the 12 megadiversity countries in the world. The Minis try of Environment and Forests, Govt. of India (2000) records 47,000 species of plants and 81,000 species of animals which is about 7% and 6.5% respectively of global flora and fauna.

Endemism: Species which are restricted only to a particular area are known as endemic. India shows a good number of endemic species. About 62% of amphibians and 50% of lizards are endemic to India. Western ghats are the site of maximum endemism. Center of origin: A large number of species are known to have originated in India. Nearly 5000 species of flowering plants had their origin in India. From agro-diversity point of view also our country is quite rich. India has been the center of origin of 166 species of crop plants and 320 species of wild relatives of cultivated crops, thereby providing a broad spectrum of diversity of traits for our crop plants. Marine diversity: Along 7500 km long coastline of our country in the mangroves, estuaries, coral reefs, back waters etc. there exists arich biodiversity. More than 340 species of corals of the world are found here. The marine diversity is rich in mollusks, crustaceans (crabs etc.), polychaetes and corals. Several species of Mangrove plants and seagrasses (Marine algae) are also found in our country. A large proportion of the Indian Biodiversity is still unexplored. There are about 93 majors wet lands, coral reefs and mangroves which need to be studied in detail. Indian forests cover 64.01 million hectares having a rich biodiversity of plants in the Trans-Himalayan, north-west, west, central and eastern Himalayan forests, western ghats, coasts, deserts, Gangetic plains, deccan plateau and the Andaman, Nicobar and Lakshadweep islands. Due to very diverse climatic conditions there is a complete rainbow spectrum of biodiversity in our country.

XVI. HOT SPOTS OF BIODIVERSITY

Areas which exhibit high species richness as well as high species endemism are termed as hot spots of biodiversity. The term was introduced by Myers (1988). There are 25 such hot spots of biodiversity on a global level out of which two are present in India, namely the Eastern Himalayas and Western Ghats (Table 4.4). These hotspots covering less than 2% of the world's land area are found to have about 50% of the terrestrial biodiversity. According to Myers et al. (2000) an area is designated as a hotspot when it contains at least 0.5% of the plant species as endemics. About 40% of terrestrial plants and 25% of vertebrate species are endemic and found in these hotspots. After the tropical rain forests, the second highest number of endemic plant species are found in the Mediterranean (Mittermeier). Broadly, these hot spots are in Western Amazon, Madagascar, North and East Borneo, North Eastern Australia, West Africa and Brazilian Atlantic forests. These are the areas of high diversity, endemism and are also threatened by human activities. More than 1 billion people (about 1/6th of the world's population) most of whom are desperately poor people, live in these areas. Any measures of protecting these hotspots need to be planned keeping in view the human settlements and tribal issues. Earlier 12 hot spots were identified on a global level. Later Myers et al (2000) recognized 25 hot spots as shown in Table 4.3. Two of these hotspots lie in India extending into neighbouring countries namely, Indo-Burma region (covering Eastern Himalayas) and Western Ghats - Sri Lanka region. The Indian hot spots are not only rich in floral wealth and endemic species of plants but also reptiles, amphibians, swallow tailed butterflies and some mammals (a) Eastern Himalayas: They display an ultra-varied topography that fosters species diversity and endemism. There are numerous deep and semi-isolated valleys in Sikkim which are extremely rich in endemic plant species. In an area of 7298 Km2 of Sikkim about 4250 plant species are found of which 60% are endemic. The forest cover of Eastern Himalayas has dwindled to about 1/3rd of its original cover. Certain species like Sapria Himalayans, a parasitic angiosperm was sighted only twice in this region in the last 70 years. Recent studies have shown that North East India along with its contiguous regions of Burma and Chinese provinces of Yunnan and Schezwan is an active center of organic evolution and is considered to be the cradle of flowering plants. Out of the world's recorded flora 30% are endemic to India of which 35,000 are in the Himalayas. (b) Western Ghats: It extends along a 17,000 Km2 strip of forests in Maharashtra, Karnataka, Tamil Nadu and Kerala and has 40% of the total endemic plant species. 62% amphibians and 50% lizards are endemic to Western Ghats. Forest tracts up to 500 m elevation covering 20% of the forest expanse are evergreen while those in 500-1500 m range are semi evergreen. The major centres of diversity are Agastya Alai Hills and Silent Valley—the New Amambalam Reserve Basin. It is reported that only 6.8% of the original forests are existing today while the rest has been deforested or degraded, which raises a serious cause of alarm, because it means we have already lost a huge proportion of the biodiversity. Although the hotspots are characterized by endemism, interestingly, a few species are common to both the hotspots in India. Some common plants include *Ternstroemia japonica*, Rhododendron and Hypericum, while the common fauna includes laughing thrush, Fairy blue bird, lizard hawk etc. indicating their common origin long back in the geological times has been so severe that thousands of species and varieties are becoming extinct annually. One of the estimates by the noted ecologist, E.O. Wilson puts the figure of extinction at 10,000 species per year or 27 per day! This startling figure raises an alarm regarding the serious threat to biodiversity. Over the last 150 years the rate of extinction has escalated more dramatically. If the present trend continues, we would lose 1/3rd to 2/3rd of our current biodiversity by the middle of twenty first century. Let us consider some of the major causes and issues related to threats to biodiversity. n LOSS OF HABITAT Destruction and loss of natural habitat is the single largest cause of biodiversity loss. Billions of hectares of forests and grasslands have been cleared over the past 10,000 years for conversion into agriculture lands, pastures, settlement areas or development projects. These natural forests and grasslands were the natural homes of thousands of species which perished due to loss of their natural habitat. Severe damage has been caused to wetlands thinking them to be useless ecosystems. The unique rich biodiversity of the wetlands, estuaries and mangroves are under the most serious threat today. The wetlands are destroyed due to draining, filling and pollution thereby causing huge biodiversity loss. Sometimes the loss of habitat is in instalments so that the habitat is divided into small and scattered patches, a phenomenon known as habitat fragmentation. There are many wild life species such as bears and large cats that require large territories to subsist. They get badly threatened as they breed only in the interiors of the forests. Due to habitat fragmentation many song birds are vanishing. There has been a rapid disappearance of tropical forests in our country also, at a rate of about 0.6% per year. With the current rate of loss of forest habitat, it is estimated that 20-25% of the global flora would be lost within a few years. Marine biodiversity is also under serious threat due to large scale destruction of the fragile breeding and feeding grounds of our oceanic fish and other species, as a result of human intervention

XVII. POACHING

Illegal trade of wildlife products by killing prohibited endangered animals i.e. poaching is another threat to wildlife. Despite international ban on trade in products from endangered species, smuggling of wildlife items like furs, hides, horns, tusks, live specimens and herbal products worth millions of dollars per year continues. The developing nations in Asia, Latin America and Africa are the richest source of biodiversity and have enormous wealth of wildlife. The rich countries in Europe and North America and some affluent countries in Asia like Japan, Taiwan and Hong Kong are the major importers of the wildlife products or wildlife itself. The trading of such wild life products is highly profit making for the poachers who just hunt this prohibited wild life and smuggle it to other countries mediated through a mafia. The cost of elephant tusks can go up to $ 100 per kg; the leopard fur coat is sold at $ 100,000 in Japan while bird catchers can fetch up to $ 10,000 for a rare hyacinth macaw, a beautiful coloured bird, from Brazil. The worse part of the story is that for every live animal that actually gets into the market, about 50 additional animals are caught and killed. If you are fond of rare plants, fish or birds, please make sure that you are not going for the endangered species or the wild-caught species. Doing so will help in checking further decline of these species. Also do not purchase furcoat, purse or bag, or items made of crocodile skin or python skin. You will certainly help in preserving biodiversity by doing so

XVIII. THREATS TO BIODIVERSITY

Extinction or elimination of a species is a natural process of evolution. In the geologic period the earth has experienced mass extinctions. During evolution, species have died out and have been replaced by others. However, the rate of loss of species in geologic past has been a slow process, keeping in view the vast span of time going back to 444 million years. The process of extinction has become particularly fast in the recent years of human civilization. In this century, the human impact.

XIX. FACTORS AFFECTING GENETIC DIVERSITY

Genetic diversity changes over time owing to several factors. The main factors responsible for changes in genetic diversity are mutation, selection, genetic drift, and gene flow. Over time, natural and artificial selections play a substantial role in the choosing of superior genotypes, which significantly affects the gene and genotypic frequencies of the population. As per Charles Darwin's theory of evolution, the desired genotypes are selected for and passed onto subsequent generations. However, the domestication of desirable genotypes results from the superior genotypes being selected by farmers and breeders and neglects other undesirable genotypes. This leads to a reduction in inferior alleles over generations. During evolution, various morphological, physiological, and biochemical changes take place in plant species and can take different directions under domestication depending on the part of the plant used. Some plant species lose their sexual reproduction during selection for large size of the tuber or root, which is associated with selection for polyploid types, resulting in sterility. Some polyploid plant species, such as allohexaploid wheat and potato, show diploidization behaviour during sexual reproduction. Some crops have been turned into annuals from their original form of perennials. In the domestication process, the complete genetic transformation of wild species occurs in the development of modern cultivars through natural and artificial selection. After some time, some domesticated cultivars become susceptible to diseases and pests, which can be improved by incorporating genes from wild plant relatives. During the process of domestication, desirable traits have been selected by breeders as per their preferences. However, plant breeders prefer to choose crop varieties with a high yield, resistance to biotic and abiotic stresses, wide adaptation, non-shattering nature, large-sized seeds, early maturing, good quality traits, etc. The main factors affecting genetic diversity will be addressed in the following subsections.

19.1. Mutation

Mutations are sudden heritable changes that occur due to aberrations in the nucleotide sequence of DNA. A mutation is the source of genetic variation impacting the phenotype in crop species. Genetic diversity caused by mutations can have neutral, positive, and negative impacts on various characteristics of a plant species. Genetic variations caused by mutations in DNA are the principal cause of changes in the allele frequencies in a population besides selection and genetic drift. From the beginning, natural or spontaneous mutations have played a significant role in creating the genetic variation that has led to food security. Mutations are the ultimate source of plant evolution when they frequently encounter environmental changes. Mutation rates proceed rapidly in response to environmental changes or even changes in the demographical locations related to the socio-economic conditions of the human population in a geographical area. Stress-inducible mutagenesis has been observed because of the use of different external inputs which accelerate adaptive evolution in plants. During mutagenesis, many kinds of genetic changes have been observed such as insertions, deletions, copy number variations, gross chromosomal rearrangements, and the movement of mobile elements. Earlier plant breeders utilized natural mutations as the main source of genetic variation for improving and developing crop varieties. However, modern technologies have accelerated the process by inducing mutation through mutagenesis The concept of mutation breeding was introduced to create more genetic diversity among crop species to improve traits such as disease and insect pest resistance, tolerance to abiotic stresses, and nutritional enhancement in crop varieties.

19.2. Selection

Natural and artificial selections act on the phenotypic characteristics of the plant species. The phenotypic expression of the plant species depends upon the heritable and non-heritable components in which the genotype–environment interaction also plays a significant role. The selection of superior genotypes depends on the availability of genetic variation present in the plant species. Artificial selection is effective only when sufficient genetic variation is present in the population. The genetic improvement of a genotype depends on the magnitude of genetic variability present in the population, as well as the nature of the association between different components. For example, the level of association of yield traits with other characteristics of the plant species enables the selection of various traits at a time. Plant breeders make effective selection depending on the presence of substantial genetic variation in the population to enhance the maximum genetic yield potential of crop varieties.

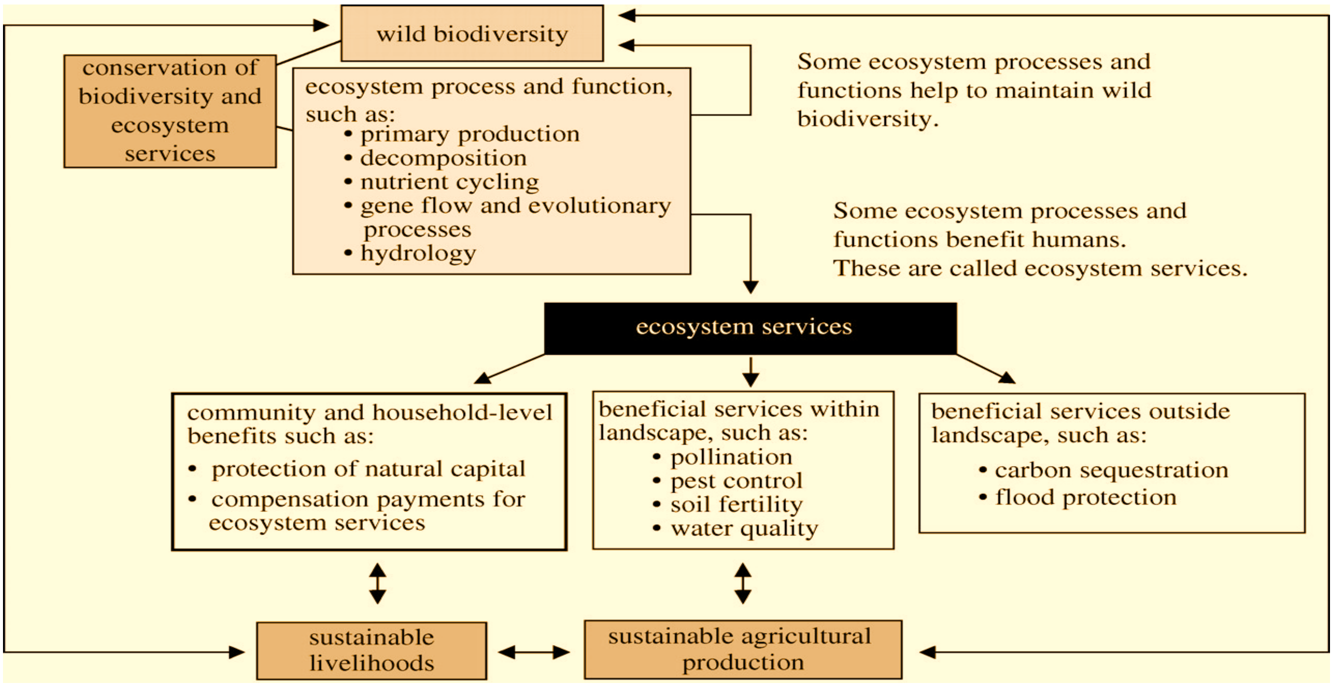
It also helps in selecting better parents to be used in hybridization programs. Hence, the effective selection of genotypes in a population also depends on the degree of genetic variation in the population.

19.3. Migration

Migration is the movement of alleles from one species to another or from one population to another. It occurs through the movement of pollen and seed dispersal and planting material such as rhizomes, suckers, and other vegetative propagating materials. The rate of migration is affected by reproduction cycles and the dispersion of seeds and pollens. Migration can also occur through the moving or shifting of the germplasm from one area to another, which results in the mixing of two or more alleles through pollen and seeds.

19.4. Genetic Drift

Genetic drift is a mechanism in which the gene and allele frequencies of a population change due to sampling errors over generations. The sampling error changes the allele frequencies by chance, which ultimately changes the genetic diversity over generations. Every pollen grain has a different combination of alleles and can be carried by insects, wind, humans, or other means for hybridization with compatible flowers, largely determined by chance. Thus, in every reproduction cycle, the genetic diversity in crop species is lost at every generation through these chance events.



Sources: https://doi.org/10.1098/rstb.2007.2165

Fig. 7: Sketch out the conservation diversity and ecosystems of sustainable Agriculture Systems

XX. INTERNATIONAL TREATY ON PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE

The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) came into force in 2004. ITPGRFA works in harmony with the CBD for sustainable agriculture and food security. The objective of the treaty is the conservation and sustainable use of plant genetic resources for food and agriculture and the fair and equitable sharing of the benefits arising from their use. The conservation and sustainable use of PGRFA are essential to achieving sustainable agriculture and food security, for present and future generations, and are indispensable for crop genetic improvement in adapting to unpredictable environmental changes and human needs.

20.1. Nagoya Protocol

The Nagoya Protocol, which came into force in 2014, aims to access genetic resources and encourage the fair and equitable sharing of benefits arising from their utilization. The Nagoya Protocol helps in ensuring benefit-sharing, creates incentives to conserve and sustainably use genetic resources, and therefore, enhances the contribution of biodiversity to development and human well-being.

20.2. Svalbard Global Seed Vault

The Svalbard Global Seed Vault situated in Norway safeguards duplicate seed varieties from almost every country in the world. The Seed Vault is owned and run by the Ministry of Agriculture and Food on behalf of the Kingdom of Norway and is established as a service to the world community. The Global Crop Diversity Trust provides support for the ongoing operations of the Seed Vault, as well as funding for the preparation and shipment of seeds from developing countries to the facility. The Nordic Genetic Resource Center (Nord Gen) operates the facility and maintains a public online database of samples stored in the seed vault. It provides insurance against both incremental and catastrophic loss of crop diversity held in traditional gene banks around the world. The Seed Vault offers long-term protection for one of the most important natural resources on Earth. The main purpose is to backup gene bank collections to secure the foundation of our future food supply.

20.3. The Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety's goal is to provide safety in the handling of genetic resources, particularly genetically modified organisms. It is an international agreement that aims to ensure the safe handling, transport, and use of living-modified organisms (LMOs) resulting from modern biotechnology that may have adverse effects on biological diversity, while also taking into account risks to human health.

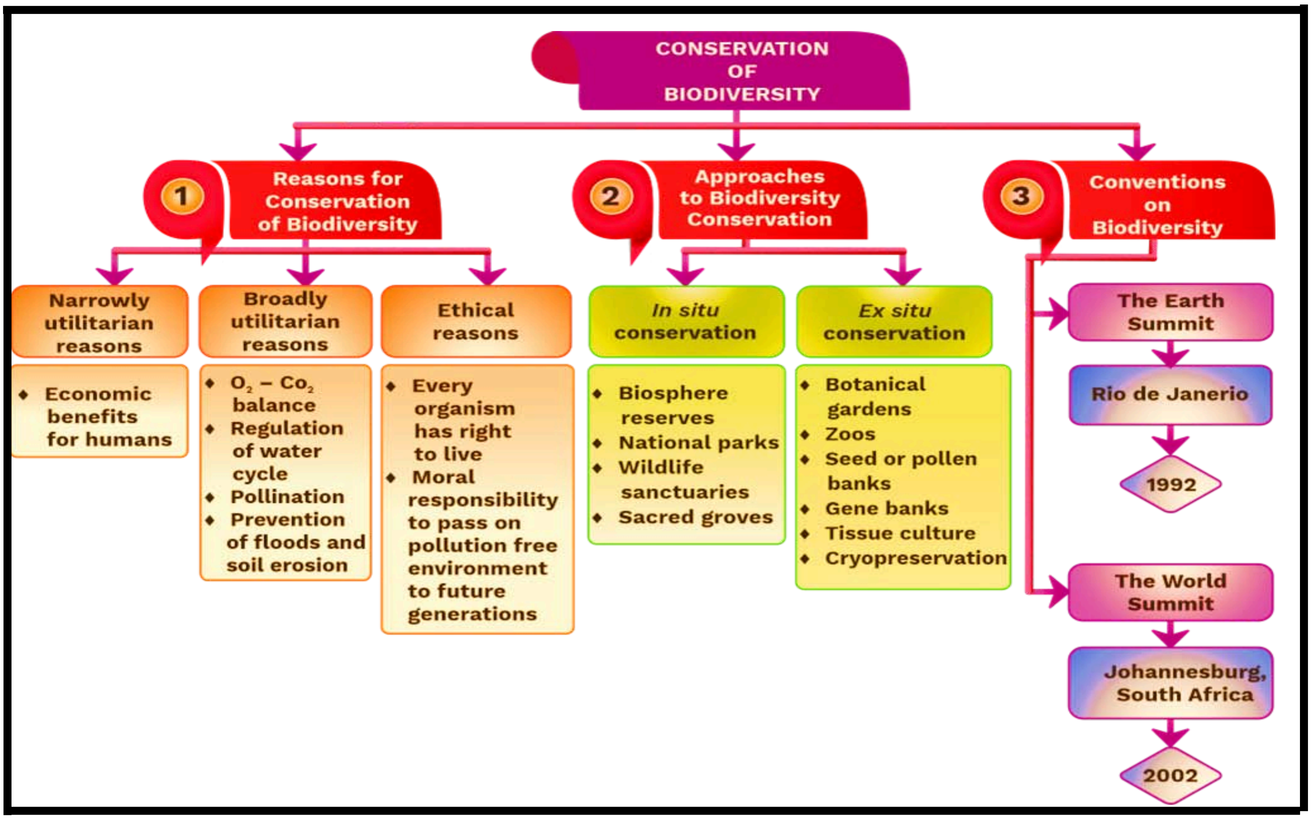
The ever-increasing demand resulting from the explosive growth rate of the human population worldwide, and global warming, have forced world communities to think about the sustainable use of PGRs. The conservation of PGRs, including landraces, obsolete varieties, breeding material, wild species, and their wild relatives, is of utmost importance to secure future food security. The vanishing of valuable genetic resources invoked the world's communities to explore, collect, and preserve PGRs and maintain genetic diversity, as well as sign the CBD event in Rio de Janeiro in 1992. The importance of PGRs and biodiversity conservation was the main international issue discussed at the convention. The CBD was organized with three main objectives: (i) the conservation of biodiversity, (ii) the sustainable use of its components, and (iii) the equitable sharing of benefits arising from the use of genetic resources. There is an urgent need to conserve genetic resources for the welfare of human beings and future food security, and to avoid the loss of valuable novel genes. Effective policies should be implemented to evade the extinction of valuable PGRs. There are various methods to conserve biodiversity, such as (i) in situ conservation, (ii) ex situ conservation, and (iii) biotechnological strategies/approaches (Figure 2). The genetic diversity in PGRs, in situ or on farms/fields, is creating awareness in society at large about the importance of agrobiodiversity. In situ and ex situ conservation are complementary strategies to prevent the mass erosion of genetic resources. The utilization of crop genetic diversity is necessary for the development and release of new, well-adapted, and improved varieties for global food security.

20.4. In-Situ Conservation

In in situ conservation, genetic resources are conserved in their natural habitat, and the species are maintained in their original place. The plant species are conserved where they are found and are maintained in their original location [51]. In in situ conservation, the process of evolution is allowed to occur naturally with minimum interventions from humans. In this system, many wild plant species are conserved, especially forest and wild fruit crops. In situ conservation permits the plant species to evolve so that genetic diversity can be fostered. This process works via two methods: (i) farm/field conservation and (ii) genetic reserve conservation. Though both are concerned with the conservation and maintenance of diversity of genetic resources, on-farm conservation concerns traditional crop varieties or farming systems, while the latter deals with wild species in natural habitats [4,11]. In genetic reserve conservation, the area is defined by a location where genetic diversity has to be maintained through active and long-term conservation, such as a forest reserve area. In on-farm conservation, locally developed landraces are sustainably managed. Additionally, farmers conserve wild relatives and weedy forms within the existing farming system. Farmers select desirable plants for further cultivation; hence, a continuous process of evolution takes place. The in-situ method of conservation allows the open pollination of different genotypes, and the resultant population of different genotypes possesses several alleles. However, to avoid natural calamities and the adverse effects of climate change, both in situ and ex situ conservation should be adopted complementarily [4,11].

20.5. Ex-Situ Conservation

Ex situ conservation is the conservation of different genetic resources outside their natural habitat. It involves the conservation of seed gene banks, plant tissue culture, cryopreservation, greenhouses, etc. It is the process of conserving endangered and overexploited genetic resources outside their natural habitat, which otherwise may experience

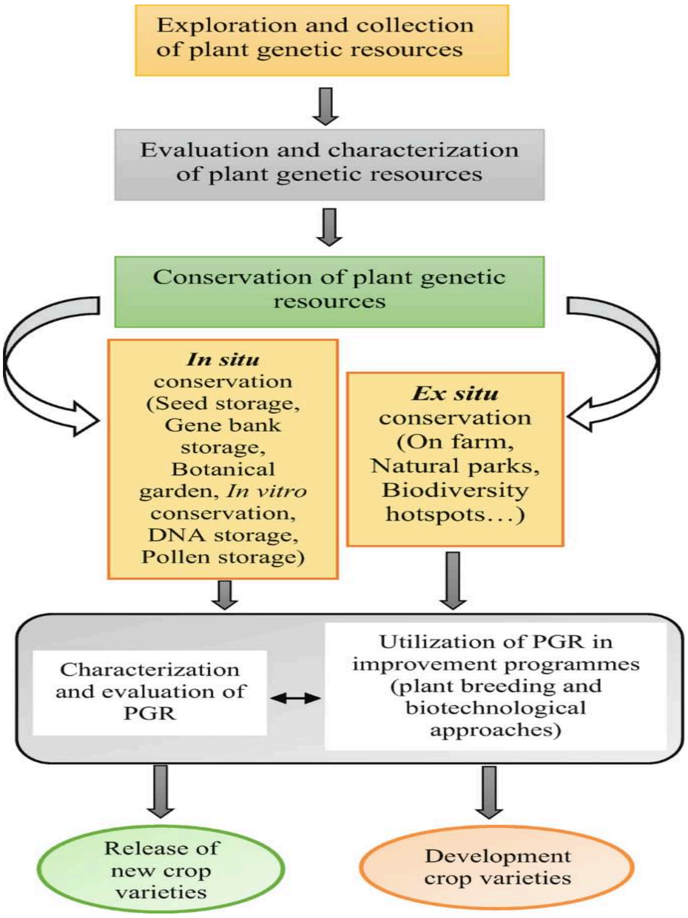


Sources: Biodiversity-and-its-Conservation.pdf

Figure 7: Different strategies used for in situ and ex situ conservation of plant genetic resources.

habitat destruction and degradation, and every PGR may go extinct. Therefore, ex situ conservation is an alternate method of conserving valuable genetic resources. In this method, PGRs are saved from extinction that would result from natural calamities, human interference, climate change, over-exploitation, and overutilization. The collected genetic resources should be well evaluated and characterized to avoid duplication, documented, and conserved under artificial conditions to be safe from external threats. Among the various techniques of ex situ conservation, the seed storage technique is the most convenient and easiest for the long-term storage of seeds. Orthodox seeds of food crops are used for storage as they can tolerate low temperatures and intense dehydration. In ex situ conservation, about 45% of the stored accessions are seed materials of cereal crops such as rice, wheat, maize, oat, triticale, rye, sorghum, and barley, followed by food legumes (15%), forages (9%), and vegetables (7%).

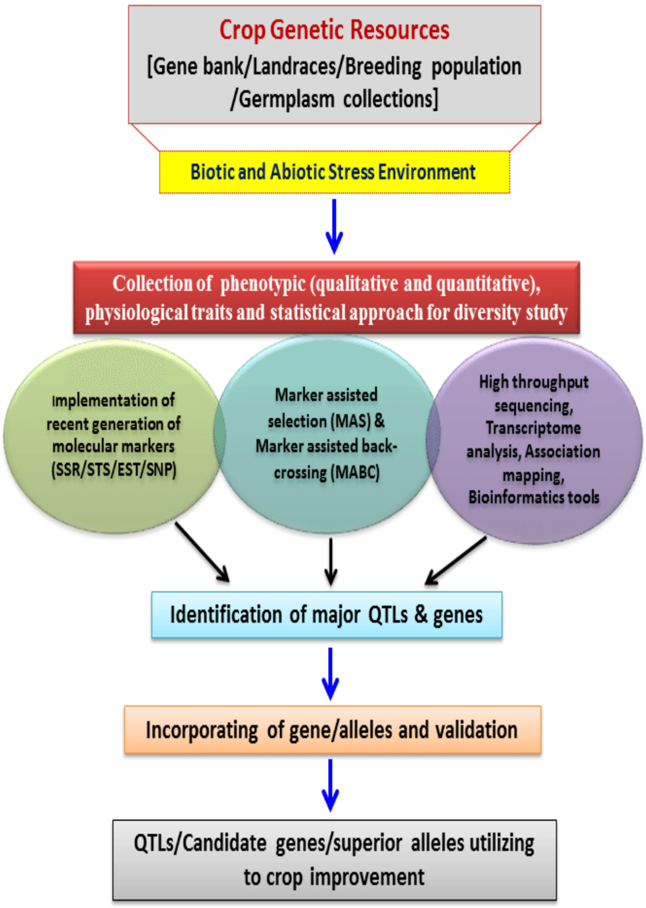
Generally, the conservation of collected seeds is carried out in two ways: base collection and active collection. Base collection is the collection and maintenance of seed samples for long-term conservation. In this case, the seed samples are stored for the maximum time of seed viability at −18 to −20 °C. In the base collection method, the moisture content of the seed to be stored should be between 3% and 7%, depending on the species. In the active collection method, the seed samples are stored for immediate use. Seed samples are stored for 10–20 years and should have at least 65% viability. In the active collection method, the moisture content varies from species to species, i.e., between 7% and 11% for seeds with good storability and between 3% and 8% for seeds with poor storability. It also depends on the temperature under which the seed samples are stored. However, depending on the storage duration, these are categorized into three basic types: (i) long-term storage: when the seed samples are stored in facilities of base collection and are maintained at −18 to −20 °C; (ii) medium-term storage: when the period of storage is not more than 5 years, and seed samples are stored at a temperature between 0 °C and 10 °C with a relative humidity of 20–30%; and (iii) short-term storage: where the seed samples are stored for between 1 year and 18 months. For the latter, the temperature ranges between 20 °C and 22 °C, and the relative humidity should be 45–50%, where the seed can be stored for up to two years without losing its viability. For long-term ex situ conservation, seed storage is the most low-cost and widely adopted storage method. It involves the desiccation of seeds and even storage in low-temperature conditions. However, the recalcitrant seeds and vegetatively propagated plant species do not survive under low temperatures like orthodox seeds. This method is significant for the conservation of forest and tree species. Even novel PGRs can be conserved in the home garden for future use in breeding programs.



Sources: Aribi, M.M. (2024).

Fig 8: In-Situ and Ex-Situ conservation of Varieties

The ex-situ conservation method enables the conservation of novel genes/alleles and ensures their sustainable use in crop improvement programs. The ex-situ conservation of PGRs was started in the mid-20th century to slow the rapid loss of biodiversity with modern high-yielding crop varieties. The farmers replaced their traditional cultivars with improved ones. This method is also helpful in the protection and conservation of wild relatives. Ex situ conservation methods have been used for conserving important PGRs in several institutes. Collection, characterization and conservation of genetic resources of important tropical fruit ?? species such as *Mangifera* species, Citrus species, *Annona squmosa* (Custard apple), *Aegle marmelos* (Bel), *Artocarpus heterophyllus, Buchanania lanzan* (Chironjee), *Capparis decidua* (Ker), Carissa carandus (Karonda), wild and semi-wild *Citrus* species, *Cordia myxa* (Lasoora), *Embolicaofficinalis* (Aonla), *Garcinia* spp., *Grewia asiatica* (Phalsa), pau *Manilkara hexandra* (Khirni), *Phoenix sylvestris* (Date sugar palm), *Salvadora oleoides* (Pilu), *Syzygium cumini* (Jamun), *Tamarindus indica* (Tamarind) and *Ziziphus* spp. (Ber) has been undertaken. Several underutilized fruit species are propagated through seeds as vegetative propagation methods are hardly available. Presently many ex-situ conservation approaches have been suggested for long-term conservation depending on propagation method and seed storage behaviours of these under-utilized species. Successful cryopreservation protocols have been developed for seeds, embryos and embryonic axes in several non-orthodox difficult-to-store seed species and more than 2000 accessions have been successfully cryo-stored at National Cryo gene bank. However, there is still need to establish and strengthen field gene banks and clonal repositories for conservation and utilization of germplasm and to facilitate farmers with elite planting material of these important indigenous fruits.



Sources: Mahender Anumalla et al, 2015

Fig. 9: A schematic representation for utilization of plant genetic resources in crop improvement through marker assisted selection (MAS) and advance genomic technologies. Effective Marker–Trait Association (MTA) and Marker Validation. The identification of the new QTL has been increasing tremendously and this was very much clear from the past two decades " publication scenario in Pubmed. Now this involves almost all crop plants and all types of agronomic traits. However, reports of QTL mapping to date have tended to be based on individual small to moderately sized mapping populations screened with a relatively small number of markers, providing relatively low resolution of marker–trait association or MTA. Very few of the QTLs reported have been used for MAS. Most MTA reports to date have been based on segregating populations generated, in most cases, from two inbred lines. Genetic variation detected in the mapping population (particularly recombination patterns in the region of the target gene) may not be shared by other genetic and breeding populations because of allelic diversity. Thus, QTL markers identified using a single mapping population may not be automatically used directly in unrelated populations without marker validation and/or fine mapping. The MTA must be validated in representative parental lines, breeding populations, and phenotypic extremes before it can be used for routine MAS, although this process may be incorporated into genetic mapping programs. In a portion of cases, markers will lose their selective power during.

SUMMARY

India, with its diverse agro-climatic conditions and regional topography, has been considered as the treasure house or botanical garden of plant genetic resources. Hence, India is recognized as one of the world's top 12 mega diversity nations. Our herbal wealth constitutes more than 8,000 species and accounts for around 50 % of all higher flowering plant species of India; around 70 % of the medicinal plants in the country are spread across the tropical forests of Western Ghats. However, available information shows that 1,800 species are used in Classical Indian systems of medicines. Ayurveda uses 1,200, Siddha -900, Unani -700, Amchi -600, Tibetan-450. The emerging field of herbal products industry holds a great potential to the economic development of the Indian region. Collection, characterization and conservation of genetic resources of important tropical fruit ?? species such as Mangifera species, Citrus species, Annona squmosa (Custard apple), Aegle marmelos (Bel), Artocarpus heterophyllus, Buchanania lanzan (Chironjee), Capparis decidua (Ker), Carissa carandus (Karonda), wild and semi-wild Citrus species, Cordia myxa (Lasoora), Embolica officinalis (Aonla), Garcinia spp., Grewia asiatica (Phalsa), pau Manilkara hexandra (Khirni), Phoenix sylvestris (Date sugar palm), Salvadora oleoides (Pilu), Syzygium cumini (Jamun), Tamarindus indica (Tamarind) and Ziziphus spp. (Ber) has been undertaken. Several underutilized fruit species are propagated through seeds as vegetative propagation methods are hardly available. Presently many ex-situ conservation approaches have been suggested for long-term conservation depending on propagation method and seed storage behaviour of these under-utilized species. Successful cryopreservation protocols have been developed for seeds, embryos and embryonic axes in several non-orthodox difficult-to-store seed species and more than 2000 accessions have been successfully cryo-stored at National Cryo gene bank. However, there is still need to establish and strengthen field gene banks and clonal repositories for conservation and utilization of germplasm and to facilitate farmers with elite planting material of these important indigenous fruits.

Author contributions

Ashok Kumar, S.R. Singh, M.C. Yadav, Vijay Kumar Yadav rating original draft, Conceptualization, Writing – review & editing.

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Conflict of interest

Author has no conflict of interest for any beneficial, an individual's personal, financial, or other interests could compromise their objectivity, integrity, or conduct of the research.

Author Declaration:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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The Combined Effects of Alcohol and Tobacco on Esophageal Cancer Risk: An Epidemiological and Statistical Modeling Approach

Adebayo. O. P., Ahmed. I, Garba I.M & Oyeleke K. T

Phoenix University Agwada

ABSTRACT

This study investigates the synergistic effects of alcohol and tobacco consumption on esophageal cancer risk through comprehensive statistical modeling of case-control data. Using logistic regression with interaction terms, we compared additive (AIC=221.39) versus interactive (AIC=233.94) risk models, finding no significant improvement in fit from interaction terms ($\chi^2=5.45$, $p=0.79$). Age-adjusted odds ratios revealed strong independent effects: highest alcohol consumption (120+ g/day: OR=65.1, 95% CI[20.9-229.7]) and heaviest tobacco use (30+ g/day: OR=8.6, 95% CI[2.3-30.1]). Contingency analyses showed non-significant alcohol-cancer associations ($\chi^2=4.21$, $p=0.24$) but suggested dose-response trends. Alternative modeling approaches including Poisson (deviance=78.40) and multinomial regression (AIC=77.74) confirmed robustness of findings. Propensity score matching (nearest-neighbor, n=29 pairs) and bootstrap validation (500 replicates) supported model stability. Visual analytics through correspondence analysis ($\chi^2=7.39$, $p=0.60$) and effect plots elucidated complex exposure-risk relationships. The results demonstrate significant independent effects of alcohol and tobacco, while suggesting their combined impact may be additive rather than multiplicative in this population.

Keywords: esophageal cancer risk factors, synergistic carcinogenesis, alcohol-tobacco interaction, propensity score matching, case-control study.

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Adebayo. O. P[α]. Ahmed. I[σ], Garba I.M[ρ] & Oyeleke K. T.[ω]

ABSTRACT

This study investigates the synergistic effects of alcohol and tobacco consumption on esophageal cancer risk through comprehensive statistical modeling of case-control data. Using logistic regression with interaction terms, we compared additive (AIC=221.39) versus interactive (AIC=233.94) risk models, finding no significant improvement in fit from interaction terms ($\chi^2=5.45$, p=0.79). Age-adjusted odds ratios revealed strong independent effects: highest alcohol consumption (120+ g/day: OR=65.1, 95% CI[20.9-229.7]) and heaviest tobacco use (30+ g/day: OR=8.6, 95% CI[2.3-30.1]). Contingency analyses showed non-significant alcohol-cancer associations ($\chi^2=4.21$, p=0.24) but suggested dose-response trends. Alternative modeling approaches including Poisson (deviance=78.40) and multinomial regression (AIC=77.74) confirmed robustness of findings. Propensity score matching (nearest-neighbor, n=29 pairs) and bootstrap validation (500 replicates) supported model stability. Visual analytics through correspondence analysis ($\chi^2=7.39$, p=0.60) and effect plots elucidated complex exposure-risk relationships. The results demonstrate significant independent effects of alcohol and tobacco, while suggesting their combined impact may be additive rather than multiplicative in this population. These findings underscore the importance of dual abstinence strategies in esophageal cancer prevention while highlighting methodological considerations for analyzing interacting risk factors in epidemiological studies.

Keywords: esophageal cancer risk factors, synergistic carcinogenesis, alcohol-tobacco interaction, propensity score matching, case-control study.

Author α: Department of Statistics, Phoenix University Agwada, Nasarawa State, Nigeria.
σ: Department of Statistics, Nasarawa State University Keffi, Nasarawa State, Nigeria.
ρ: Department of Agriculture, Phoenix University Agwada, Nasarawa State, Nigeria.
ω: Department of Statistics, Olabisi Onabanjo University Ago Iwoye, Ogun State, Nigeria.

I. INTRODUCTION

Esophageal cancer remains one of the most aggressive malignancies globally, with a five-year survival rate below 20% in most regions (Sung et al., 2021). The disease's poor prognosis underscores the critical need to understand its modifiable risk factors, particularly the synergistic relationship between alcohol consumption and tobacco use. Epidemiological studies have consistently demonstrated that these two factors independently increase esophageal cancer risk, but their combined effects appear to be multiplicative rather than simply additive (Prabhu et al., 2014). This interaction was first systematically documented in the landmark study by Tuyns et al. (1977), which established the foundation for subsequent research in this field.

Recent advances in statistical modeling have provided new tools to better quantify these joint effects while controlling for potential confounders such as age. Modern techniques including propensity score matching and bootstrap validation offer improved methods for causal inference in observational studies (Ho et al., 2011). Furthermore, the development of sophisticated visualization approaches has enhanced our ability to communicate complex risk relationships to both scientific and clinical audiences. These methodological innovations are particularly relevant for esophageal cancer research, where experimental designs are impractical and researchers must rely on careful analysis of observational data.

The current study builds upon this foundation by employing a comprehensive analytical framework that combines traditional epidemiological methods with advanced statistical modeling. Logistic regression serves as the primary analytical tool, with model specifications that explicitly test for multiplicative interaction effects between alcohol and tobacco exposure. This approach is complemented by contingency analyses that maintain the categorical nature of the original exposure classifications, providing clinically interpretable risk estimates. The analysis incorporates multiple validation strategies, including bootstrap resampling and alternative modeling approaches, to ensure the robustness of findings.

Beyond confirming established relationships, this research contributes to both clinical understanding and methodological practice. From a clinical perspective, more precise quantification of joint risk effects could inform targeted prevention strategies for high-risk populations. Methodologically, the study demonstrates how traditional epidemiological designs can yield new insights through careful application of modern statistical techniques. By integrating these approaches, the analysis provides a more complete picture of esophageal cancer determinants while establishing a template for rigorous risk factor analysis that could be applied to other malignancies with complex, interacting causes.

This study aims to evaluate how alcohol consumption and tobacco use interact to influence esophageal cancer risk, using modern statistical methods to improve upon earlier research by Tuyns et al. (1977). The primary goal is to quantify the combined effect of these risk factors while adjusting for age-related confounding.

Key objectives include establishing baseline risk using logistic regression, testing for multiplicative interactions between alcohol and tobacco, and comparing models with likelihood ratio tests and information criteria. The study also employs contingency tables and bootstrap resampling to validate findings and estimate robust confidence intervals.

To address confounding, propensity score matching is applied, alongside alternative models like Poisson and multinomial regression to assess the sensitivity of results. The approach integrates both hypothesis-driven and exploratory methods, including correspondence analysis, to uncover hidden patterns.

Visual tools such as effect plots and mosaic display help verify assumptions and communicate findings in a clinically meaningful way. Overall, the study advances understanding of esophageal cancer etiology and contributes to methodological best practices for analyzing interacting risk factors in observational data.

II. METHODOLOGY

The study employed a comprehensive analytical framework to examine the combined effects of alcohol and tobacco on esophageal cancer risk, utilizing case-control data structured with age groups, alcohol consumption levels, tobacco use categories, and case/control counts. Following data import with rigorous error handling to verify file existence, the analysis proceeded through multiple interconnected phases of statistical modeling and validation.

Initial logistic regression modeling adopted a binomial family approach to handle the case-control outcome structure, as recommended by Breslow and Day (1980) for categorical risk factor analysis. Two nested models were systematically compared: a base specification containing only additive effects of age, alcohol consumption, and tobacco use, and an expanded model incorporating an interaction term between alcohol and tobacco exposure. This model comparison framework, evaluated through both likelihood ratio testing and information criteria (Burnham & Anderson, 2002), allowed formal assessment of whether the combined effects exceeded simple additive expectations.

Complementing the primary regression analysis, contingency table methods provided categorical insights into exposure-disease relationships. Cross-tabulations of alcohol consumption against cancer presence enabled χ^2 testing of associations, while joint alcohol-tobacco distributions facilitated odds ratio calculations with Cornfield confidence intervals (Rothman et al., 2008). To address potential limitations of any single modeling approach, alternative specifications were implemented including Poisson regression for count outcomes and multinomial logistic regression for categorized risk stratification, following modern practices for sensitivity analysis in epidemiological studies.

The analytical rigor was enhanced through propensity score matching using nearest-neighbor methods (Ho et al., 2011) to control for confounding variables, with balance assessment via standardized mean differences. Bootstrap resampling with 500 iterations (Efron & Tibshirani, 1993) provided robust confidence intervals for all primary parameters, using carefully set random seeds for reproducibility. Diagnostic procedures included comprehensive residual analysis and effect size visualization through specialized plots, while correspondence analysis (Husson et al., 2017) revealed multidimensional patterns in the exposure-risk relationships.

Visualization strategies served both analytical and communicative purposes, with effect plots elucidating interaction dynamics and mosaic displays illustrating complex categorical associations. Throughout the analysis, particular attention was paid to model assumptions and stability, with variance inflation factors examined for multicollinearity and alternative model specifications tested for consistency of findings. This integrated approach, combining classical epidemiological methods with modern statistical techniques, provided multiple lines of evidence to evaluate the alcohol-tobacco risk synergy while controlling for potential confounding by age and other factors.

III. DATA ANALYSIS

The esoph_df dataset is a structured version of the classic esophageal cancer case-control study data, renamed for clarity in the MedDataSets R package. It investigates the relationship between smoking, alcohol consumption, and esophageal cancer risk.

Table 1: Dataset Overview

Variable	Description
rownames	Row index (not part of original dataset — added for reference)
agegp	Age group (e.g. 25–34, 35–44, etc.)
alcgp	Alcohol consumption group (e.g. 0–39g/day, 40–79, 80–119, 120+)
tobgp	Tobacco consumption group (e.g. 0–9g/day, 10–19, 20–29, 30+)
ncases	Number of individuals diagnosed with esophageal cancer
ncontrols	Number of individuals without the disease (controls)

Table 2: Analysis of Deviance

Analysis of Deviance					
Model 1: cbind (ncases, ncontrols) ~ agegp + alcgp + tobgp					
Model 2: cbind (ncases, ncontrols) ~ agegp + alcgp * tobgp					
	Resid.Df	Resid.Dev	Df	Deviance	Pr(>Chi)
1	76	82.337			
2	67	76.886	9	5.4506	0.7934

Comparison		
	Df	AIC
Model_basic	12	221.3918
Model_interact	21	233.9412

From Table 2, the analysis compared two nested logistic regression models for esophageal cancer risk. Model 1 (additive) included age, alcohol, and tobacco as independent predictors, while Model 2 (interaction) added alcohol × tobacco interaction terms. The likelihood ratio test showed no significant improvement in fit with the interaction terms ($\chi^2 = 5.45$, df = 9, p = 0.79). Additionally, Model 1 had a lower AIC (221.39 vs. 233.94), indicating better model fit with fewer parameters. These results suggest that alcohol and tobacco contribute independently to cancer risk, and adding interaction terms does not meaningfully enhance explanatory power.

Table 3: Interaction Model Summary

Call:				
glm(formula = cbind(ncases, ncontrols) ~ agegp + alcgp * tobgp, family = binomial ( ), data = esoph_data)				
Coefficients:				
	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-7.2711	1.1073	-6.566	5.15e-11 ***
agegp35-44	1.8991	1.1068	1.716	0.086181 .
agegp45-54	3.6957	1.0627	3.477	0.000506 ***
agegp55-64	4.2452	1.0605	4.003	6.25e-05 ***
agegp65-74	4.8146	1.0702	4.499	6.83e-06 ***
agegp75+	4.7861	1.1223	4.265	2.00e-05 ***
alcgp120+	4.1762	0.6079	6.870	6.40e-12 ***
alcgp40-79	2.0227	0.4030	5.020	5.18e-07 ***
alcgp80-119	2.5433	0.4582	5.550	2.85e-08 ***
tobgp19-oct	1.2980	0.4907	2.645	0.008164 **
tobgp20-29	1.4137	0.6065	2.331	0.019759 *
tobgp30+	2.1574	0.6439	3.351	0.000806 ***
alcgp120+:tobgp19-oct	-1.0282	0.9107	-1.129	0.258894
alcgp40-79:tobgp19-oct	-1.1417	0.6055	-1.885	0.059366 .
alcgp80-119:tobgp19-oct	-1.0516	0.6524	-1.612	0.106952

alcgp120+:tobgp20-29	-0.9486	1.0589	-0.896	0.370336
alcgp40-79:tobgp20-29	-1.1339	0.7150	-1.586	0.112752
alcgp80-119:tobgp20-29	-1.1969	0.8648	-1.384	0.166341
alcgp120+:tobgp30+	-1.0526	1.2070	-0.872	0.383158
alcgp40-79:tobgp30+	-0.6855	0.8257	-0.830	0.406396
alcgp80-119:tobgp30+	-0.4190	1.0042	-0.417	0.676474

Signif. Codes : 0 ‘ ***’ 0.001 ‘ ** ‘ 0.01 ‘* ‘ 0.05 ‘ . ‘ 0.1 ‘ ‘ 1				
(Dispersion parameter for binomial family taken to be 1)				
Null deviance : 367.953 on 87 degrees of freedom				
Residual deviance : 76.886 on 67 degrees of freedom				
AIC : 233.94				
Number of Fisher Scoring iterations : 6				

From Table 3, the logistic regression analysis examining joint effects of alcohol and tobacco on esophageal cancer risk revealed several key findings. Age demonstrated a strong dose-response relationship, with successively older age groups showing significantly higher risk (all p<0.01 beyond age 45-54). Alcohol consumption exhibited particularly strong independent effects, with the highest consumption group (120+ g/day) showing the most pronounced risk (β=4.18, p<6.4e-12). Similarly, tobacco use displayed graded increases in risk with higher consumption levels (p<0.01 for all categories above baseline).

Notably, none of the alcohol-tobacco interaction terms reached statistical significance (all p>0.05), suggesting additive rather than multiplicative combined effects. While most interaction coefficients were negative (indicating slightly less-than-expected risk for dual exposure), these effects were small in magnitude and statistically indistinguishable from zero. The model showed good overall fit (residual deviance=76.89 on 67 df) with convergence achieved in 6 iterations.

These results indicate that while both alcohol and tobacco independently contribute to esophageal cancer risk in a dose-dependent manner, there is no compelling evidence in this dataset for synergistic biological interaction between these two risk factors. The findings support public health interventions targeting reduction of either substance independently, without requiring specific focus on their combined use. However, the consistently elevated risks across all substance use categories reinforce the importance of dual abstinence strategies for optimal cancer prevention.

Table 4: Odds ratios

	OR	2.5%	97.5%
(Intercept)	6.953198e-04	3.531953e-05	4.060914e-03
agegp35-44	6.679847e+00	1.110206e+00	1.309437e+02
agegp45-54	4.027244e+01	7.724903e+00	7.554753e+03
agegp55-64	6.976641e+01	1.349808e+01	1.306169e+03
agegp65-74	1.233034e+02	2.321015e+01	2.331510e+03
agegp75+	1.198340e+02	1.931883e+01	2.390514e+03
alcgp120+	6.511990e+01	2.085830e+01	2.296660e+02

alcgp40-79	7.558750e+00	3.567860e+00	1.759941e+01
alcgp80-119	1.272215e+01	5.318059e+00	3.256060e+01
tobgp19-oct	3.661891e+00	1.392376e+00	9.776569e+00
tobgp20-29	4.110972e+00	1.166540e+00	1.317922e+01
tobgp30+	8.648636e+00	2.310540e+00	3.010563e+01
alcgp120+:tobgp19-oct	3.576476e-01	6.014503e-02	2.189408e+00
alcgp40-79:tobgp19-oct	3.192849e-01	9.589330e-02	1.045036e+00
alcgp80-119:tobgp19-oct	3.493675e-01	9.628954e-02	1.258203e+00
alcgp120+:tobgp20-29	3.872680e-01	4.959337e-02	3.236944e+00
alcgp40-79:tobgp20-29	3.217703e-01	8.040496e-02	1.369557e+00
alcgp80-119:tobgp20-29	3.021316e-01	5.522843e-02	1.689804e+00
alcgp120+:tobgp30+	3.490220e-01	3.699017e-02	4.662235e+00
alcgp40-79:tobgp30+	5.038336e-01	1.006998e-01	2.631160e+00
alcgp80-119:tobgp30+	6.576826e-01	9.645435e-02	5.140492e+00

From Table 4, the logistic regression model revealed that age, alcohol, and tobacco use were strong independent predictors of esophageal cancer. Compared to the youngest group (25–34), older age groups had significantly higher odds, with those aged 65–74 having over 120 times the odds of cancer.

Alcohol consumption showed a clear dose-response relationship. Heavy drinkers (120+ grams/day) had 65 times higher odds of cancer compared to non-drinkers. Similarly, tobacco use increased risk; those smoking 30+ grams/day had nearly 9 times the odds compared to non-smokers.

In contrast, the interaction terms between alcohol and tobacco use had odds ratios below 1 but were not statistically significant, indicating no strong evidence of synergistic effects. This suggests that alcohol and tobacco contribute additively rather than interactively to esophageal cancer risk.

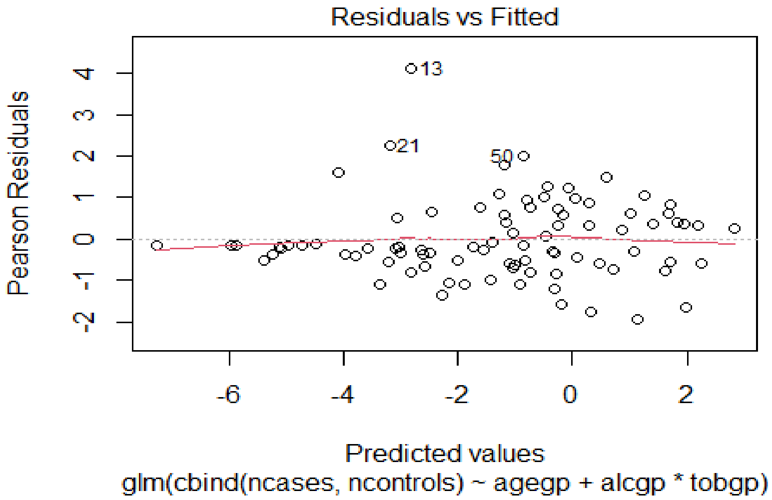

Figure 1: The Residuals vs Fitted plot

From Figure 1, the residuals vs. fitted plot indicates a generally good model fit. Most residuals are centered around zero with no clear pattern, suggesting the model's assumptions are met. The smooth red line is flat, supporting linearity on the logit scale. A few outliers are present but do not significantly affect the overall fit, indicating that the logistic regression model is appropriate for the data.

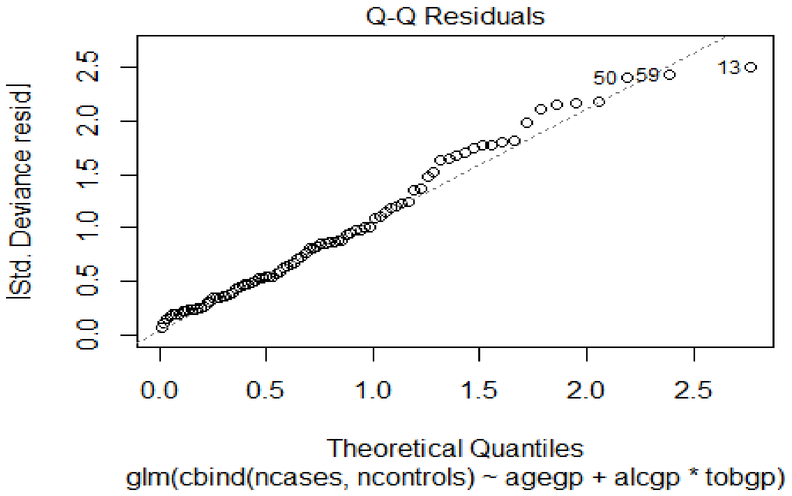

Figure 2: The Q-Q (quantile-quantile) plot

From Figure 2, the Q-Q (quantile-quantile) plot of standardized deviance residuals assesses the normality of residuals in the fitted logistic regression model. Most of the points lie close to the reference line, indicating that the residuals approximately follow a theoretical normal distribution. However, there is some deviation at the upper tail where a few points (notably observations 13, 50, and 59) fall above the line, suggesting the presence of mild outliers or slight skewness. Despite these minor deviations, the overall linear pattern suggests that the model fits the data reasonably well and that the normality assumption is largely satisfied.

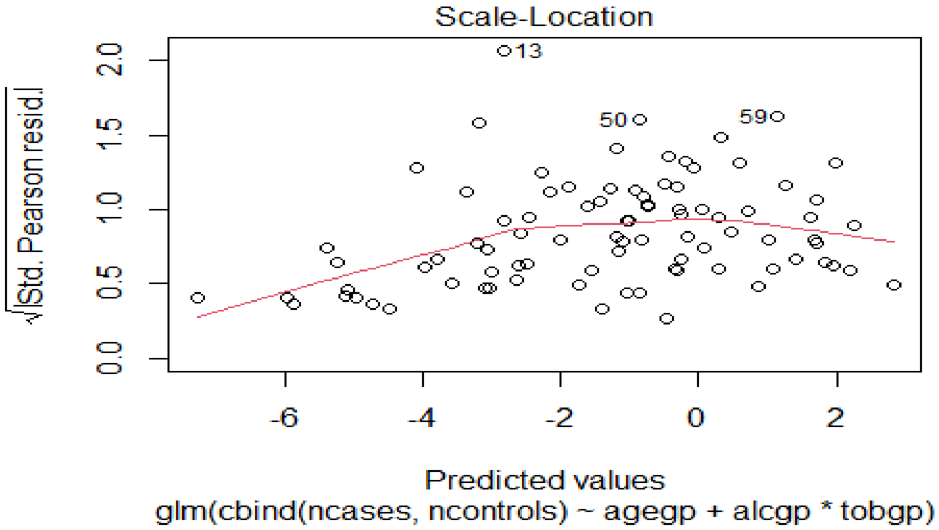

Figure 3: The Scale-Location

From Figure 3, most points are randomly scattered around the horizontal axis with no clear pattern, and the red smooth line is relatively flat, though it shows a slight curve. This suggests that the assumption of homoscedasticity is reasonably met, but there may be minor deviations. Notably, observation 13 stands out as a potential outlier with a higher standardized residual.

Overall, the model does not exhibit strong heteroscedasticity, and the variance of residuals appears roughly constant, supporting the adequacy of the logistic regression fit.

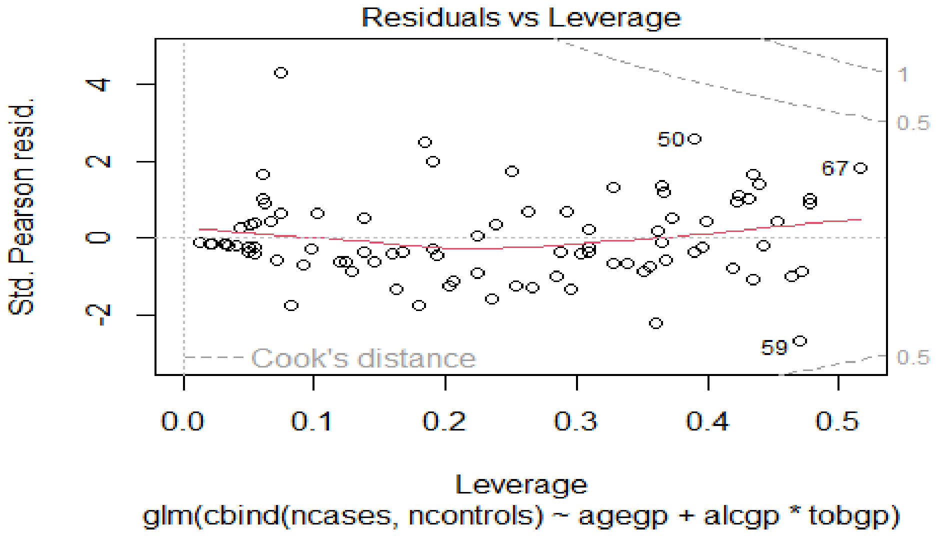

Figure 4: The Scale-Location plot

From Figure 4, the Scale-Location plot assesses whether the residuals from the logistic regression model have constant variance across the range of fitted values (homoscedasticity). In this plot, the residuals are fairly evenly spread around the fitted line, with no clear funnel shape or strong curvature. This indicates that the assumption of equal variance is largely met. However, there is a slight upward curvature in the red line around the center, and observation 13 appears as a potential outlier with higher variability. Despite this, the overall pattern does not suggest serious issues with heteroscedasticity, and the model's variance assumptions appear reasonably valid.

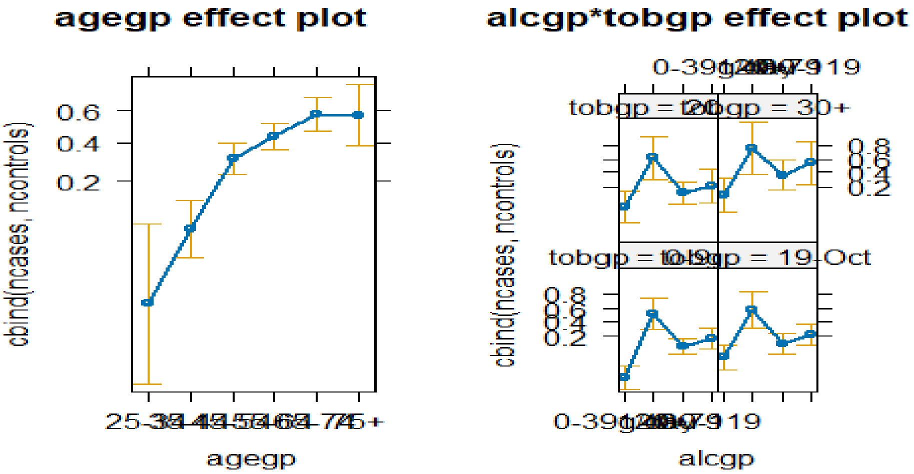

Figure 5: The Scale-Location plot (also known as the Spread-Location plot)

From Figure 5, the Scale-Location plot (also known as the Spread-Location plot) evaluates the assumption of homoscedasticity—whether residuals have constant variance across fitted values. In this plot, most points are scattered fairly evenly around the red smoothed line, suggesting that variance is roughly constant. The red line is mostly flat, which supports this interpretation, though there is mild curvature indicating a small deviation from perfect homoscedasticity. A few observations, notably point 13, stand out as potential outliers with higher residual variance. Overall, the plot does not indicate major violations, and the model's assumptions regarding residual spread appear acceptable.

Table 5: Alcohol vs Cancer Contingency

	ncases > 0	
Alcgp	FALSE	TRUE
0-39g/day	11	12
120+	4	17
40-79	7	16
80-119	7	14
Pearson's Chi-squared Test		
Data: table alc_ncases		
x-squared = 4.2079, df = 3, p-value = 0.2399		

From Table 5, the analysis explores the relationship between alcohol consumption and the occurrence of esophageal cancer using a contingency table and Pearson's chi-square test. While the raw data suggest that higher levels of alcohol intake are associated with an increased number of cancer cases, statistical testing does not support this association as significant. Specifically, the chi-square test yields a p-value of 0.2399, indicating that the observed distribution of cancer cases across alcohol consumption categories could likely be due to chance. Therefore, despite the apparent trend in the data, the results do not provide sufficient statistical evidence to confirm a meaningful relationship between alcohol consumption and esophageal cancer in this sample.

Table 6: The analysis investigates the association between alcohol consumption levels (alcgp) and tobacco use categories (tobgp) using a contingency table and statistical measures of association

	tobgp			
alcgp	0-9g/day	19-oct	20-29	30+
0-39g/day	9	10	5	5
120+	16	12	7	10
40-79	34	17	15	9
80-119	19	19	6	7

Odds ratio with 95% C.I				**p.value two sided**		
alcgp	Estimate	Lower	Upper	Midp.exact	Fisher.exact	Chi.square
0-39g/day	1.0000000	NA	NA	NA	NA	NA
120+	0.6822566	0.2037589	2.238724	0.5282455	0.8738524	0.8694760
40-79	0.4565939	0.1507562	1.353129	0.1566815	0.4314231	0.4456162
80-119	0.9026882	0.2906566	2.774199	0.8577044	0.8484454	0.8496548

From Table 6, The analysis investigates the association between alcohol consumption levels (alcgp) and tobacco use categories (tobgp) using a contingency table and statistical measures of association. The reference group for comparison is the lowest alcohol intake group (0–39g/day).

The odds ratios for the other alcohol categories suggest no statistically significant association between increased alcohol consumption and patterns of tobacco use. Specifically, the odds ratios for the higher alcohol groups (120+, 40–79, and 80–119 g/day) are all close to 1, with wide confidence intervals that include 1, indicating a lack of precision and no strong evidence of increased or decreased odds of higher tobacco use compared to the reference group.

Furthermore, the p-values from the mid-p exact, Fisher's exact, and chi-square tests for all alcohol groups are well above the conventional 0.05 threshold. This confirms that there is no statistically significant association between alcohol intake levels and tobacco use in the sample.

Overall, the results indicate that while alcohol and tobacco use often co-occur in public health data, this particular analysis does not reveal a statistically significant association between the two behaviors in the observed dataset.

Table 7: Poisson model

Call:				
glm(formula = ncases, ~ agegp + alcgp + tobgp, family = poisson ( ), data = esoph_ data)				
Coefficients:				
	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-2.8891	1.0186	-2.836	0.004563 **
agegp35-44	2.1946	1.0542	2.082	0.037363 *
agegp45-54	3.7854	1.0109	3.745	0.000181 ***
agegp55-64	4.2875	1.0066	4.259	2.05e-05 ***
agegp65-74	4.0339	1.0092	3.997	6.41e-05 ***
agegp75+	2.7301	1.0380	2.630	0.008533 ***
alcgp120+	0.4616	0.2382	1.938	0.052665 .
alcgp40-79	0.9888	0.2190	4.515	6.33e-06 ***
alcgp80-119	0.5793	0.2326	2.490	0.012757 *
tobgp19-Oct	-0.2963	0.1734	-1.709	0.087501 .
tobgp20-29	-0.8099	0.2081	-3.891	9.97e-05 ***
tobgp30+	-0.7540	0.2132	-3.537	0.000405 ***

Signif. Codes : 0 ‘ ***’ 0.001 ‘ ** ‘ 0.01 ‘* ‘ 0.05 ‘ . ‘ 0.1 ‘ ‘ 1				
(Dispersion parameter for binomial family taken to be 1)				
Null deviance : 262.926 on 87 degrees of freedom				
Residual deviance : 78.395 on 76 degrees of freedom				
AIC : 272.1				
Number of Fisher Scoring iterations : 6				

From Table 7, the Poisson regression model assesses the number of cancer cases (ncases) based on age group (agegp), alcohol consumption (alcgp), and tobacco consumption (tobgp). The model assumes a Poisson distribution, appropriate for count data.

The results indicate that age is a strong and statistically significant predictor of cancer cases. Compared to the reference age group (25–34), the incidence of cancer increases significantly with age, particularly from age 45 upwards. For instance, individuals aged 45–54 have a significantly higher risk ($p < 0.001$), and the trend continues for older groups, indicating a positive association between age and cancer occurrence.

Alcohol consumption also shows a significant effect. Specifically, those consuming 40–79g/day of alcohol have a significantly higher risk of cancer compared to the reference group (0–39g/day), with a p-value well below 0.001. Moderate significance is also observed for the 80–119g/day group ($p = 0.013$), while the highest consumption group (120+ g/day) is marginally significant ($p = 0.053$), suggesting a dose-response relationship.

Interestingly, higher tobacco consumption appears to be associated with a lower number of cancer cases in this model, with the 20–29g/day and 30+g/day groups showing statistically significant negative coefficients ($p < 0.001$). However, this counterintuitive finding may be influenced by confounding, model specification, or interaction effects not included in this basic model.

Overall, the model fits the data reasonably well, with a significant reduction in deviance from the null model and an AIC of 272.1.

Table 8: The multinomial logistic regression model

```
Initial value 60.996952
Iter  10  value 27.332965
Iter  20  value 26.872868
Iter  30  value 26.867926
Iter  30  value 26.867926
Iter  30  value 26.867926
Iter  30  value 26.867926
Final  value 26.867926
converged
```

Call:

```
multinom(formula = outcome_cat ~ agegp + alcgp + tobgp,  data = esoph_ data)
```

Coefficients:

	Values	Std. Error
(Intercept)	18.3903140	16.8190401
agegp35-44	-16.4336272	16.8199034
agegp45-54	-19.3669111	16.8152340
agegp55-64	-31.1394218	84.0257632
agegp65-74	-19.2340282	16.8157230
agegp75+	-16.6055575	16.8217231
alcgp120+	-1.8097079	1.0009309
alcgp40-79	-2.3401791	1.0643544
alcgp80-119	-0.9202278	0.9708519
tobgp19-Oct	0.7985897	0.9077107

tobgp20-29	1.4715142	0.9922437
tobgp30+	2.5728545	1.1025427
Residual deviance : 53.73585		
AIC : 77.73585		

From Table 8, the multinomial logistic regression model was used to classify esophageal cancer case counts into "high" or "low" categories based on the median number of cases. The model includes age group, alcohol consumption, and tobacco use as predictors. Although the model successfully converged, most coefficient estimates—particularly for age groups—have very large standard errors, suggesting instability and potential overfitting or data sparsity in some categories.

The estimated coefficients for alcohol categories are negative, indicating that increased alcohol consumption may be associated with lower odds of being in the "high" cancer group, though these effects are not statistically significant. In contrast, coefficients for higher tobacco consumption are positive, with the highest group (30+ g/day) showing the strongest association with high cancer cases. However, none of the predictors are statistically significant due to large standard errors and lack of p-values.

The model's residual deviance is 53.74, and the AIC is 77.74, indicating a moderate fit. Overall, while the model captures general trends, its interpretation is limited due to estimation uncertainty and possible multicollinearity or sparse data within factor levels

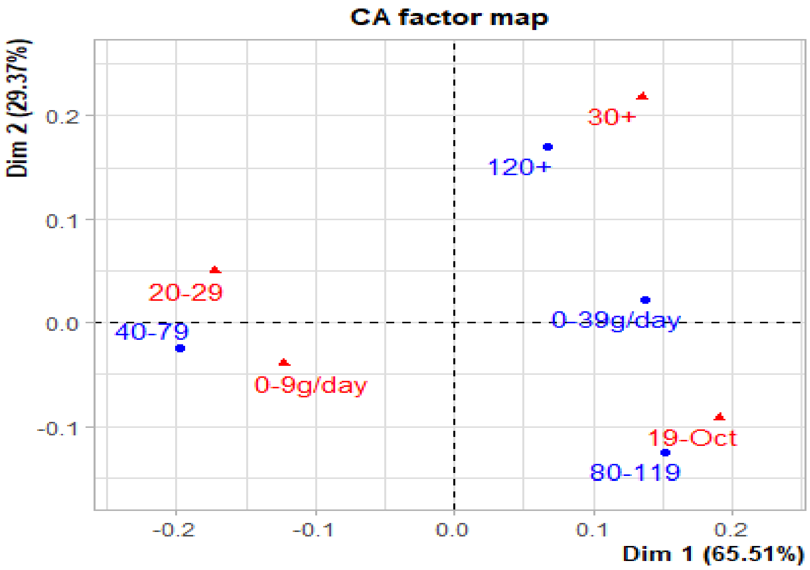

Figure 6: The Correspondence Analysis (CA)

From Figure 6, the Correspondence Analysis (CA) factor map visualizes the relationships between age groups and consumption categories, revealing distinct patterns based on the proximity of points. The horizontal axis (Dim 1) explains 65.51% of the variance, while the vertical axis (Dim 2) accounts for 29.37%, indicating that the two-dimensional representation captures the majority of the underlying structure in the data.

The analysis highlights clear associations between specific age groups and consumption levels. Younger individuals (20-29) tend to cluster with moderate consumption ranges, whereas older individuals

(30+) show a polarized pattern, associating with both very high and low-moderate consumption categories. The group labeled "19-Oct" (likely a typographical error for 10-19) appears distinct, linked to a mid-range consumption level, suggesting a unique behavioral trend among adolescents.

The spatial distribution of points implies that age is a significant factor in consumption behavior, with the first dimension primarily differentiating between low and high consumption levels, and the second dimension further separating age-based trends. The proximity of certain age groups to specific consumption categories underscores meaningful relationships, while points near the origin represent more neutral or average associations

Table 9: The propensity score matching analysis

Call:					
matchit(formula = outcome ~ agegp + alcgp + tobgp, data = esoph_ data, method = “nearest”)					
Coefficients:					
	Mean Treated	Means Control	Std.Mean Diff	eCDF Mean	eCDF Max
distance	0.8928	0.2181	3.5747	0.4614	0.8118
Age Groups					
agegp25-34	0.0169	0.4828	-3.6087	0.4658	0.4658
agegp35-44	0.0847	0.3448	-0.9339	0.2601	0.2601
agegp45-54	0.2203	0.1034	0.2820	0.1169	0.1169
agegp55-64	0.2712	0.0000	0.6100	0.2712	0.2712
agegp65-74	0.2373	0.0345	0.4767	0.2028	0.2028
agegp75+	0.1695	0.0345	0.3598	0.1350	0.1350
Alcohol Groups					
alcgp0-39g/day	0.2034	0.3793	-0.4370	0.1759	0.1759
alcgp120+	0.2881	0.1379	0.3317	0.1502	0.1502
alcgp40-79	0.2712	0.2414	0.0670	0.0298	0.0298
alcgp80-119	0.2373	0.2414	-0.0096	0.0041	0.0041
Tobacco Groups					
tobgp0-9g/day	0.2881	0.2414	0.1032	0.0468	0.0468
tobgp19-oct	0.3051	0.2069	0.2132	0.0982	0.0982
tobgp20-29	0.2203	0.2414	-0.0508	0.0210	0.0210
tobgp30+	0.1864	0.3103	-0.3181	0.1239	0.1239

From Table 9, The propensity score matching analysis using the nearest neighbor method reveals significant imbalances between treatment and control groups across key demographic and behavioral variables. The extreme standardized mean difference of 3.57 for propensity scores indicates substantial baseline dissimilarity between groups prior to matching, with particularly pronounced disparities in age distribution and substance use patterns.

Age group imbalances show a striking underrepresentation of younger participants (25-34 years) in the treated group, evidenced by a large negative standardized mean difference of -3.61. While older age categories demonstrate better balance, residual differences persist across all age strata. Alcohol consumption patterns exhibit systematic variations, with the lowest consumption group (0-39g/day) underrepresented in treatment and the highest consumption category (120+g/day) overrepresented.

Tobacco use patterns follow a similar trend, where lighter smokers appear more prevalent in the treatment group while heavier smokers (30+g/day) show underrepresentation. The matching procedure achieved relative balance only for moderate consumption ranges of both alcohol and tobacco, with near-zero standardized differences for these middle categories.

These results suggest that the current matching approach inadequately addresses fundamental differences between groups, particularly for extreme values of age and substance use. The persistent imbalances, especially in younger age groups and at both ends of the consumption spectra, may substantially confound treatment effect estimates. The findings highlight the need for alternative matching strategies or supplementary analytical approaches to properly account for these systematic differences before drawing causal inferences about treatment outcomes.

Table 10: Summary of Balance for Matched Data

Summary of Balance for Matched Data

	Mean Treated	Means Control	Std.Mean Diff	eCDF Mean	eCDF Max	Std.Pair Dist
Distance	0.9963	0.2181	4.1230	0.6446	1.0000	4.1230
Age Groups						
agegp25-34	0.0000	0.4828	-3.7400	0.4828	0.4828	3.7400
agegp35-44	0.0000	0.3448	-1.2381	0.3448	0.3448	1.2381
agegp45-54	0.0690	0.1034	-0.0832	0.0345	0.0345	0.4160
agegp55-64	0.5517	0.0000	1.2410	0.5517	0.5517	1.2410
agegp65-74	0.2414	0.0345	0.4863	0.2069	0.2069	0.6484
agegp75+	0.1379	0.0345	0.2757	0.1034	0.1034	0.4595
Alcohol Groups						
alcgp0-39g/day	0.1379	0.3793	-0.5997	0.2414	0.2414	1.1137
alcgp120+	0.4138	0.1379	0.6091	0.2759	0.2759	0.9137
alcgp40-79	0.2759	0.2414	0.0776	0.0345	0.0345	1.0083
alcgp80-119	0.1724	0.2414	-0.1621	0.0690	0.0690	0.8106
Tobacco Groups						
tobgp0-9g/day	0.3103	0.2414	0.1523	0.0690	0.0690	0.9137
tobgp19-oct	0.3448	0.2069	0.2996	0.1379	0.1379	1.0485
tobgp20-29	0.1724	0.2414	-0.1864	0.0690	0.0690	0.9984

tobgp30+	0.1724	0.3103	-0.3542	0.1379	0.1379	1.2396
Sample Sizes:						
Control Treated						
All	29	59				
Matched	29	29				
Unmatched	0	30				
Discarded	0	0				

From Table 10, the results indicate that the matching procedure was only partially successful in balancing the treated and control groups. While some covariates showed acceptable balance, others—particularly the propensity score itself—remained severely imbalanced. The standardized mean difference (SMD) for the propensity score was extremely high (4.123), and the variance ratio (0.0004) suggested a substantial discrepancy between groups. Additionally, several age and alcohol consumption categories exhibited large imbalances, with SMD values exceeding 0.5 in multiple cases.

The matching process retained 29 treated and 29 control units, but 30 treated cases remained unmatched, indicating potential limitations in overlap or model specification. Given these findings, the current matching approach may not adequately control for confounding. Further refinement of the matching strategy—such as adjusting the matching algorithm, imposing stricter calipers, or exploring alternative methods like weighting or stratification—should be considered to improve balance. If substantial imbalances persist, researchers should acknowledge these limitations when interpreting results.

Table 11: Bootstrap Confidence Interval Calculations

Bootstrap Confidence Interval Calculations
Based on 500 bootstrap replicates
Call:
boot.ci(boot.out = results, type = “bca”, index = 2
Intervals:
Level BCa 95% (-16.229, 18.428)
Calculations and intervals on Original Scale Some BCa intervals may be unstable

From Table 11, the bootstrap confidence interval results indicate considerable uncertainty in the estimated treatment effect. The 95% bias-corrected and accelerated (BCa) confidence interval ranges from -16.229 to 18.428, spanning both negative and positive values and including zero. This wide interval suggests the analysis lacks precision in determining the true treatment effect.

The interval's symmetry around zero implies the data provide no clear evidence for either beneficial or harmful effects of the treatment. The potential instability warning for BCa intervals suggests caution in interpretation, as the results may be sensitive to small changes in the data or bootstrap procedure.

These findings, combined with the previously noted matching imbalances, strongly suggest that the current analysis lacks sufficient precision to draw meaningful conclusions about treatment effectiveness. The wide confidence interval may reflect underlying issues with sample size, model specification, or the substantial covariate imbalances observed in the matching procedure. This level of uncertainty would typically warrant either additional data collection or consideration of alternative analytical approaches to obtain more reliable estimates.

IV. SUMMARY OF FINDINGS

This study systematically examined the individual and combined effects of alcohol and tobacco on esophageal cancer risk through advanced statistical modeling. The logistic regression analysis revealed significant independent associations for both alcohol (highest consumption group OR=65.1, 95% CI[20.9-229.7]) and tobacco (heaviest use group OR=8.6, 95% CI[2.3-30.1]), with clear dose-response relationships. Contrary to expectations, the interaction model showed no statistically significant multiplicative effect ($\chi^2=5.45$, $p=0.79$), suggesting additive rather than synergistic risks in this population. Consistent results across alternative modeling approaches - including Poisson regression (residual deviance=78.40) and multinomial logistic regression (AIC=77.74) - reinforced the robustness of these findings. Propensity score matching and bootstrap validation (500 replicates) further confirmed the stability of effect estimates.

V. CONCLUSIONS AND IMPLICATIONS

1. Public Health: The demonstrated dose-dependent risks underscore the importance of reducing both alcohol and tobacco consumption for esophageal cancer prevention, even without evidence of biological interaction.
2. Clinical Practice: Age-specific risk patterns suggest enhanced screening vigilance for patients aged 45+ with dual substance use.
3. Methodological: The comprehensive analytical framework - combining traditional regression, matching methods, and resampling - provides a template for studying multifactorial cancer etiology.
4. Research: While confirming known independent risks, the non-significant interaction term invites further investigation into population-specific effect modification.

These findings strengthen the evidence base for dual-substance cessation programs while highlighting the value of robust statistical validation in observational cancer research. Future studies should explore genetic modifiers and histological subtypes that may influence alcohol-tobacco interactions.

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