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PLANT SCIENCE: FROM FUNDAMENTALS TO ADVANCED RESEARCH

VOLUME I

Editors:
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Dr. Manoj R. Kakpure
Dr. Sumita Dasgupta
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PREFACE

The study of plant science has been a cornerstone of scientific inquiry since the earliest days of human civilization. From the ancient practices of agriculture and herbal medicine to the cutting-edge biotechnological advancements of today, the exploration of the plant kingdom has continually expanded our understanding of life and its intricate systems.

"Plant Science: From Fundamentals to Advanced Research" is the first in a series that aims to bridge the gap between foundational knowledge and the latest research developments in plant biology. This volume is designed to serve as a comprehensive resource for students, educators, and researchers alike, offering insights into the fundamental principles of plant science while also delving into the most recent scientific discoveries and technological innovations.

The chapters in this volume are authored by experts in various subfields of plant science, each bringing their unique perspective and expertise to the topics covered. From the cellular and molecular mechanisms that drive plant growth and development to the ecological interactions and environmental challenges that shape plant life, this book provides a thorough exploration of the diverse and dynamic world of plants.

As the global community faces unprecedented challenges such as climate change, food security, and biodiversity loss, the importance of plant science has never been more critical. By understanding the fundamental processes that govern plant life, we can better appreciate the role that plants play in sustaining life on Earth and develop strategies to harness their potential for the benefit of humanity.

It is our hope that this volume will inspire a deeper interest in plant science and encourage further research and innovation in this vital field. Whether you are a student beginning your journey in plant biology or an experienced researcher seeking to expand your knowledge, we believe that "Plant Science: From Fundamentals to Advanced Research" will be an invaluable addition to your library.

We extend our gratitude to the contributors whose expertise and dedication have made this volume possible, and we look forward to the continued exploration and discovery that future volumes in this series will bring.

Editors

TABLE OF CONTENT

Sr. No.	Book Chapter and Author(s)	Page No.
1.	**MAJOR DISEASES OF SUGARCANE AND THEIR INTEGRATED MANAGEMENT** Dinesh K. Pancheshwar, B. K. Sharma, Ramesh Amule, Ashish Tiwari, Pawan K. Amrate, Monika Singh Thakur, Mayank Mehra and Shubham Mishra	1 – 13
2.	**ADVANCED TECHNOLOGIES OF MANGO CULTIVATION WITH ULTRA HIGH-DENSITY PLANTING** Hemant Dnyaneshwar Chandore	14 – 28
3.	**THE SECONDARY METABOLITES OF PLANTS AS MICROBIAL PRODUCTS** Ashok Kr. Singh	29 – 35
4.	**SUMMER POLLEN SOURCES TO *APIS DORSATA* HONEY BEES COLLECTED FROM NAGBHID FOREST AREA OF CHANDRAPUR DISTRICT OF MAHARASHTRA STATE (INDIA)** Laxmikant Borkar, Ankit Nakade and Devendra Mate	36 – 45
5.	**ALLELE MINING: PRINCIPLES, PROSPECTS AND POTENTIAL** Sourav Ranjan Nanda, M Usha and Shreya Singh	46 – 51
6.	**EXPLORING PLANT SECONDARY METABOLITES PRODUCTION WITH EMERGING TECHNIQUES IN PLANT BIOTECHNOLOGY** Jyoti Raju Kadam, Piyush Mandal, Nada Raees and Safa Khot	52 – 58
7.	**SEED SURFACE CHARACTERISTICS AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *CUSCUTA REFLEXA* ROXB. SEEDS OF FAMILY CONVOLVULACEAE** P. P. Ulhe	59 – 64
8.	**STRATEGIES FOR PLANT DISEASE RESISTANCE** Mamta Ghasil, Deepak Gupta and Anju Ghasil	65 – 79
9.	**ADVANCEMENTS AND CHALLENGES IN BREEDING SELF-POLLINATED CROPS: MECHANISMS, METHODS, AND FUTURE PROSPECTS** Anita, Deepak Gupta and Anil Kumar	80 – 91

10.	**ASSESSING THE IMPACT OF NOISE POLLUTION IN NANDED CITY: SOURCES, EFFECTS, AND SOLUTIONS** M. A. Bangar	92 – 99
11.	**PHARMACOLOGICAL ACTIVITIES OF LEAVES OF *PHASEOLUS VULGARIS* LINN. A COMPRESSIVE REVIEW** Amrin Shaikh and Madhavi Badole	100 – 103
12.	**METABOLOMICS AND PLANT SECONDARY METABOLITES** Rosy Khatoon and Kumar Prabhat	104 – 115

MAJOR DISEASES OF SUGARCANE AND THEIR INTEGRATED MANAGEMENT

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Abstract:

Sugarcane is a significant commercial crop farmed in tropical and subtropical regions of the country. Over the past century, the country has experienced numerous epidemics. Diseases include red rot, smut, wilt, rust, leaf scald, and yellow leaf (YLD). Sugarcane damage during epidemics varies depending on the disease and its transmission across affected kinds. Sugarcane cultivars were replaced due to new diseases or pathogenic strains. Red rot is the most common sugarcane disease in India, followed by wilt and smut. YLD, a new disease, significantly reduces cane productivity in certain states. The severity of grassy shoot disease, pokkah boeng, and rust is increasing in various regions of the country. Sugarcane propagation via. vegetative cuttings promote disease transmission through planting materials. Disease spread through seed canes significantly impacts sugarcane development and performance. Developing disease-resistant sugarcane varieties is vital for managing illnesses and has been done in the past. Sugarcane diseases are effectively managed by various agronomical procedures and physical methods, including heat therapy. Sugarcane tissue culture is being promoted as a method for producing virus and phytoplasma-free plant components. ELISA and tissue-blot techniques are widely employed for detecting bacterial, phytoplasmal, and viral diseases in sugarcane. Molecular approaches such as polymerase chain reaction (PCR), reverse transcriptase (RT)-PCR, and nested PCR tests are more sensitive than serological techniques for detecting sugarcane viruses and phytoplasmas in low concentrations. To effectively manage sugarcane diseases, it is recommended to use disease-resistant cultivars and maintain robust seed nursery programs.

Keywords: Sugarcane, Diseases, Symptoms, Pathogen, Epidemiology, Diagnosis, Integrated Management.

Introduction:

Sugarcane is a perennial grass crop of the Poaceae family, scientifically known as *Saccharum officinarum*, primarily cultivated in tropical and subtropical regions for its sweet juice. This versatile plant, which takes 9-24 months to reach maturity, is propagated using sections of stalks called setts and requires warm temperatures, abundant sunlight, and ample water. Major producing countries include Brazil, India, China, and Thailand. Sugarcane's primary use is sugar production, but it's also utilized for ethanol fuel, molasses, and bagasse (a fibrous byproduct). As a significant cash crop, it supports millions of farmers worldwide but faces environmental challenges due to its water-intensive nature and potential for soil depletion if not managed properly. Harvesting can be done manually or mechanically, with stalks cut close to the ground. While economically important, sugarcane cultivation also raises concerns about sustainable farming practices and environmental impact.

Sugarcane production in India faces numerous constraints, including water scarcity, soil degradation, pest and disease outbreaks, labor shortages, climate change impacts, fragmented landholdings, inadequate mechanization, fluctuating market prices, limited access to quality inputs, inefficient irrigation systems, lack of proper storage facilities, transportation challenges, outdated farming practices, insufficient research and development policy inconsistencies, high production costs, limited processing capacity, unreliable power supply, inadequate extension services, low adoption of improved varieties, delayed payments to farmers, competition from other crops, limited crop insurance coverage, poor post-harvest management, lack of valueadded product diversification, insufficient credit availability, inadequate infrastructure in rural areas, limited use of precision agriculture techniques, soil salinity issues, waterlogging problems, inefficient use of fertilizers, lack of farmer organizations, limited access to market information, inadequate cold storage facilities, poor road connectivity in some regions, limited awareness of sustainable farming practices, lack of crop rotation, mono-cropping leading to pest resistance, inadequate trash management, limited use of organic farming methods, insufficient drought-resistant varieties, lack of proper ratoon management, limited use of drip irrigation, inadequate pest surveillance systems, limited use of integrated pest management, lack of proper weed control measures, insufficient use of bio-fertilizers, limited adoption of tissue culture technology, inadequate field preparation techniques, limited use of intercropping, lack of proper harvesting equipment, insufficient use of remote sensing technology, limited access to weather forecasting services, inadequate soil testing facilities, lack of proper planting material, insufficient use of mulching techniques, limited adoption of trash mulching, inadequate use of crop modeling tools, limited use of micro-irrigation systems, insufficient use of bio-control agents, lack of proper nutrient management, limited use of green manuring, inadequate use of balanced fertilization, insufficient attention to micronutrient deficiencies, limited use of foliar sprays, lack of proper

seed treatment, inadequate use of growth regulators, limited adoption of conservation agriculture practices, insufficient attention to soil health management, lack of proper crop residue management, limited use of organic amendments, inadequate attention to root health, insufficient use of bio-stimulants, limited adoption of precision nutrient management, lack of proper water management in ratoon crops, inadequate attention to planting time optimization, insufficient use of crop rotation with legumes, limited adoption of integrated farming systems, lack of proper trash shredding equipment, inadequate attention to soil moisture conservation, insufficient use of vermicomposting, limited adoption of site-specific nutrient management, lack of proper farm record keeping, inadequate attention to farm mechanization for small holders, insufficient use of solar pumps for irrigation, limited adoption of climate-smart agriculture practices, lack of proper agro-advisory services, inadequate attention to soil and water conservation measures, insufficient use of farm ponds for water harvesting, limited adoption of alternate furrow irrigation, lack of proper crop insurance schemes, inadequate attention to value chain development, insufficient use of farmer producer organizations, limited adoption of contract farming models, and lack of proper market linkages for small and marginal farmers.

About 55 diseases of sugarcane caused by fungi, bacteria, viruses, phytoplasmas and nematodes have been reported from India (Rao *et al.,* 2002). Sugarcane cultivation is challenged by numerous diseases, including red rot *(Colletotrichum falcatum*), smut (*Sporisorium scitamineum*), wilt (*Fusarium sacchari*), leaf scald (*Xanthomonas albilineans*), ratoon stunting disease (*Leifsonia xyli* subsp. *xyli*), yellow leaf disease (Sugarcane yellow leaf virus), pokkah boeng (*Fusarium moniliforme*), grassy shoot disease (phytoplasma), mosaic (Sugarcane mosaic virus), yellow spot (*Mycovellosiella koepkei*), eyespot (*Bipolaris sacchari*), rust (*Puccinia melanocephala*), chlorotic streak (unknown virus), pineapple disease (*Ceratocystis paradoxa*), root rot (*Pythium arrhenomanes*), top rot (*Fusarium moniliforme*), basal stem rot (*Marasmius sacchari*), sett rot (various fungi), red stripe (*Acidovorax avenae* subsp. *avenae*), leaf blight (*Leptosphaeria sacchari*), stalk rot (*Fusarium moniliforme*), brown spot (*Cercospora longipes*), ring spot (*Leptosphaeria sacchari*), purple spot (*Dimeriella sacchari*), black spot (*Phyllachora sacchari*), leaf speckle (*Cercospora koepkei*), downy mildew (*Peronosclerospora sacchari*), whip smut (*Ustilago scitaminea*), banded sclerotial disease (*Rhizoctonia solani*), red leaf spot (*Cercospora vaginae*), orange rust (*Puccinia kuehnii*), white leaf disease (phytoplasma), leaf scorch (*Stagonospora sacchari*), gumming disease (*Xanthomonas vasculorum*), root knot (Meloidogyne spp.), leaf fleck (Sugarcane bacilliform virus), sugarcane streak mosaic (Sugarcane streak mosaic virus), sugarcane streak disease (Sugarcane streak virus), fiji disease (Fiji disease virus), chlorotic streak (unknown virus), ramu stunt (unknown virus), sugarcane yellow leaf syndrome (Sugarcane yellow leaf virus), sugarcane white leaf (phytoplasma), sugarcane grassy shoot (phytoplasma), sugarcane downy mildew (*Peronosclerospora sacchari*),

sugarcane mosaic (Sugarcane mosaic virus), sugarcane mild mosaic (Sugarcane mild mosaic virus), sugarcane bacilliform virus disease (Sugarcane bacilliform virus), sugarcane chlorotic fleck (unknown virus), sugarcane yellow spot (*Mycovellosiella koepkei*), sugarcane brown rust (*Puccinia melanocephala*), sugarcane orange rust (*Puccinia kuehnii*), sugarcane common rust (*Puccinia kuehnii*), sugarcane leaf scald (*Xanthomonas albilineans*), sugarcane red rot (*Glomerella tucumanensis*), sugarcane smut (*Sporisorium scitamineum*), sugarcane wilt (*Cephalosporium sacchari*), sugarcane pineapple disease (*Ceratocystis paradoxa*), and sugarcane leaf speckle (*Cercospora koepkei*), all of which can significantly impact crop yield, quality, and overall productivity, requiring vigilant monitoring, proper management practices, and the development of resistant varieties to ensure sustainable sugarcane production.

Table 1: List of major diseases of sugarcane in India (Rott *et al.*, 2000)

S.No	Major Diseases of Sugarcane	Causal Organism
1	Red rot	*Colletotrichum falcatum* Went
2	Smut	*Sporosorium scitamineuma*
3	Wilt	*Fusarium sacchari*
4	Pineapple disease	*Ceratocystis paradoxa*
5	Pokkah boeng	*Giberella moniliformis,* *Fusarium moniliforme*
6	Rust	*Puccinia melanocephala, P. kuehnii*
7	Seedling rot (damping-off)	*Pythium, Rhizoctonia*
8	Gumming	*Xanthomonas campestris* pv.*vasculorum* (Cobb) Dye
9	Ratoon stunting	*Leifsonia xyli* subsp. xyli
10	Red stripe	*Pseudomonas rubrilineans*
11	Mosaic	Sugarcane mosaic virus, Sugarcane streak mosaic virus
12	Yellow leaf	Sugarcane yellow leaf virus
13	Grassy shoot	Grassy shoot phytoplasma

Yellow leaf disease

Viswanathan *et al.,* (2006) established that disease and the associated virus (SCYLV) in infected sets are the primary source for the disease in the field. Duplex and Multiplex-RT PCR were developed for the detection of SCMV, SCSMV and SCYLV, three of the major RNA viruses widely prevailing in the sugarcane growing regions in India (Singh *et al.,* 2011).

Symptoms- This disease affects the 5 to 6 months crop. Yellowing of midrib and adjacent laminar region also yellowing. Subsequent leaf drying along the mid rib in 3 to 5 leaves from top. In some cases reddish discoloration is also seen. In severe cases drying of spindle along with leaves. Stunted growths of cane, particularly internodes are affected. Identify the disease in long distance itself some times in severe cases it shows the bunchy top appearance at top of cane.
In matured cane this disease will spreads heavily.


Pathogen- The virus is transmitted by aphids, *Melanaphis sacchari* and *Rhopalosiphum maidis*, in a semi-persistent manner. SCYLV is a member of the Luteoviridae family. The virus is localized within the phloem cells of the plant.

Management strategies-

Cultural method: Growing of resistant and moderately resistant varieties viz., Co 09004 (Amritha) Selection of disease free setts for planting; Field should be maintain with proper hygiene; Application proper nutritional management and use resistant varieties; To avoid this disease first plant the setts in nursery and then transplant to main field; Selection of tissue culture plant especially meristem culture plant is used for planting in field.

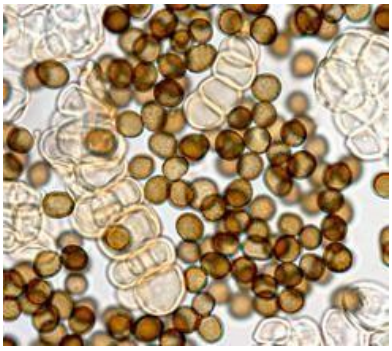
Chemical method: Secondary transmission of the disease by insect vectors can be controlled by application of Acetamiprid (0.2%) or Malathion (0.1%); Soil application of quinalphos @ 2.0 kg/ha or carbosulfan @ 6.0 kg/ha; Two sprayings of malathion @ 1.5 kg/ha at monthly intervals during September and October after detrashing of dry leaves.

Smut: *Ustilago scitaminea*

The disease is widespread in all the sugarcane growing states and is one of the major biotic constraints affecting sugarcane production in India. The disease caused severe yield loss to sugarcane for long time in Maharashtra and Northern Karnataka regions till Co 740 was under cultivation. The disease was responsible for the elimination of many commercial varieties in the country. Detailed studies on pathogenicity of smut pathogen conducted by Alexander and Ramakrishnan (1978) established parasitism, teliospore germination, dikaryotisation and infection. Infected setts serve as a major source for the transmission of the disease in the field.

Symptoms- Production of whip like structure (25 – 150 cm) from the growing point of the canes. Whip covered by translucent silvery membrane enclosing mass of black powdery spores. Initial thin canes with elongated internodes later become reduced in length. Profuse sprouting of lateral buds with narrow, erect leaves especially in ratoon crop.

Identification of pathogen- The fungal mycelium spores are echinulate, light brown and spherical, measuring 6.5 – 8.5 µ in diameter. They germinate readily in water, producing 2-3 celled promycelia. Sporidia arise terminally or laterally and are hyaline, thin walled, single celled and elliptical to linear.



Management strategies:

Cultural method: Growing of resistant and moderately resistant varieties viz., Co 12009 (Sankalp), CoM 12085, Co 14005 (Arunima), MS 14082 (Phule Sugarcane 13007), Co 94008 (Shyama), CoPb 95, Co 85004 (Prabha), Co 86032 (Nayana), Co 87025 (Kalyani), Co 87044 (Uttara), Co 8371 (Bhima), CoM 88121 (Krishna), CoSnk 05103, CoV 15356 (Ranga), Co 86249, CoG 93076, CoC 22, CoSi 6 and CoG 5; Discourage ratooning of the diseased crops having more than 10 per cent infection; *Cajanus cajan* can be grown as a companion crop between rows of sugarcane, and the secondary spread of the disease is substantially reduced.

Physical method: Treating the seed setts with Areated Steam Therapy (AST) at 50 ºC for 1 hour or in hot water at 50 ºC for 30 minutes or at 52 ºC for 18 minutes; Roguing of smut whips with gunny bags/polythene bag and dipped in boiling water for 1 hour, and diseased clums must be uprooted and burnt.

Chemical method: Sett treatment with fungicides viz., Triadimefon @ 1gm in 1 litre of water or Carbendazim @ 1gm in 1 litre of water for 10 minutes; Spray on infected stools with a small amount of a 10% solution of roundup, using a small hand held sprayer; In severe cases spray the entire block with glyphosate (360 G/L) at 5-7 lit/ha.

RUST: PUCCINIA ERIANTHI

Symptoms- The earliest symptoms are small, elongated yellowish spots that are visible on both leaf surfaces. The spots increase in length, turn brown to orange-brown or red-brown in color, which coalesced and formed large, irregular necrotic areas, thus it shows rusty appearance of leaf. This eventually resulted in premature death of the leaves.

Pathogen: Uredinia are elongate, reddish-brown, with capitate, hyaline to light brown paraphyses. Urediniospores are thick-walled, orange-brown, obovoid, measuring 26-34 x 16-20 µm. The urediniospore surface is echinulate with 4-5 equatorial pores. Teliospores are dark brown and measure 30-43 x 17-23 µm, clavate, two-celled and slightly constricted at the septum.

MANAGEMENT STRATEGIES

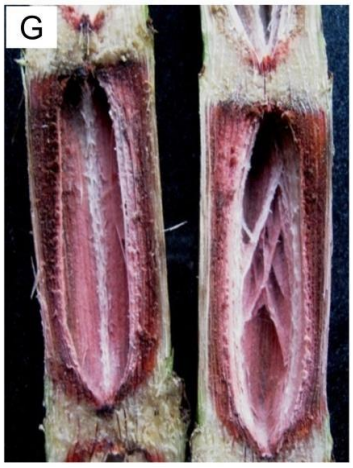
Cultural method: The best means of control for sugarcane rust is to grow resistant varieties

Use resistant varieties like Co 91010 (Dhanush), Co 87025 (Kalyani), Co 87044 (Uttara); Affected leaves should be removed and burn immediately; Sugarcane grown in fields receiving recent applications of mill mud is typically very prone to rust.

Chemical method: Spray Tridemorph 1.0 litres or Mancozeb 2.0 kg/ha. Application of Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC fungicide @ 1 ml/ lit of water.

Wilt: *Fusarium sacchari*

Symptoms-The first symptoms of the disease become apparent only when the plant has grown for about 4-5 months. Then gradual yellowing and drying of foliage, shrinkage/withering of canes. If the affected canes are cut and examined, the pith will be Light to dark purplish or brown discolouration of ground tissue, pithiness and boat shaped cavities in the middle of the internodes. A characteristic disagreeable odour is also associated with this disease. Often a cottony white mycelium is seen in the pith region.



Pathogen- The fungal mycelium is hyaline, septate and thin walled. The conidiophores are simple or branched and produce single celled, hyaline, oval to elliptical microconidia. Macroconidia are straight with 3-5 septae measuring 27-73 × 3.4-5.2 mm. Blastoconidia are either straight, sickle shaper or pike shaped with 2-3 septae. Moreover will be found as elliptical cell shaper structure with 1.-43x3.0-4.5 mm in size.

Management strategies:

Cultural method: Selection of healthy seed setts from disease-free area for planting**.** Grow resistant varieties like Co 86032 (Nayana), CoSnk 05103, Co 617 and B.P.17 are more resistant than other varieties**.** Crop rotation, managing root borer, avoiding prolonged drought and water logging and hygienic practices.

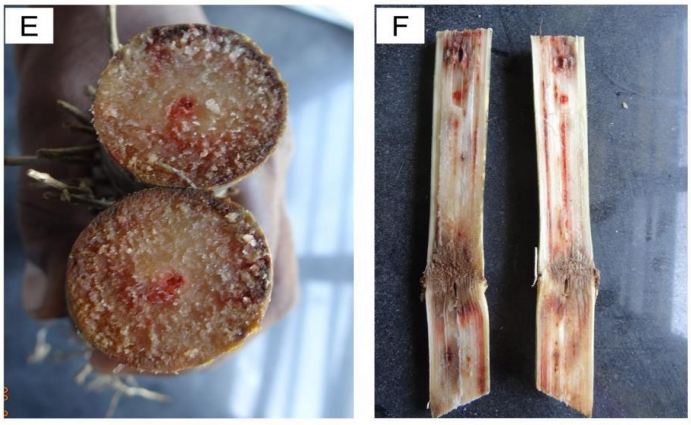
Chemical method: Dipping the setts in 40 ppm of boron or manganese, or spraying the plants with either of these minor elements reduces the disease intensity. Sett treatment with fungicide like Bavistin, 0.1 per cent before planting. Apply carbendazim @ 2gm/lit of water at the root zone area and same as follow at 15 days interval.

Red rot: *Glomerella tucumanensis*

It is one of the most widespread sugarcane diseases in the country and has been a constraint for sugarcane productivity for the past 100 years in India. The disease is responsible for the elimination of many commercial varieties in India in the earlier decades. Epidemics of the disease have been very common ever since its occurrence in India. Recently reports of red rot occurrence in CoC 671 in parts of Kolhapur and Solapur Districts in Maharashtra were reported. In the recent years we have witnessed break down of the cvs CoS 8436, CoSe 95422, 92423 and BO 138, the important commercial varieties of subtropical region to red rot. Severe damage to crop stand is found in these varieties due to disease epidemics in the states of Haryana, Uttar Pradesh and Bihar. The red rot infection reduced total carbohydrates in the diseased canes and the reduction was more in the highly susceptible varieties (Agnihotri 1990). Higher production of acid invertases in the highly susceptible varieties was recorded upon pathogen infection as compared to resistant varieties (De Silva *et al.*, 1977). Pathogen infection also results in increased levels of total soluble salts, acidity, reducing sugars and gum and simultaneously decrease in pH, sucrose and purity of cane juice in affected canes (Singh and Waraitch 1977).

Symptoms- The affected canes exhibit leaf colour change, from green to orange and then to yellow in the third or fourth leaf. Then the leaves start drying from bottom to top. If the fungal spores enter the leaf sheath through the leaf midrib, then reddish spots can be seen on the back side of the leaf midrib also. The external symptoms appear only after16 - 21 days after infection and drying of entire cane takes another 10 days' time. When the affected cane is split opened, the inner region is reddish in colour with intermittent white tinges across the cane length.

Sometimes, the pith inside the cane is filled with blackish brown liquid and exhibited alchohol odour.



Pathogen- Red rot disease is caused by the fungus *Glomerella tucumanensis*. An older name, *Colletotrichum falcatum*, is still preferred by some pathologists. Pathogen present on leaf sheaths and blades, solitary or aggregated, often forming short lines between vascular bundles, globose, immersed, dark brown to black 65-250 µm dia.; wall up to 8 cells thick, sclerotia on outside, pseudoparenchymatous within, ostiole slightly papillate, circular.

Management strategies:

Cultural method: The best way to control red rot is to select setts for planting from healthy plants in a disease- free area. The red rot affected field must be rotated with rice for one season and other crops for two seasons. Growing of recommended resistant and moderately resistant varieties viz., CoLk 12209 (Ikshu-7), CoLK 09204 (Ikshu-3), Co 86032 (Nayana), CoPb 95, CoPb 96, Co 15023 (Karan 15), Co 87025 (Kalyani), CoSnk 05103, Co 86249, CoSi 95071, CoG 93076, CoC 22, CoSi 6 and CoG 5.

Physical method: Removal of the affected clumps at an early stage and soil drenching with Carbendazim 50 WP (1 gm in 1 litre of water). The cut ends and entire setts should be dipped in a fungicide solution, such as one per cent Bordeaux mixture. If the disease is noticed in the field, the leaves and canes should be collected and destroyed by burning.

Chemical method: Adopt sett treatment with Carbendazim before planting (Carbendazim 50 WP (0.5 gm in 1 litre of water) or Carbendazim 25 DS (1gm in 1 litre of water) along with 2.5 kg of Urea in 250 litre of water. Use fungitoxic chemicals like Bavistan at 0.1 per cent for 18 min. at 52ºC for dipping setts which gave almost complete elimination of rot infection. Using a drone technique, spray Azoxystrobin 18.2% + Difenoconazole 11.4% w/w SC at 1 ml/lit er of water on a standing crop.

Grassy shoot disease

Symptoms- Initial symptom appears in the young crop of 3 – 4 months age as thin papery white young leaves at the top of the cane. Later, white or yellow tillers appear in large number below these leaves (profuse tillering). The cane becomes stunted with reduced internodal length with axillary bud sprouting. This disease appears in isolated clumps.

Pathogen-

The disease is caused by Mycoplasma like organisms. Mycoplasma cells are physically small – less than 1 µm – and they are therefore difficult to detect with a conventional microscope.

Management strategies-

Cultural method: Growing resistant varieties viz., Co 86249, CoG 93076 and CoC 22. Avoid ratooning if Grassy Shoot Disease incidence is more than 15 % in the plant crop. If disease symptoms are visible within two weeks after planting, such plants can be replaced by healthy plants. Uprooted infected plants need to disposed of by burning them.

Physical method: Rogue out infected plants in the secondary and commercial seed nursery. Treat the setts with aerated steam at 50°C for 1 hour to control primary infection. Treating them with hot air at 54^0C for 8 hours.

Chemical method: Spray dimethoate 30% EC @ 1ml in 1 litre of water to control insect vector. Apply pesticide oxydemeton-methyl 25% EC @ 2ml/lit of water for controlling aphids.

Pokkah boeng

Pokkah boeng is a re-emerging disease in sugarcane. Pokkah boeng is a Javanese term denoting a malformation or distorted top. Pokkah boeng is caused by the *Fusarium* species complex. Severity of Pokkah boeng disease incidence increases with the occurrence of the crown mealybug *Phenacoccus saccharifolii* coincides with summer months of April-July. The mealybug *P. saccharifolii* colonizes the crown region of the crop and associated with black ant *Camponotus compressus*. Profuse honey dew secretion by the crown mealybugs leads to extensive sooty mould (*Capnodium* sp.) growth on the leaves. Three to seven months old plant crops are most susceptible to the disease, where as in ratoon one month old crops itself got affected by the Pokkah boeng disease.

Symptoms: Chlorotic phase (chlorotic, rough, wrinkling, twisting and shortening of the leaves). Top rot phase (growing point is killed and the entire top of the plant dies). Knife cut phase (Its look like the tissues of the stem is removed with a sharp knife). Shortening of internodes. Bud sprouting (it occurs during before maturation of crop).

Pathogen: Pokkah boeng is caused by the Fusarium species complex (*Fusarium sacchari*, *Fusarium proliferatum*, *Fusarium verticillioides* etc.). The pathogen (*Fusarium*) can survive for 12 months in the plant debris under natural conditions.

Management:

Cultural methods: Use moderately tolerant variety- Swarna mukhi (CoT 10367), Co 86032; Highly susceptible varieties may be avoided- CoV 09356, CoV 94101, Co 11015, Co 06022 and 87 A 298**.**

Physical methods: Before spraying ensure detrashing. Monitor the movement of ants regularly. Removal of infected plant parts showing top rot and knife cut symptoms.

Chemical methods: Sett treatment with Propiconazole 25% EC (1ml/lit.) + Imidacloprid 70 WS (1ml/lit.) for 20 minutes as prophylactic measures (Plant crop). Application of recommended dose of fertilizers and micro nutrients after every ratoon and at the time of earthing up. Spraying with Propiconazole 25% EC 1ml + Imidacloprid 17.8% SL 0.4 ml + sticking agent 1ml + water 1lit. soon after the appearance of symptom. If incidence noticed again, spraying with Propiconazole 25% EC (1ml/l) + Flonicamid 50 WG (0.3g/l) or Clothianidine 50 WDG (0.5g/l) @ 20 days interval.

Conclusion:

The diseases like red rot, smut, wilt and grassy shoot were mainly responsible for the elimination of many elite commercial varieties in the past in different epidemics. Additionally, many of the non-fungal diseases contribute to decline in their performance which is referred as ‘varietal degeneration’. Lack of awareness on seed cane health and ignoring quarantine regulations resulted in introduction of diseases, their epidemics and varietal degeneration in the country. To increase sugarcane productivity in India, supply of healthy seed canes is to be ensured in the field. Research personnel and development workers should be actively involved in creating awareness on supply of healthy seed. In addition to detecting sugarcane pathogens in seed canes, the recent approaches in the disease diagnosis using serological and molecular approaches have applications in the field of developing virus-free seedlings, germplasm exchange and quarantine, disease surveillance and integrated disease management in sugarcane.

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ADVANCED TECHNOLOGIES OF MANGO CULTIVATION WITH ULTRA HIGH-DENSITY PLANTING

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Abstract:

India, the largest producer and exporter of mangoes, faces challenges in mango productivity, with an average yield of 6 metric tonnes per hectare, far behind other countries like Brazil. The low productivity is attributed to various factors including low plant population per hectare, inefficient irrigation and nutrient management, improper orchard practices, pest and disease losses, and unscientific harvesting methods. To address these issues and enhance mango yield, Ultra-High-Density Planting (UHDP) has emerged as a promising technique. UHDP optimizes resource use, increases production per unit area, and enhances profitability for farmers. This approach involves practices such as formative pruning, canopy management, drip irrigation, and fertigation, ensuring uniformity in fruit shape, color, and quality. Particularly relevant for regions like Maharasthra, UHDP can significantly boost mango productivity and quality, positioning farmers to better meet both domestic and export demands.

Introduction:

Mango cultivation in India spans approximately 2.45 million hectares, producing about 22.55 million tonnes annually, with an average productivity of 9.4 tonnes per hectare in 2023-24 (Source; https://www.indiastat.com). This productivity is notably lower compared to countries like Israel, which achieves 30 tonnes per hectare. In Maharasthra, mango is cultivated on around 169,300 hectares, yielding approximately 0.522 MT, with an average productivity of 3.08 MT per hectare in 2023-24 (https://www.indiastat.com). Factors contributing to this low productivity include poor orchard management, suboptimal water and nutrient management, wide tree spacing with dense canopies, inadequate sunlight interception, and insufficient ventilation, which encourage pest and disease incidence.

The primary mango-growing states in India are Uttar Pradesh (Leads in mango production with a 25.76% share and the highest productivity in 2023-24 as per 2nd advance estimate; https://www.indiastat.com), Andhra Pradesh, Bihar, Karnataka, Telangana, West Bengal, India is a significant exporter of fresh mangoes worldwide. In the year 2023-24, the country exported 32,104.09 metric tonnes of fresh mangoes, valued at Rs. 495.46 crores

(approximately 60.14 million USD). Major Export Destinations (2023-24) are United Arab Emirates, United Kingdom, United States, Kuwait, Qatar

Ultra-High-Density Planting (UHDP) offers a viable solution to these challenges. UHDP involves planting mango trees at closer spacings i.e. 3x2 (Kumar N. 2019, Gopu *et al.*, 2014) or 4 x 2 meters, maximizing land use and achieving higher yields throughout the orchard's lifespan. Key practices associated with UHDP include formative pruning to establish desirable plant architecture, annual canopy management to promote vegetative growth post-harvest, and stopping vegetative growth in September to favor fruit bud initiation. Additionally, drip irrigation and fertigation are essential to maintain optimal moisture levels and provide balanced nutrients, thereby enhancing yield and fruit quality.

Research institutes in India have successfully demonstrated the benefits of high-density planting in mangoes, but field implementation by farmers has faced challenges due to incomplete adherence to UHDP protocols. This paper discusses the prospects and challenges of adopting UHDP in Maharashtra, emphasizing the importance of integrating related technologies and practices to achieve higher mango productivity and quality.

Origin and adoption of Ultra-High-Density Planting (UHDP) technique:

The concept of ultra-High-Density mango plantation originated from observations made in South Africa, where farmers successfully planted 900 mango trees per acre. This stark contrast to traditional orchards, which typically accommodated only 40 to 70 trees per acre, caught the attention of Indian scientists attending international seminars. Impressed by the productivity and uniformity of fruits achieved through this method, Indian scientists began adapting and refining the technique to suit local climatic conditions.

1. Historical development:

The idea of high-density planting (HDP) itself dates back to Europe in the late 19th century, marking a shift away from traditional low-density orchards. The fundamental principle behind HDP is to maximize productivity per unit area by optimizing both vertical and horizontal space, while ensuring efficient use of inputs.

2. Evolution in India:

In India, the concept evolved further with the introduction of the Meadow Orchard System, initially developed for guava cultivation at CISH, Lucknow. This modern approach involves using small or dwarf trees with modified canopies, coupled with precise management practices such as fertilizer application, spacing optimization, and rigorous training and pruning techniques. These methods not only enhance growth regulation but also facilitate mechanization, thereby boosting the rate of photosynthesis and ultimately increasing yield per unit area.

3. Adoption and benefits

The adoption of UHDP in mango cultivation aims to maximize profitability by accommodating the maximum number of plants per unit area without depleting soil fertility. By harnessing scientific advancements in planting density and management, farmers can achieve higher yields, uniform fruit quality, and efficient resource utilization suited to Indian agricultural contexts.

Mechanization and water management in Ultra-High-Density mango orchards:

Mechanization plays a crucial role in achieving high production levels in mango orchards, especially in ultra-High-Density planting (UHDP) systems. Automated irrigation and fertigation are key operations that significantly contribute to the success of high-density orchards. Ensuring consistent irrigation and nutrient supply through fertigation is essential to prevent stress on plants, particularly after pruning and during fruit development stages. In UHDP mango orchards, drip irrigation systems are indispensable for effective water management. Initially, seedlings receive 4 liters of water per hour at a distance of 1.5 feet, which is gradually increased to 10 liters after the first year and up to 20-25 liters for mature, yielding trees. This precise delivery of water directly to the root zone optimizes water usage and enhances tree health and productivity. Additionally, water-soluble fertilizers are administered through the drip system at recommended intervals, further boosting nutrient uptake and overall orchard productivity.

Land preparation and planting methods:

1. Adopting efficient planting techniques: Traditionally, mango planting involves 1m x 1m x 1m pits at specified spacing. However, in UHDP systems, trenching is preferred for its convenience and effectiveness. Alternating one-meter-deep and one-meter-wide trenches every four meters, facilitated by machinery like JCB, simplifies establishment and promotes optimal root development. Before planting, these trenches should be allowed to settle for several weeks. The planting media, comprising 40-50 kg native soil, 0.5-1.0 kg Single Super Phosphate (SSP), 0.25 kg Neem cake, 20 kg compost (or 10 kg vermicompost), and 50g Carbofuran granules, ensures nutrient-rich conditions for seedling growth. Using black polythene mulch over raised beds further aids in moisture retention and weed suppression.

Strategies to Enhance Orchard Productivity

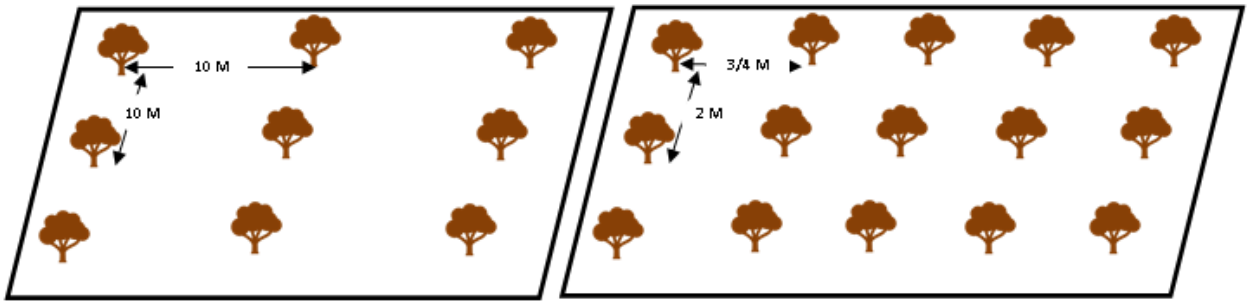
2. Optimizing orchard management: Effective water and nutrient management are pivotal in optimizing orchard productivity. Implementing drip and fertigation systems allows for precise control and efficient utilization of resources. High-Density Planting (HDP) practices, coupled with proper canopy management techniques, are effective in rejuvenating older orchards with dense canopies, thereby improving fruit quality and yield. These strategies collectively enhance productivity and sustainability in mango cultivation under UHDP systems.

3. Planting material for UHDP mango orchards

In Ultra High-Density Planting (UHDP) systems for mango cultivation, selecting the right planting material is crucial for ensuring successful establishment and high productivity. Soft wood grafted mango plants with at least three flushes are ideal for UHDP. Plants younger than three flushes or older than one to two years may not respond well to early pruning and training, potentially leading to poor growth and yield. It is essential to use planting material that can adapt well to the intensive management practices required in UHDP, including formative pruning and annual canopy management.

Planting geometry and spacing:

The geometry and spacing of planting in UHDP mango orchards significantly influence orchard management and productivity. Common planting systems include single hedge row, double hedge row, and square systems, which provide sufficient alleyways for movement of farm machinery and ensure optimal light distribution and interception. Spacings commonly used in UHDP are 2.5 m × 2.5 m or 2.0 m × 2.0 m, effectively increasing plant density by reducing both inter-row and intra-row spacing. For instance, a spacing of 3.0 m × 2.0 m allows for 674 trees per acre, significantly higher compared to traditional methods which accommodate only 40 to 70 trees per acre i.e. 9.63 to 16.85 times more trees over.



Conventional Planting Distance 10x10 M **Ultra High Desnity Plating Method 3x2 or 4x2 M**

Planting method and management practices:

Planting mango trees in UHDP begins with careful preparation of planting pits during the rainy season in moderately rainy areas or after the cessation of rains in regions with heavy rainfall. Pits of approximately 1.0 m × 1.0 m × 1.0 m size are dug, exposed, and filled with a mixture that includes 50 kg well-decomposed farmyard manure (FYM) and 2kg super phosphate. If there is a white ant problem,100-150mg polydol powder is added to the pit to prevent infestation. Cow dung is avoided in areas prone to white ants due to its heat-producing properties. Cow dung if applied produces too much heat and attracts white ants and hence should not be applied where white ants are a serious problem. Manure is applied 2 months before planting or 6 months after planting.

Before planting, nitrogen application accelerates plant growth, ensuring vigorous establishment. Grafted plants are positioned with the graft joint or union approximately 20 cm above the soil surface to prevent disease entry. Planting is ideally done on cool days, preferably in the evenings, and plants are immediately watered and staked for support. Proper planting methods and management practices are crucial for the initial growth phase of mango trees in UHDP, setting the foundation for optimal orchard development and high fruit yield.

Soil management in Ultra High-Density Planting (UHDP) mango orchards:

Mango cultivation in Ultra High-Density Planting (UHDP) systems requires careful soil management practices to optimize growth and yield. Mangoes can thrive on a variety of soils, including alluvial and lateritic soils, as long as they are deep (2.0-2.5m) and well-drained. The ideal soil pH for mango cultivation ranges from 5.5 to 7.0. It is crucial that the soil has good drainage and permeability to prevent waterlogging, which can be detrimental to mango roots. Additionally, a soil with a fair water-holding capacity is beneficial to sustain the tree during dry periods. Groundwater should ideally be accessible at a depth of 3 to 4 meters, ensuring adequate water availability for the mango trees.

In UHDP mango orchards, soil preparation plays a vital role in ensuring optimal root development and nutrient uptake. Prior to planting, the soil should be deeply tilled and well-prepared to loosen compacted layers and improve aeration. Organic matter, such as farmyard manure (FYM), should be incorporated into the soil to enhance its structure, water retention capacity, and nutrient content. This helps in establishing a healthy root system and supports vigorous vegetative growth, especially during the early stages of orchard establishment.

Soil fertility management in UHDP mango orchards typically involves regular soil testing to monitor nutrient levels and pH. Based on soil test results, appropriate fertilizers are applied through fertigation systems to ensure balanced nutrition throughout the growing season. This precise nutrient management not only promotes healthy growth but also enhances fruit quality and yield. It is essential to avoid soils that are very poor, shallow, alkaline, rocky, or calcareous, as these conditions can limit root development and overall tree health in UHDP mango orchards.

Proper soil management practices in UHDP mango cultivation contribute significantly to sustainable orchard productivity, ensuring that mango trees thrive and produce high-quality fruit consistently.

Mango cultivars suitable for Ultra-High-Density Plantation (UHDP)

Mango cultivation in UHDP systems can be successful with specific cultivars that are suitable for dwarfing and respond well to intensive pruning and management practices. Here are some recommended mango cultivars for UHDP:

Cultivars suitable for UHDP

Alphonso (Hapus) - Known as the "King of Mangoes," Alphonso is highly favored for its excellent flavor and is well-suited to UHDP due to its response to repeated pruning.

Amrapali - This variety is ideal for UHDP with its dwarfing characteristics. It can be planted at a spacing of 2.5 m × 2.5 m, accommodating up to 1600 plants per hectare.

Langra - A popular variety known for its unique flavor, Langra is suitable for UHDP in regions where its growth can be managed effectively through pruning.

Himsagar - Another favored mango variety for UHDP, Himsagar is appreciated for its sweet taste and adapts well to the intensive cultivation practices.

Banganapalli - Known for its large size and sweet flavor, Banganapalli is suitable for UHDP in regions like Tamil Nadu and Andhra Pradesh.

Baneshan - A traditional variety that responds well to UHDP practices, particularly in South India.

Kesar - This mango variety, with its distinct aroma and flavor, is recommended for UHDP in Maharashtra and Gujarat.

Ratna - Developed by Indian agricultural research centers, Ratna is valued for its disease resistance and suitability to intensive cultivation methods.

Mallika - Known for its excellent flavor and adaptability to UHDP, Mallika is a hybrid mango variety developed to meet the demands of modern orchard practices.

Regional recommendations

Andhra Pradesh: Alphonso, Alampur, Baneshan, Totapuri

Bihar: Bombay, Himsagar, Langra, Chausa

Goa: Mankaurad

Gujarat: Alphonso, Kesar

Karnataka: Alphonso, Bangalora, Neelum, Mallika

Tamil Nadu: Alphonso, Banganapalli, Neelum

Uttar Pradesh: Bombay Green, Dashehari, Langra

Maharashtra: Alphonso, Kesar, Ratna

These cultivars have been selected based on their ability to thrive in UHDP systems, responding well to pruning, disease resistance, and regional suitability. Choosing the right cultivar is essential for optimizing yield and quality in mango orchards under UHDP practices.

Management practices for consistent mango bearing:

To ensure regular bearing in mango trees, proper management practices must be followed post-harvest. Typically, pruning should be done in the first week of June. From July to September, encourage vegetative growth for about three months. In the second half of September, halt vegetative growth and promote the development of reproductive shoots and

flower buds by applying Paclobutrazole (PBZ). PBZ works by inhibiting the synthesis of Gibberellic Acid (GA), which, if present, continues to drive vegetative growth. Applying PBZ stops this process, allowing shoots that are 3-4 months old to initiate and differentiate flower buds in October-November, preparing them for flowering.

After the Northeast monsoon ends, suspend drip fertigation for one month. This practice induces profuse flowering in December-January, followed by fruiting in April-May, making the mangoes ready for harvesting within 3-4 months.

Effective training and pruning strategies for UHDP mango cultivation:

Canopy management in UHDP: Proper canopy management is crucial in the Ultra High-Density Planting (UHDP) system to control tree size and balance vigor and productivity. This process begins early, starting from the initial months after planting.

1. Training stages

1.1 First training: When the plant reaches a height of 45-60 cm (approximately four months after planting), pinch the terminal bud 5-6 cm below the apex to stimulate the growth of auxiliary buds. Retain 3-4 healthy and vigorous shoots from the auxiliary buds at 15-20 cm intervals, which will develop into primary branches.

1.2 Second training: Three months after the first pruning, when the primary branches reach 60-70 cm in height, cut them back to 35-40 cm from the base below the whorl. Retain only two healthy shoots from each primary branch, forming the secondary branches. Depending on the initial number of primary branches, this results in either six or eight secondary branches per plant.

1.3 Third training: Three to four months after the second training, when the secondary branches reach 60-70 cm, cut them back to 35-40 cm from the base below the whorl. Retain only two healthy shoots from each secondary branch, forming the tertiary branches. This results in 12 or 16 tertiary branches per plant, depending on the number of secondary branches. By this stage, the plant will have a minimum of 12 branches or a maximum of 16 branches, forming the framework for the entire plant. Pruning should always be done below the whorl to ensure optimal growth and canopy shape. Properly trained and pruned trees will form a dome-shaped hedge, facilitating sunlight penetration and reducing pest and disease incidence.

2. Pruning practices in Ultra-High-Density Plantation (UHDP) mango orchards

Importance of Pruning in UHDP Systems

Pruning plays a crucial role in ultra-High-Density planting (UHDP) mango orchards to maintain optimal canopy structure, enhance fruiting efficiency, and manage orchard health effectively.

2.1 Timing and Techniques

a) Initial pruning strategy: Pruning should commence approximately 2-3 months after the third training phase, focusing on tertiary branches. These branches are pruned back to 35-40 cm to stimulate the growth of multiple new shoots, each capable of producing panicles or inflorescences, thereby maximizing flowering and fruiting per unit area.

b) Seasonal pruning schedule:

i) Post-harvest pruning: Ideally completed before June 15th in regions like Maharashtra, tertiary branches are pruned back to maintain a plant height of approximately 1.5 meters, ensuring 10-15 tertiary shoots per plant. This practice prevents excessive growth and facilitates easier orchard management.

ii) Long-term pruning (After 5 years): As trees mature, individual shoot pruning becomes challenging. Utilizing a pruning saw to reduce the tree's height to 5 feet is recommended, ensuring that 50% of the shoots receive normal pruning. This approach promotes canopy health and allows for adequate sunlight penetration.

2.2 Post-pruning care and precautions

1. **Tree height management:** Keeping tree height below 7 feet is crucial for maintaining orchard density and facilitating management operations.
2. **End treatment:** Immediately after pruning, apply Bordeaux paste, 2% Copper Oxychloride (COC) suspension, or 0.2% Blitox-50 to cut ends to prevent fungal infections.
3. **Shoot thinning:** One month after pruning, thin out newly emerged shoots to prevent overcrowding and promote balanced growth.

2.3 Levels of pruning

1. **Light pruning:** Involves removing 30% of the previous season's growth (brown wood) to maintain canopy structure.
2. **Annual back pruning:** Removes 50% of the previous year's growth, alternating annually to balance canopy growth and maintain productivity in UHDP systems.

2.4 Enhancing growth and disease prevention

To stimulate vegetative growth and safeguard against fungal infections post-pruning, apply a spray of 1% urea combined with 0.2% copper fungicide. This application not only enhances vegetative vigor but also protects against fungal diseases, ensuring robust and healthy tree development in UHDP mango orchards.

3. Benefits of training and pruning

1. **Shape and size control:** Training and pruning effectively control tree shape and size, aiding in canopy management. Proper tree form, branch angles, and limb spacing contribute to growth control and enhanced productivity.

2. **Maintenance of fruiting shoots:** Regular pruning ensures the maintenance of fruiting shoots, essential for consistent bearing and high yield.
3. **Disease prevention:** Pruning and subsequent spraying help prevent fungal infections, ensuring the health and vigor of the trees.

Irrigation management for mango trees

1. Young and non-bearing trees

Young and non-bearing mango trees require different irrigation management compared to bearing trees. The primary goal during this period is to ensure rapid tree growth and the development of a robust leaf canopy, necessitating more frequent irrigations throughout the year. Newly planted young mango plants should be irrigated twice a week during hot weather for the first 4-6 months. For the first 4-5 years, regular and frequent irrigations of light intensity are essential due to the limited root spread. During the rainy season, irrigation intervals should be adjusted based on the intensity and distribution of rainfall.

2. Bearing trees

For bearing trees, it is crucial to manage irrigation carefully, especially in the months leading up to the flowering season (October-December), where excessive irrigation should be avoided to promote flowering and curb vegetative growth. From February to June, irrigation is beneficial as it helps reduce fruit drop and supports fruit size development. Generally, 2-3 irrigations are recommended between fruit set and fruit development stages for trees in full bearing. To ensure better fruit quality, it is advisable to avoid irrigation before harvesting. The basin system of irrigation is typically used to economize water, with basins connected in series or to a channel dug between rows.

3. Drip irrigation in UHDP technology

i) Importance of drip irrigation

In Maharashtra, mango is traditionally not considered an irrigated crop. However, to enhance productivity and income under Ultra High-Density Planting (UHDP) technology, it is imperative that mango growers adopt a complete drip irrigation system. Drip irrigation precisely delivers the required volume of water to the root zone at regular intervals, keeping only the root zone wet and leaving the rest of the field dry. This method significantly saves water (up to 50%), fertilizers (up to 30%), power, and labor.

ii) Water requirements by plant age

The water requirements for mango plants vary with their age and season:

1st year: Approximately 1 liter of water per day.

2nd year: 2-3 liters of water per day.

3rd year: 8-9 liters of water per day.

Bearing period (3rd year onwards): Around 15 liters of water per day per plant.

Irrigation is particularly important for bearing trees starting from the 3rd week of September to October to induce flowering. Rainfall events can be erratic, and the general recommendation is to suspend drip irrigation for 2-3 days if rainfall exceeds 10 mm in a single day.

iii) Drip system setup

The online drip system is highly suitable for mango cultivation, with drip laterals spaced according to the relevant row spacing. Each tree should be provided with one dripper of 4 L/h during the initial two years and two drippers of 4 L/h from the 3rd year onwards. When using two drippers, they should be placed 45 cm away from the trunk to ensure optimal water distribution (Annon.2015).

12. Fertilizer management for mango cultivation

12.1 Conventional vs. UHDP fertilization methods

In traditional mango cultivation, fertilizers are applied in two split doses during June-July and October-November. However, under Ultra High-Density Planting (UHDP), fertilization is rationalized over the entire growing season. Fertigation is scheduled over 9-10 months annually, resulting in high fertilizer use efficiency and excellent yields.

12.2 Fertilization recommendations for UHDP

For UHDP mango plants, it is recommended that each plant receives 120g of Nitrogen (N), 75g of Phosphorus (P), and 100g of Potassium (K), along with 15 kg of Farm Yard Manure (FYM) per year, starting from the fifth year onwards. These fertilizers are applied across different seasons (Table 1 & 2):

- **June to August:** Fertilizers are applied weekly for 12 weeks.
- **September:** Fertilizers are applied weekly for 4 weeks.
- **January to March:** Fertilizers are applied weekly for 12 weeks.
- Fertigation is stopped just before harvest in April and May.

Table 1: Fertiliser recommendation for UHDP mango (Soman 2009)

Age	g/tree			FYM Kg/ Tree
	N	P	K	
1st Yr	35	15	25	5
2nd Yr	45	25	50	5
3rd Yr	75	50	75	10
4th Yr onwards	120	75	100	15

Table 2: Fertigation schedule and quantity (Kg/dose/acre) for mango (Soman 2009)

Age	Month	No. of doses (Weekly once)	Urea	H_3PO_4	MOP	$MgSO_4$
1st Yr	July-Sept	12	1.4	0.5	0.8	0.000
	Jan-May	20	1.7	0.6	0.9	0.000
2nd Yr	July-Sept	12	2.7	1.5	2.3	0.278
	Jan-May	20	1.6	0.7	1.4	0.167
3rd Yr	15 June-Aug	12	4.5	2.3	3.5	0.555
	September	4	1.4	1.2	3.1	0.000
	Jan-May	20	3.2	1.2	1.5	0.333
4th Yr onwards	15 June-Aug	12	7.2	3.5	4.6	0.833
	September	4	2.2	1.7	4.2	0.000
	Jan-March	12	5.1	1.7	3.2	0.833

3. Manuring practices

While mango orchards are not generally manured, doing so can significantly increase yields. During the pre-bearing stage, the primary objectives are rapid growth and the development of a strong framework. Nitrogen is particularly needed in heavy quantities to support healthy and fast growth. Applying nitrogen in the form of organic matter can improve soil texture, moisture-holding capacity, and root development. Phosphorus is essential for root development, respiration, and carbohydrate translocation, while Potassium helps in fruit development, quality, and control of fruit drop.

4. Fertilization during bearing stage

During the bearing stage, the fertilization program aims to ensure sufficient vegetative growth early in the season to support the next year's growth and to maintain regular bearing with superior fruit quality. This is achieved by applying a heavy dose of nitrogen slightly earlier than flowering in the on-year to initiate vegetative growth and suppress bud differentiation. This practice reduces cropping in the on-year, promotes the production of vegetative shoots, and ensures regular bearing with high-quality fruit. By adopting these comprehensive fertilizer management practices, mango growers can optimize tree growth, fruit quality, and overall orchard productivity.

Advantages of Ultra High-Density Planting (UHDP) in mango cultivation

- **Maximized land utilization and yield**: As cultivation land becomes increasingly scarce, UHDP in mango cultivation enables the best use of available land, significantly raising per acre yield.
- **Shortened gestation period**: UHDP mango orchards start commercial bearing from the 3rd to 4th year, compared to the 7th to 9th years required in traditional planting. This provides farmers with earlier returns and promotes export opportunities.

- **Enhanced productivity**: By controlling excessive vegetative growth through dwarfing rootstocks, UHDP significantly increases productivity. Mature UHDP orchards can yield up to10-12 to 18-20 MT per hectare, two to three times higher than traditional systems, within ten years (Table 4, Singh *et al.*, Soman, 2009)..
- **Efficient resource Use**: UHDP allows for maximum yield in the shortest span, enabling farmers to switch to other crops or replant more quickly. Additionally, pruning technology under UHDP can produce off-season crops in varieties like Bangalora.
- **Labour efficiency**: UHDP simplifies orchard maintenance, allowing farmers to manage the trees and hand-pick fruits independently, which is beneficial during labour shortages.
- **Improved fruit quality**: Training and pruning in UHDP ensure an optimal and balanced fruit load on the trees, resulting in a higher percentage of export-quality fruits.
- **Water conservation**: UHDP reduces water usage for irrigation by up to 50%, promoting more sustainable farming practices.
- **Enhanced fertilizer uptake**: Practicing fertigation in UHDP increases the plants' fertilizer uptake capacity, leading to better growth and yield.

Demerits of Ultra High-Density Planting (UHDP) in mango cultivation

- **Higher Initial Costs**: Establishing a UHDP orchard can be more expensive initially compared to conventional systems due to the need for additional infrastructure and inputs.
- **Shorter Economic Lifespan**: The economic lifespan of a UHDP orchard tends to be lower, necessitating more frequent replanting or crop rotation.
- **Potential Reduction in Fruit Size and Weight**: There is a risk of decreased fruit size and weight under UHDP, which can affect marketability and profitability.
- **Challenges in Intercultural Operations**: The dense planting pattern of UHDP makes intercultural operations, such as weeding, soil management, and pest control, more challenging.
- **Complex Maintenance of Plant Architecture**: Maintaining the desired plant architecture through regular pruning and canopy management is labor-intensive and requires meticulous attention.
- **Strict Adherence to Recommended Practices**: Failure to follow recommended UHDP practices can lead to several problems, including vigorous and overcrowded growth, poor sunlight penetration, and low yields (1.5-2.0 MT per hectare). Additionally, issues such as harvesting difficulties, and increased susceptibility to diseases like powdery mildew, white scale, and anthracnose can arise.
- **Limited market:** UHDP mangoes are a premium product and may have limited market demand. Farmers need to identify and target specific markets to sell their produce.

Table 3: Comparison between UHDP, HDP and conventional method of planting (Singh *et al.*, 2017)

Parameters	UHDP	HDP	Conventional Planting
Gestation period (Year)	3	5	10-15
Plant population (trees acre^{-1})	674	200	40-70
Plant geometry	2.5m×2.5m	2.5m×2.5m to 5m×3m	7m×7m to 12m×12m
Pruning operation	Easy	Manageable	Very difficult
Spray operation	Easy	Manageable	Difficult
Probability of Insect, Pest and Diseases attack	Low	Moderate	High
Technology required	High	Medium	Low
Resource use efficiency	High	Medium	Low
Fruit uniformity	Highly Uniform	Uniform	Not Uniform
Yield at maturity (t acre^{-1})			
Prolific bearing varieties	10-12	7-8	4-5
Shy-bearing varieties	2-2.5	3-4	5-6
Remunerative price	High	Medium	Low
Establishment costs	High	Medium	Low
Opportunity cost	Low	Medium	Low
Market force of fruit in global market	High	Medium	Low
Marketable surplus	High	Medium	Low
Marketability	High	Medium	Low
Cost of production	Low	Medium	High
Optimality condition	High	Medium	Low
Quality of fruit			
Expected annual income (Lakh Rs. acre^{-1})			
Prolific bearing varieties @ Rs. 5 kg^{-1}	0.50-0.60	0.35-0.40	0.20-0.25
Shy-bearing varieties @ Rs. 12 kg^{-1}	0.60-0.72	0.36-0.48	0.24-0.30
Commercial orchard life (years)	25-30	30-50	Up to 50
Cost of orchard till it comes to commercial bearing (Lakh Rs. acre^{-1})	1.53	0.60	0.50
Management practices	Easy to manage due to small tree size	Easy to manage due to small tree size	Difficult to manage due to large tree size
Labour intensification	Less labour	Less labour	More labour

Table 4: A Comparison of UHDP with traditional planting system (Soman 2009)

S. No.	Particulars	Planting method	
		Traditional	UHDP
1	Pre bearing period	7-8 years	4 years
2	Duration to reach full yield potential	15 years	5 years
3	Yield potential	Medium	Very high
4	Pruning	Very difficult	Easy
5	Spray operation	Difficult	Easy
6	Spray efficiency	Very poor	Good
7	Harvest	Very difficult	Very easy
8	Control of fruit quality	Impossible	Easy
9	Expected yield /Ha		
	a. High volume varieties	12	20-25tonnes/Ha
	b. Low volume varieties	6	10-12
10	Incidence of Pests and diseases	Very high	Low/No
12	Commercial orchard life	Up to 50 years	20-25 years

Application of Paclobutrazole (PBZ):

To ensure consistent and early flowering, manage alternate bearing, and achieve higher yields, the application of Paclobutrazole (PBZ) is recommended. In the context of UHDP, apply PBZ at a rate of 2ml per meter of plant canopy, mixed in 2 liters of water. For 3-year-old plants, the dosage should be 3ml per 2.5 meters of canopy, also mixed in 2 liters of water and applied to the basin. Follow this application with continuous drip irrigation for a period of 15 days to ensure optimal absorption and effectiveness.

Harvesting practices in Ultra High-Density Planting (UHDP) mango orchards

Harvesting mangoes in UHDP orchards requires careful practices to ensure fruit quality and minimize post-harvest losses. Unlike traditional methods that involve knocking or shaking trees vigorously, which can damage the fruit, UHDP orchards are designed with trees of manageable height, slightly taller than a person. This setup facilitates easier access to fruit bunches using step ladders, ensuring minimal fruit injury during harvesting. The uniformity in fruit types within tree bunches further simplifies the picking process. To maintain fruit quality, it is recommended to harvest mangoes with a small fruit stalk attached, as this helps preserve the fruit's condition during transportation and storage. Harvested fruits should be gently placed in canvass bags or padded baskets to prevent bruising and maintain their marketability, especially for export purposes. Proper handling from harvest to packing house ensures that mangoes retain their freshness and appeal to consumers

Conclusion:

Ultra High-Density Planting (UHDP) represents a revolutionary approach to mango cultivation, offering a sustainable and highly productive alternative to traditional methods. By optimizing tree spacing, implementing systematic training and pruning techniques, and utilizing

advanced irrigation and nutrient management practices, UHDP significantly enhances both the yield and quality of mangoes. This method not only ensures a higher density of fruit-bearing trees per unit area but also facilitates better sunlight penetration, air circulation, and efficient resource utilization, thereby reducing the incidence of pests and diseases. The structured canopy management in UHDP allows for easier harvesting, minimizing fruit damage and improving overall fruit quality, which is crucial for both domestic consumption and export markets.

Moreover, UHDP promotes early bearing and increased fruit production, providing farmers with higher and more consistent income. The system's adaptability to various climatic conditions and its ability to withstand environmental stresses further underscore its viability as a sustainable agricultural practice.

In conclusion, UHDP is a transformative technique for mango cultivation that promises enhanced productivity, better fruit quality, and improved economic returns for farmers. Its adoption marks a significant step forward in achieving sustainable agricultural growth and meeting the increasing global demand for high-quality mangoes.

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THE SECONDARY METABOLITES OF PLANTS AS MICROBIAL PRODUCTS

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Introduction:

The secondary metabolites are the microbial products which are produced during the idiophase of microbial growth These are secreted when depletion of one or more nutrients is caused in the culture medium. Secondary metabolites are not further required by the microorganisms their growth. Therefore, these are called growth-independent metabolites. They can act as chemical messenger across organisms or species.

1. Toxins

In 1988, E. Roux and A. Yersin first observed the presence of toxin in a disease caused by *Corynebacterium diphtheriae*. Gaumann (1954) stated "microorganisms are pathogenic only if they are toxigenic". He used the term "toxin" and "microbial poison" to denote all substances produced by the pathogens. Therefore, there is a correlation between toxin and development of disease resulting in the death of hosts. Toxins interrupt the metabolic processes of the living host cell. Toxin toxin is "an organic poison secreted by a microorganism causing some disease". It clearly differs from a poison, as it is a substance which destroys life or injures health, when taken up in small quantity by a living organism. In 1960, A.R. Ludwing regarded a toxin as a microbial product or a microbe-host complex which acts on living protoplasts influence the development of disease. Toxins are generally proteins which are antigenic and therefore, have antitoxins. As far as host specificity is concerned, toxins are of two types, host specific and host non-specific toxins. Host specific toxins show specific relation between its production and pathogenicity, whereas host non-specific toxins do not.

Toxins secreted by microorganism lead to the development of infection in plants and animals as the affected cells lose their resistance.

1.1. Bacterial toxins

Toxins secreted by bacteria also play a key role in disease development in animals. In recent years, use of toxin secreting microorganisms in control of disease-causing flies, has become a Seld of major interest in formulation of biological pesticides. However, screening programme for discovery of new secondary metabolite from *Streptomyces* having insecticidal activity has been carried out in Japan,

Chemical nature of the toxin

It is mentioned that generally toxins are proteinaceous in nature, they interrupt the metabolism of host cells. It differs from enzyme in action as the latter destroys structural

integrity of the cell. It is interesting to note that crystals of toxin secreted by *B. thuringiensis* are water soluble, and heat stable. This toxin has been termed as B-exotoxin which is of molecular weight of 900.

Production of B-exotoxin

Production of B-exotoxin on a large scale is easy. Pure culture of *B. thuringiensis* is grown on a nutrient medium in submerged culture supplemented with starch because this bacterium secretes amylolytic enzymes. When bacterial growth is over the culture fluid is filtered. The final product is obtained from the filtrate by vacuum drying

Microbial insecticides

B-exotoxin is active against flies and mosquitos, beetles, termites, butterflies and moths. Mammals including man are not affected because of secretion of acid in stomach and most probably degradation by pepsin to non-toxic form. Similarly, an anaerobic spore forming rod shaped bacterium, *B. papillae* feeds upon the larvae of the Japanese beetle, Popillia japonica

2. Mycotoxins

The fungi play a significant role in production of many products such as enzymes, organic acids, antibiotics, SCP, cheese, etc. However, the biotechnological hazards caused by them have also been realised in recent years. Mycotoxin refers to secondary metabolites of certain fungi which are toxic to human animals and plant health as well. Chemically, mycotoxin are non-antigenic compounds of low molecular weight. These toxins are generally detected in milk, cheese, corn, peanuts, cotton seeds, copra, almond, figs, spices and other foods and feeds. Depending on concentrations, these may be carcinogenic, mutagenic, teratogenic or oestrogenic with the result of reduced immune response and acute disease syndrome.

Mycotoxicosis is the poisoning by ingestion of mycotoxins through food contaminated by toxigenic fungi. Historically, mycotoxicosis has been known for hundreds of years 9th and 18th century due to death of thousands of people in Europe caused by food-borne and a toxin as ergot producing fungus by *Claviceps purpurea* in rye grains. In 1978, Ethiopian epidemic occurred due to consumption of grains contaminated with *C. purpurea* infected wild oats where about 50% infected people died and the remaining suffered from many side effects.

In India, a similar episode is reported following human ingestion of bajra Pearl millet infected with *C. fusiforms*. Moreover, the cases of liver carcinoma North Bihar have been found more frequent in post monsoon period and these have been suspected to be due to consumption of mouldy maize. In the coastal Ine of Karnataka high incidence of liver enlargement was found associated with heavy fungal infestation of foodgrains Though there is many mycotoxins yet some of them which contaminate different food materials are aflatoxins, ochratoxins, zearalenone, citrinin, sterigmatocystin, trichothecenes. patulin, penicillic acid, etc. In 1988, a new group of water-soluble toxins, fumonisins, produced by certain stains of *Fusaarium*

moniliforme was discovered in 1988. These were associated with contaminated corn and commercial corn-based food stuff. If contaminated corns are used in fermentation for ethanol production, a little degradation of toxin occurs. However, most of the fumonisin B, could be recorded through distillation in spent grains, and stillage. When spent grains are used as animal feed without detoxification, it causes serious problems. Similarly, patulin was found in detectable levels in wines, beers and fruit juices.

The toxigenic fungi

Mostly all the fungi are equipped with toxin producing ability depending on environmental conditions. However, a few of them associated with food and feed materials pose a great hazard, for example, species of *Alternaria, Aspergillus Claviceps, Fusarium, Penicillium, Boletus. Agaricus, Amantia*, *Myrothecium, Pithomyces*.

The biochemical pathways through which mycotoxins are produced are: (1) the polykatide pathway (aflatoxins), (3) the terpene pathway (trichothecenes), (iii) the tricarboxylic acid pathway rubratoxin and (iv) the amino acid pathway gliotoxin.

Action of mycotoxins

Mode of action involves the biochemical reaction of mycotoxins with molecular receptors in animal cells. The molecular receptors are DNA RNA, functional proteins, enzyme co-factor and membrane constituents. The reactions between mycotoxins and their receptors may be either co-valent irreversible or non-covalent reversible In the first reaction, the reactive forms of mycotoxins conjugate with receptors to form adducts whereas in the second reaction mycotoxin receptor complexes get dissociated as the metabolic processes remove the toxin from the receptor sites,

After ingestion mycotoxins enter in human body and encounter various molecules. Toxins interact with gastrointestinal microflora, epithelial cells of intestine, liver, bile, blood, kidney reproductive and nervous systems, skin and lungs. Some of the effects of mycotoxins are briefly described below:

Effect on energy production

There are several mycotoxins which inhibit certain enzymes involved in Krebs cycle. Moniliformin, a toxin of *Fusarium*, inhibits the oxidation of pyruvate and a-ketoglutarate.

Inhibition of immune systems

Inhibition in the synthesis of DNA RNA and protein these macromolecules lead to cell death. Aflatoxin B inhibits DNA synthesis in liver cells. This is caused due to covalent binding of aflatoxin B, to DNA and proteins. Aflatoxin B is also known as to inhibit the synthesis of nuclear RNA in liver cells of rat. Since the mycotoxins inhibit DNA synthesis, the other products expressed by genes are also inhibited. Aflatoxin B causes delay in interferon production in turkeys. However, at high doses, it reduces Ig G and Ig A in chicks with the consequences of

decreased acquired immunity. Aflatoxin B reduces the cell mediated immune response in animals.

Ochratoxin A inhibits the activity of phenylalanyl - tRNA synthetase which is required in the first step of protein synthesis. It also reduces the renal mRNA coding for certain enzymes such as phosphoenolpyruvate carboxylase. Due to inhibition in protein synthesis several consequences occur. One of these is the changes in composition of serum protein which results in the suppression of non-specific humoral substances.

Effects on nervous systems

Mycotoxins are grouped into three on the basis of mode of action:

1. Mycotoxins causing paralysis and inhibition in respiratory system e.g. citreoviridin. They kill nerve cells disrupting energy supply as they inhibit ATPase activity.
2. Mycotoxins inducing trembling in animals e.g. fumitremorgin A, penitrem A. They alter functional states of neurotransmitters and disrupt nervous system.
3. Mycotoxin causing vomiting in animals e.g. vomitoxins such as trichothecenes. They act on chemoreceptor trigger zone in medulla oblongata and change the biogenic amines.

Effects on hormones' activities

In target cells, steroid hormones regulate the functions. Steroid forms the complexes with receptor which are then activated and transported to the nucleus. They bind to the activator sites of chromatin and induce protein synthesis selectively. Aflatoxin B, binds covalently with acceptor sites of chromatin and thus, reduces the nuclear acceptor sites of hormone-receptor complexes. Consequently, hormonal activities are reduced.

Carcinogenic effects

Aflatoxins, sterigmatocystin, versicolorin and luteoskyrin are known as carcinogenic mycotoxins. These are genotoxic also. The chemicals which cause gentle damage and initiate the carcinogenic process are known as genotoxic or initiators, whereas those which promote transformation of genetically modified cells to cancerous cells are known as promoters. Most of the chemicals are both genotoxic and promoters. Aflatoxin B causes liver cancer. It binds with DNA at the guanine base in liver cells, corrupting the genetic code that regulates cell growth.

Effects on reproductive systems

The urogenital systems of swine cattle and poultry animals are known to be affected by zearalenone which at I ppm produces hyperastrogenism in pigs. In young male swine it produces testicular atrophy and mammary gland enlargement. It has also been observed that at high concentrations zearalenone is associated with infertility, teat enlargement and under secretion in cattle. Zearalenone causes embryonic death and inhibition of development in swines.

Ergot ingestion may cause abortion in animals. Moreover, ergot is also associated with reduced weight gain and milk production in animals.

Management of mycotoxins

Although total elimination of mycotoxin from human food and animal feed is far difficult, yet the hazards from high concentrations can be minimised in some cases by adopting certain precautionary steps.

i. Residues of susceptible crops serves as a substrate for inoculum production of certain toxigenic fungi such as Claviceps, Aspergillus flavus, etc. Therefore, management of crop residues and use of resistant varieties should be done.
ii. Crop rotation should be adopted to lower the primary inoculum of toxigenic fungi.
iii. Since grain and oilseed crops are susceptible to fungal deterioration, these must be harvested when the crops have reached to their optimum maturity. At this time, presence of low moisture content in grains will minimise the risk of field contamination. As most of the contaminants get associated from the field.
iv. Prior to storage grains and oilseeds must be sundried to lower the moisture content. Fungicides and insecticides of low toxicity must be used.
v. Electronic or hand sorting of contaminated peanuts and other seeds, wherever possible should be done.
vi. Biological method of detoxification should be adopted by using certain strains of yeasts. moulds or bacteria e.g. *Flavobacterium aurantiacum* (NRRL B-184) from the liquid medium. These catalyse the hydration of aflatoxins (Marth and Doyle, 1979).
vii. Chemical detoxification is also one of the methods for inactivating the aflatoxins. Ammonisation use of ammonia gas resulted in significant reduction in levels of aflatoxins in contaminated peanuts, cotton seed meals, corn, etc.

Antibiotics

In the beginning of 20th century, the idea of growth inhibition of one micro-organism presents in the vicinity of other one came into existence. Later, it was demonstrated that growth inhibition of the former micro-organism was mediated by secretion of toxic metabolites by the latter. This toxic metabolite was termed as 'antibiotic and the phenomenon of act of growth inhibition by antibiotics as antibiosis". The antibiotics are defined as "the complex chemical substances, the secondary metabolites which are produced by microorganisms and act against other micro-organisms".

In nature, there is universal distribution of antibiosis among the microorganisms owing to which they are involved in antagonism. Those microorganisms which have capacity to produce more antibiotics can survive for longer time than the others producing antibiotics in less amount. However, antibiotics produced by microorganisms have been very useful for the cure of certain human diseases caused by bacteria, fungi and protozoa. Due to continuous endeavour made in this field, the antibiotics discovered at present are about 5,500. Total world production of

antibiotics is more than one million tonne per annum. This success has been possible only due to continuous research made during the last four decades.

Among the antibiotics discovered so far, there are four major groups which are most extremely used throughout the world the penicillin's, erythromycins, cephalosporins, tetracyclines. It has been already mentioned that antibiotics are produced in culture medium during idiophase due to depletion caused by one or more nutrient in the medium. In 1979, D. Perlman has described that the biosynthesis of antibiotics may be regarded as a result of a series of in-born errors of metabolisms. These errors may be exaggerated by subjecting the original microorganism to mutagenic substances. However, high yield of commercially important antibiotics owes much to the selection of such mutant stains as improvement of strain of *Penicillium chrysogenum* to yield benzylpenicillin about 20 mg/ml over the normal rate 720mug / ml.

Moreover, research done on this aspect have shown that the synthesis of some of the antibiotics in Streptomyces was mediated not by a plasmid. Therefore, there is possibility to produce new antibiotic by transfer of plasmids into a single cell of Streptomyces.

At present, there are thousands of antibiotics produced by microorganisms of which only a few hundreds have been marketed so far. Among this the penicillin's, cephalosporins and tetracyclines have been commercialized.

Penicillin's

It was Alexander Fleming (1929) who first discovered the bacteriostatic principle from a fungus and named it penicillin. He observed that a fungal contaminant prevented the growth of *Staphylococci*, which was later identified as *Penicillium notatum*. In 1932, P.W.J. Clutterbuck and coworkers studied the chemical nature of penicillin. They found that penicillin was an organic acid which was dissolved into organic solvents from aqueous solutions at low pH. It was vulnerable to hydrogen jon and heat. After evaporation of solution to dryness, the biological activity was lost.

The biological activity and recovery of penicillin were investigated by Chain *et al.*, (1940). They cultured Fleming's fungus in surface culture in a small pilot plant scale and recovered penicillin in 1.000-fold amount only by keeping low temperature during extraction. This was the first attempt to extract penicillin salt in the form of dry powder which could have the curative properties.

Strain improvement

The fungus *P. notation* originally used by Fleming for penicillin production, gave poor result. Moreover, many strains of this fungus were developed which produced many folds more penicillin than the original one. Besides *P. notatum, P. chrysogenum* was also tested which gave good results in submerged culture condition. Mutations in *P. chrysogenum* are generally induced

by ultraviolet radiation or other mutagenic chemicals (e.g. N-methyl-N-nitro-N-nitrosoguanidine (NTG).

The chemical nature of penicillin's

Molecular structure of penicillin's reveals that they include the 4 membered B-lactam ring. The amide bond of B-lactam ring is readily broken in both acidic and alkaline medium and can be hydrolysed by penicillinase synthesis by many bacteria. The naturally occur penicillin's differ from each other in groups. When different R group attached with penicillin it results in different types of penicillin of penicillin.

Conclusion:

This chapter emphasizes the important of secondary metabolites from various sources and its production application in various field. The secondary metabolites are one of their essential means of growth and difference. These are considering an alternative to most of the synthetic drug and their commercial valuable compounds.

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SUMMER POLLEN SOURCES TO *APIS DORSATA* HONEY BEES COLLECTED FROM NAGBHID FOREST AREA OF CHANDRAPUR DISTRICT OF MAHARASHTRA STATE (INDIA)

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Abtract:

80 pollen loads recovered directly from the honey combs of *Apis dorsata* (Rock Bee) collected in 23 April to 27 April 2012 from Girgaon and Kachepar forest area of Nagbhid Tahsil of Chandrapur District of Maharashtra State, were analysed. 30 (37.5%) pollen loads were found to be Uniforal, 16 (20%) biforal and 34 (42.5%) multfloral. The Unifloral pollen loads were contained *Terminalia* sp. The pollen of *Terminalia* sp. was recovered from 73(91.25%) of the total pollen loads studied. The study high lights *Terminalia* sp. (combretrceace) does the major pollen source and *Mangifera indica* (Anacardeaceae), *Blumea* sp. (Asteraceae), and *Delonix regia* (Caesalpineaceae) as fairly important sources of pollen of the honey bees during the summer period.

Keywords: Pollen Sources, Honey Bee, Nagbhid Tahsil. forest area

Introduction:

Honey bees visit plants for nectar and pollen. Nectar consisting predominantly of sources often associated with limited quantity of glucose and pollen grains provide the chief source of protein requirement of the bees essential for building their body tissues (Rahman Khan, 1941). particularly during the early embryonic growth, bees prefer the nectar of a plant species that has the maximum sugar concentration (Ramanujam, 1991). Similarly they prefer pollen type with the maximum nutritive values and palatability. Melittopalynological investigation involving honey samples and pollen loads furnish reliable information on the relative preferences of the honey bees among the floral sources available within their foraging ranges (Ramanujam, 1994). Analysis of pollen load unravels the floral fidelity of fixity of the bees to a particular plant species in any floristic community, by highlighting the numerical status of the pollen type in the individual loads. The quantification of the data would help us to recognize the major and minor sources of pollen in any particular area (Chaudhari, 1978).

Studies involving the analysis of pollen loads are few when compared to those of honeys, in the Indian context. Sharma (1970 a & 1970 b, 1972) and Chaturvedi (1973) studied the pollen loads of *Apis cerena*, the Indian hive bee, from Kangra in Himachal Pradesh and Banthara in the vicinity of Luckhnow. Seethalakshmi and Perey (1980) recognized Borassus flabellifer as a good pollen sources in Tamilnadu by analysing 900 pollen loads of *Apis cerena* at Vijayarai in West Godawari District of Andra Pradesh and recognized potential of this region for apiculture Kalpana, Khatija and Ramanujam (1990) and Ramanujam and Kalpana (1990) provided information on the pollen sources of *Apis florea* and *Apis cerena* honey bees in Hydrabad and Ranga Reddy District. Recently Cherian *et al.* (2011) provided information on the pollen sources of *Apis cerena* honey bees in Nagpur District of Maharashtra. In recent research, Borkar Laxmikant and Mate Devendra (2014, 2023) successfully identified the origin of *Apis dorsata* pollen. Cherian *et al.* (2011) discussed pollen sources for *Apis cerena* honey bees in Nagpur District of Maharashtra, as well as in the Bramhapuri forest in Chandrapur District.

This study is aimed to recognize the major and minor sources of pollen to *Apis dorsata* bee in these forest during summer period (Honey flow season) on the basis of qualitative and quantitative analysis of numerous pollen loads recovered directly from various honey combs.

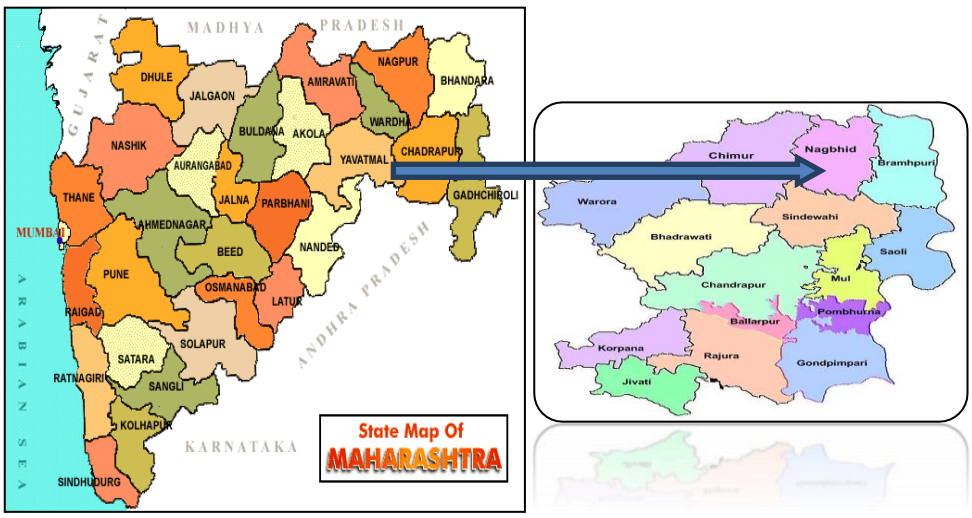


Image showing Nagbhid Tahsil of Chandrapur District from where the pollen loads were collected

Material and Methods:

Pollen loads (Comb loads) 80 in number of *Apis dorsata* were obtained from Two Honey combs collected on 24th and 26th April 2012 from Girgaon and Kachepar forest area of Nagbhid tahsil of Chandrapur District of Maharashtra State. (CHN – NAG- GIR).

The pollen grains of each pollen load were dispersed in 1 ml of glacial acetic Acid and later on subjected to acetolysis (Erdtman, 1960). One slide prepared for each pollen load and microscopically examined. All such pollen loads consisting of a single pollen type represent unifloral loads, with two pollen types bifloral and with more than two, multifloral (Sharma, 1970

a). Identification of the pollen types was based upon the reference palynoslides of the forest flora and the relevant literature. The pollen productivity of the significant taxa was computed using haemocytometer.

Result:

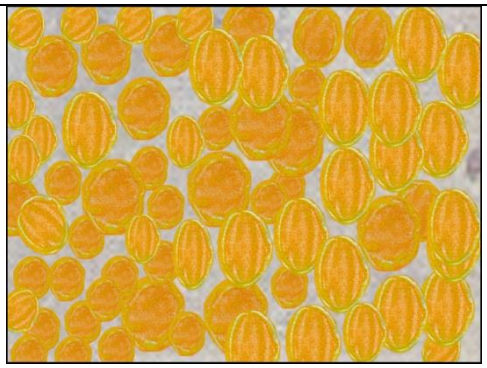
The analysis has brought to light that 30 (637.5%) loads were unifloral, 16 (20%) were bifloral and the remaining 34 (42.5%) loads multifloral (Table 2).

The pollen grain of 10 taxa referable to 09 families were recorded. These are *Terminalia* sp. (Combratrceace), *Mangifera indica* (Anacardeaceae), *Blumea* sp, *Citrus* sp. (Rutaceae), *Delonix regia* (caesalpiniaceae), *Bombax ceiba* (Bombaceae), *Carthamous tinctorius* (Asteraceae), and *Prosopis juliflora* (Mimosaceae). Of these *Blumea* sp. and *Carthamous tinctorius* are herbaceous weeds which represent the undergrowth, the remaining taxa are either arborescent member or shrub of the forest range.

The unifloral pollen loads include 27 (33.75%) of *Terminalia* sp. only 3 (3.75%) *Mangifera indica* (Fig. 1), bifloral 8 (10%) include *Terminalia* sp. & *Mangifera indica*, 2 each of *Prosopis juliflora Terminalia* sp. And *Delonix regia* and *Terminalia* sp., 1 each species of *Terminalia* sp. in combination.

The multifloral loads which are encountered showed the pollen types of *Terminalia sp.*, M*angifera indica*, *Blumea* sp., *Citrus sp.*, *Carthamus tinctorius*, *Delonix regia*, *Cappnris grandis*, *Bombax ceiba*, *Cleome gynandra* and *Prosopis juliflora* (Fig. 2).

When the representation (Irrespective of percentage) of the various pollen types in the total number of pollen loads studied was considered & the percentages of pollen types recorded in each bifloral and multifloral loads were determined by counting 200 pollen grains at random, (Sharma, 1970a) pollen of *Terminalia* sp. were noted in as many 73 loads (91.25%) followed by *Mangifera indica* in 44 loads (55%), *Delonix regia* 31 (15.01%), *Blumea* sp. 15 (18.75%).



***Terminalia* sp.**

Mangifera indica

Figure 1: Pollen types in unifloral pollen loads

Table 1: Pollen morphological characters of the Taxa recorded

S.N.	Pollen Type	Size, Shape & Symmetry	Aperture Pattern	Pollen Wall (sporoderm) structure & sculpture
Asteraceace				
01	***Blumea*** sp.	21-24 µm, Amb spheroidal, isopolar, Radially symmetrical	Tricolprate, colpi long	Exine 3 µm thick, surface echinate, spines 5-6 µm long, 4 spines in the inter apertural region interspinal area psilate
02	***Carthamus tinctorius*** Linn.	59-65 µm, Amb spheroidal: 58-62× 66-73 µm, subprolate, radially symmetrical	Tricolporate, colpi with tapering ends, ora lalongate	Exine (spinoid processes included) about 8 µm thick at poles, 10 µm at equator tectate, tectum prominently columellate, columella simple or branched, sharply undulating with supratectal solid, pointed, robust sinule like processess
Anacardeaceae				
03	***Mangifera indica*** Linn.	27-31 µm, Amb subtriangular; 29-32 ×26-28 µm, subprolate; Radially symmetrical	Tricolporate colpi long, tips acute ora prominently lanlongate	Exine 2.5 µm thick, subtectate, surface striatoreticulae, striations more or less parallel in equatorial view, lumen generally elongated in polar direction, murisimplibaculate
Bombaceae				
04	***Bombax ceiba*** Linn	51 µm (49.5×52.5) µm, peroblate, isopolar, Radially symmetrical	Tricolprate, col. length 12 (10.5-13.5) µm	Exine thick 3 µm, coarsely reticulate, mesh 4.1 µm (3-4.5 µm) in the major part except at the angles showing medium reticulations 1-8 µm (1.5 -3 µm), greater number of baculae are found in the lumen. Muri simplibaculate, faint LO pattern.

Caesalpiniaceae				
05	***Delonix regia*** (Boj. ex. Hoof.) Ref.	59.62 µm, Amb more or less spheroidal to subtriangular; 53-56× 57-60 µm, oblate to suboblate; Radially symmetrical	Tricolporate, colpi long with blunt ends, ora faint, more or less rounded	Exine 5.2 µm thick, subtectate, surface coarsely reticulate. Heterobrochate, meshes smaller near the apertural regions & larger elsewhere, lumina poly to hexagonal with a number of free bacules, muri thick, sinuous, simpli to locally duplibaculate
Capparidaceace				
06	***Capparis grandis*** Linn.	10-12 µm, Amb spheroidal; 14-16 ×9-12 µm prolate to subprolate; Radially symmetrical	Tricolporate, colpi linear to narrowly elliptic, ends tapering, tips acute, ora faint lalongate	Exine 1 µm thick, tectate, surface faintly granular to almost psilate
Combratrceace				
07	***Terminalia*** sp.	19-22 µm, Amb spheroidal; 21-24 x20-22 µm, subprolate; Radially symmetrical	Tricolporate, colpi alternating with pseudocolpi colpi linear, tips acute pseudocolpi almost equal the size of colpi, ora more of less circular	Exine 1.5 µm thick, tectae, surface psilate to locally finely granular

Mimosaceae				
08	***Prosopis juliflora*** (Sw.) DC	36-39 µm, Amb rounded triangular; 38-42× 30-35 µm, prolate to subprolate; Radially symmetrical	Tricolllporate, occasionally syncolpate, colpi tapering towards poles, tips acute, ora lalongate	Exine 3.2 µm thick, tectate surface faintly reticulate
Rutaceae				
09	***Citrus*** sp.	27-29 µm, Amb squarish, 26-30 ×25-27 µm, prolate spheroidal radially symmetrical	Tetracolporate, colpi linear, tips acute, ora lalongate	Exine 2 µm thick subtectate, surface Reticulate. Heterobrochate, meshes smaller near the apertural regions and larger elsewhere, lumina hexa to pentagonal or irregular, psilate, muri simpli to locally duplibaculate
Cleomaceae				
10	***Cleome gynandra*** Linn	19-21 µm, Amb spheroidal, 18-22 ×14-16 µm, prolate spheroidal; radially symmetrical	Tricolporate, colpi with tapering ends, ora faint, lalongate	Exine 1 µm thick, sub-tectate, surface finely reticulate, homobrochate, lumina polygonal, smooth, muri simplibaculate

Table 2: Analysis of pollen loads from honey com

Nagbhid Tahsil							
Comb	**Total Pollen Loads**	**Unifloral Loads**		**Bifloral Loads**		**Multifloral Loads**	
		Number	**Composition**	**Number**	**Composition**	**Number**	**Composition**
CHN-NAG-Gir-14	42	20	17 – Te 3 – Ma	13	5-Ma(89,11), Te(89,11) 2-Pr(20,81), Te(19,80) 2-De(14,21), Te(79,86) 1-Te(80), Bl(20) 1-Te(89), Bo(11) 1-Pr(20), Ma(80) 1-De(84), Ma(16)	09	**4-Ma(7,58),** **Te(17,51),** **Bl(6,37),** **Cl(4,20)** **2-Te(5,66),** **De(6,22),** **Ma(23,78)** **1-Te(43),** **Ma(41),** **Bl(16)** **1-Cl(14),** **Te(80),** **Bl(6)** **1-Te(56),** **De(33),** **Bl(5), Ma(6)**

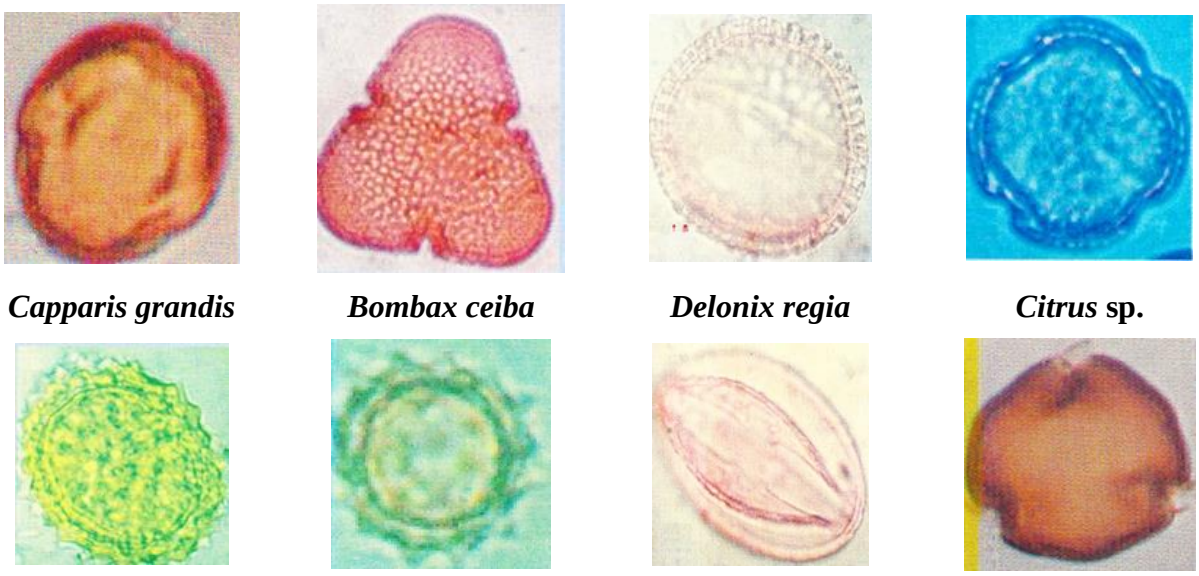
CHN-NAG-Kach-15	38	10	10 – Te	03	3-Ma(13,22), Te(78,87)	25	**10-Te(44,63), De(8,50), Ma(5,30) 7-Te(21,85), Bl(11,19), Ma(3,22), De(11,28) 5-Te(13,63), Ci(8,18), Ma(3,56), De(3,36) 1-Te(6), Pr(8), De(86) 1-Ca(8), De(42), Ma(50) 1-Cart(4), Te(87), De(9)**
Total	80	30 (37.5%)		16(20%)		34(42.5%)	

Abbreviations for pollen types recorded from pollen loads

Te- *Terminalia sp.* Ma- *Mangifera indica* Bl- *Blumea sp.* Ci- *Citrus sp.*

Car – *Carthmus tinctorius* De- *Delonix regia* Ca- *Capparis grandis* Bo- *Bombax ceiba*

Pr- *Prosopis juliflora* Cle- *Cleome gynandra*



Capparis grandis ***Bombax ceiba*** ***Delonix regia*** ***Citrus* sp.**

Carthamus tinctorius ***Blumea* sp** ***Prosopis juliflora*** ***Cleome gynandra***

Figure 2: Light Microscopic photograph of pollen grain in pollen loads

Discussion:

The analysis showed that the pollen loads obtained from the bee hives of *Apis dorsata* in the Girgaon and Kachepar forest area of Nagbhid Tahsil of Chandrapur District of Maharashtra State, originated predominantly from some of the characteristics arborescent and shrubby plants of this forest area. Viz. *Terminalia* sp, *Mangifera indica*, *Citrus* sp., *Delonix regia*, *Cappnris grandis*, *Cleome gynandra* and *Prosopis juliflora*. The contribution to herbaceous weeds such as *Blumea* sp. *Carthamus tinctorius* as pollen source to *Apis dorsata* bees is very meagre.

The quantification of the data revels unequivocally the predominance of the pollen of *Terminalia* sp. as evidenced by its very high representation of 73% in the Unifloral loads and 91.25% in the totality of the pollen loads material studied.

It can therefore be concluded that *Terminalia* sp constitutes the major source of pollen to the honey bees during the summer period. The other fairly significant source of pollen to the honey bees of this area are *Mangifera indica* 44 (55%), *Delonix regia* 31(38.75%), *Blumea* sp. 15 (18.75%), *Citrus* sp. 17 (21.21%), and *Cleome gynandra* 5 (6.25%).

All these taxa also constitute important pollen source during the summer season for the honey bees of this forest area.

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ALLELE MINING: PRINCIPLES, PROSPECTS AND POTENTIAL

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Abstract:

Allele mining refers to the identification of naturally occurring allelic variation at agronomically important genetic loci. The success of Allele mining process depends upon collection of different wild species of a crop. Core collection is a crucial step for collection of wild germplasms with less loss of diversity. Allele mining can be done by Association mapping-based allele mining, Sequencing-based allele mining, and Eco tilling-based allele mining, which is a modified tilling operation. Allele mining requires the use of bioinformatic tools like PLACE, plantCARE, TRANSFAC, JASPAR, MEME, Plantprom DB, DCPD, SCPD, BioEdit, ClustalW2 etc to know the variation present in loci. The analysis and interpretation of the data help to understand the genetic variation among the germplasms. In this chapter we will understand the Allele mining and importance of it.

Introduction:

Allele mining refers to the identification of naturally occurring allelic variation at agronomically important genetic loci (otherwise called as genes or regulatory genomic regions). Numerous methods, such as association mapping, QTL and AB-QTL analysis, mutant screening, genome-wide surveys for the signs of artificial selection, etc., can be used to complete this.

The use of differed germplasm collections—especially those rich in wild species—is crucial to the success of the allele mining process. This is because, due to the unavoidable loss of diversity throughout the domestication process, most of the allelic variation at the gene(s) of interest is thought to occur in the wild relatives of a crop (i.e., not in the farming crop types). Numerous attempts have been undertaken to identify novel, helpful alleles that exist in practically all crop plants' natural gene pool.

Unfortunately, despite those efforts, several obstacles, such as a lack of resources for analyzing large collections, have prevented entire germplasm entries from being effectively characterized for their unique phenotypes. As an alternative, germplasm core collections have been suggested as a source of allele mining resources. The term "core collection" refers to a

representative subset of the entire germplasm collection that has been refined to have the greatest amount of diversity in the fewest possible accessions.

Therefore, by reducing the number of accessions, core collections aid in the incorporation of novel, valuable alleles into traditional or molecular breeding programmes, while maintaining the maximum allelic diversity at loci affecting traits of interest. As a result, wide-ranging, varied elite breeding lines with increased yield and environment adaption will be developed. The best core collections can be created by gathering a variety of diversity-related evidence and then selecting accessions that best represent the diversity. Geographic origin is one such simple generic characteristic. Conventional accessions are likely to exhibit variations throughout the genome since they often have an autonomous history of domestication spanning thousands of years from diverse parts of the world.

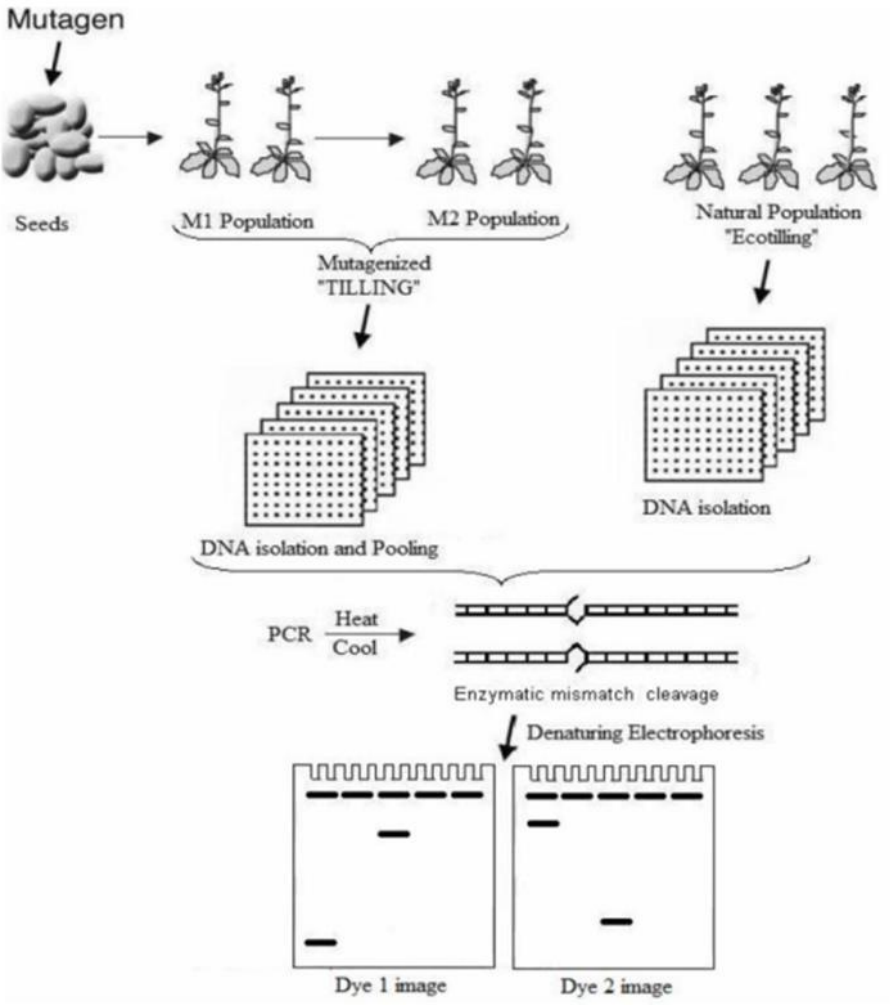
A core collection of such types can find most novel alleles in a very small number of accessions. However, it's important to keep in consideration that even a highly selected core collection won't be able to reveal the entire list of alleles in every potential combination at this point. Screening the entire germplasm is therefore crucial. This attempt wouldn't be so difficult until other mining methods that are quicker and less expensive are created. Consequently, a directory of every gene in a given crop along with its function is produced by large-scale genome sequencing projects and functional genomics initiatives on a number of important food crops. Even though the reference crop cultivar or accession was used to create this information, allele mining allows for its extension to other varieties and species as well.

Allele mining requires the use of tools like PLACE, plantCARE, TRANSFAC, JASPAR, MEME, Plantprom DB, DCPD, SCPD, BioEdit, ClustalW2, and others. Allele mining, then, is a method that analyses naturally existing allelic variations or desirable alleles for candidate genes influencing important agronomic qualities that may be used to improve crops. It aids in the production of allele-specific molecular markers for use in marker-assisted selection, the discovery of new haplotypes, and the tracking of allele change.

Approaches for allele mining:

The following techniques exist for allele mining: association mapping-based allele mining, sequencing-based allele mining, and ecotilling-based allele mining, which is a modified tilling operation. Eco-Tilling is the term for modified tilling techniques. While Eco-Tilling approach detects natural mutation, TILLING (Targeting Induced Local Lesions IN Genomes) technique can discover single base-pair allelic variation in target gene (more particularly, induced point mutations). The technique is economical as it requires only one individual to be sequenced for every haplotype, enabling the quick identification of variance in numerous individuals. Creating a mutagenized population, preparing and pooling DNA, and finding mutations are the three main phases in tilling (Figure 1). Initially, chemical mutagens are used to

randomly induce mutations in genomes. Ethylmethanesulfonate (EMS) treatment mutagenizes seeds. The resulting M1 plants are self-fertilized, and M2 individuals are used to prepare DNA samples for mutational screening.Test samples are extracted of their DNA. The DNA samples are combined and arranged into 96 microtiter plate wells. Gene-specific infrared dye-labeled primers are used to amplify a target region in PCR as the first step in the mutation screening process. IRDye 700, a fluorescent dye that can be detected at 700 nm, is used to 5-end label the forward primer, whereas IRDye 800 is used to label the reverse primer. To enable the creation of mismatches or heteroduplexes, which stand for induced and naturally occurring single nucleotide polymorphisms (SNPs), these PCR products are denatured and re-annealed. After that, samples are incubated with a nuclease specific to a single strand to break down mismatched base pairs. For mismatch-specific cleavage, several enzymes, including S1 nuclease 5, T4 endonuclease VII6 and Cel-1 7 have been used. Cleaved bands representing mutations or polymorphisms are visualized using denaturing polyacrylamide gel.



Schematic diagram of the TILLING and ECOTILLING strategy for plants. (Modified from Simsek and Kacar 2010)

Sequencing-based allele mining

In sequencing-based allele mining, alleles in various genotypes are amplified using PCR, and then DNA sequencing techniques are used to identify nucleotide variation. In other words, sequence-based allele mining allows us to identify different alleles among the cultivars as well as the multiplication of a certain section of deoxyribose nucleotides.

Next generation sequencing for allele mining

An approach that helps us understand this nucleotide's arrangement in DNA molecules is sequencing. "Massively parallel" techniques have been developed in recent years, contributing to the creation of "next generation" sequencing machines that offer higher accuracy and throughput. These techniques are applied to resequencing, sequence data alignment, and genome comparison with a reference. The first of this kind, which did not require cloning and depended on pyrosequencing, was brought to market by 454 Life Sciences. This 454-sequencing platform may extend read length by an average of more than 250 bases and generate 100 Mb of sequence with 99.5% accuracy

Association mapping-based allele mining

According to Zhu and coworkers the strategy is used to establish regions of the genome associated with critical phenotypes by association or linkage-disequilibrium mapping. The approach relies on the assumption that alleles responsible for a phenotype, along with the markers which flank the locus, are inherited as a block. Using DNA markers has been suggested to identify useful alleles in the vast reservoirs of genetic diversity.

Bioinformatic tools required for allele mining

A wide range of advanced bioinformatics tools, such as PLACE, plantCARE, TRANSFAC, JASPAR, MEME, Plantprom DB, DCPD, SCPD, BioEdit, and ClustalW, are needed for allele mining. These tools are helpful for aligning sequences so that fresh genome sequences can be compared to reference genomes, or sequenced genome data.

Some examples of allele mining for crop improvement

- Allele mining for blast resistance genes in finger millet and rice
- Association mapping-based allele mining for node of first fruiting and its height in Upland cotton (*Gossypium hirsutum* L.)
- Allele mining and haplotype discovery in barley candidate genes for drought tolerance
- Eco-Tilling for Allelic Variation of Salinity Tolerance Genes in Barley cultivars

Challenges in allele mining

- **Selection of genotypes:**

  Numerous strategies, including the creation of core/mini core collections, precise phenotyping techniques, and adaptable computational tools, can be used to prioritise genotypes for allele mining.

- **Handling genomic resources**

  Computational techniques for assessing functional nucleotide diversity and predicting nucleotide changes causing altered function must be developed in order to keep up with the rapidly accumulating nucleotide and gene expression data. The recommended approach makes use of advancements in comparative genomics, association genetics, and allele mining by merging knowledge from several fields, including molecular genetics, statistics, and bioinformatics.

- **Demarcation of promoter region**

  The positions of promoters and regulatory elements vary from gene to gene and are dispersed across the upstream region. The development of specialised software tools that can reliably forecast the core promoter area based on the over- or under-representation of regulatory motifs is imperative.

- **Characterization of regulatory region**

  Characterizing the amount of cis-acting regulatory variation is far more difficult than detecting variation in the genome's coding regions because it is impossible to detect even in completely sequenced genomes. Potential trans-acting variables or environmental variations complicate the process of screening for regulatory variants based on individual differences in transcript levels. Even software cannot accurately predict orientation since promoter regions might work in a bidirectional route.

- **Higher sequencing costs**

  Reducing the time and effort needed while lowering the cost per data point is one of the major difficulties. By using more affordable and quick sequencing platforms for high throughput allelic variation detection, some of these difficulties may be solved.

Applications of allele mining-

- **Description of allelic diversity in gene banks-** Utilizing allele variation that influences plant phenotypic is crucial for improving crop yield through the use of genetic resources. The allelic drive is characterized by several of the international crop research institutes that maintain crop quality.
- **Identification of new haplotypes-** The identification of nucleotide variation at a genomic area (candidate gene) linked to phenotypic variation for a trait may be possible with allele mining.
- **Development of allele specific marker for Marker Assisted Selection-** The development of an allele-specific marker assay for the accurate introgression of a superior or novel allele to an appropriate genetic background will be made possible by the identification of sequence variation.
- **Allelic synteny and evolutionary relationship-** Usingthe sequence information obtained

from allele mining study, syntenic relationship can be assessed among identified loci/gene across species/genera. In rye, superior homologous alleles for aluminum tolerance were isolated using syntenic allele sequence information from wheat.

Conclusion:

Allele mining is an important approach in agricultural genetics since it provides the potential to identify advantageous alleles that can be used to generate superior crop types. The success of allele mining largely depends on the utilization of diverse germplasm collections, particularly those rich in wild species. Continuous developments in sequencing technologies and bioinformatics tools, together with thorough genotype analysis, will increase the efficiency and effectiveness of allele mining in crop improvement programmes.

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EXPLORING PLANT SECONDARY METABOLITES PRODUCTION WITH EMERGING TECHNIQUES IN PLANT BIOTECHNOLOGY

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Abstract:

Plant cell culture is a potential source for the production of secondary metabolites. The chapter explores the advanced methodologies for the production of secondary metabolites using plant tissue culture and its application. There are few strategies mentioned for the improvement and getting large production for secondary metabolites like hairy root culture and use of elicitors. It highlights hairy root culture as one of the potential and modern methodologies for the production of secondary metabolites. The chapter outlines about the lab-scale production of withaferin-A, a type of withanolides from *Withania somnifera* plant using hairy root culture technique. The hairy root culture is a technique where a bacterium *Agrobacterium rhizogenes* is introduced in a plant which induces the hairy root growth in a plant containing metabolites in it and then it is extracted. If mass production is needed, then the growth of hairy roots can be cultivated in bioreactors which will give production at a higher rate. It also introduces the concept of elicitors and its significance in metabolite yield. The most important of producing metabolites under controlled conditions in the laboratory is that it can be in any climate or season. Plant cells have been potentially used as factories for the production of high yield of secondary metabolites under regulatory conditions. Secondary metabolites are able to cross the cell membranes and they perform their biological activities which makes them a suitable compound in the pharma industry.

Keywords: Secondary Metabolites, Hairy Root Culture, Withaferin-A, Elicitation, Molecular Farming

Introduction:

Secondary metabolites

Products synthesised by plants can be categorised into two categories: primary metabolites and secondary metabolites. Primary metabolites are those compounds which are produced in larger amounts and play vital roles in plants that are essentially needed by plants for their respiration, photosynthesis, reproduction, growth and development. Plants also synthesise other metabolites in small amounts as compared to primary metabolites which are not primarily

helpful for plants and required by the plants for defence mechanism, help the plants to adapt in unfavourable conditions which are known as secondary metabolites. They also attract pollinators by providing pigment, smell and taste which facilitate the dispersal of fruits and seeds.

During the stationary phase, most of the secondary metabolites are produced because during the active phase of growth primary metabolites are synthesised. Plants produce secondary metabolites when they are undergoing any kind of stress like biotic (virus, insects, fungi, bacteria) or abiotic (temperature, salinity, metals, light, water) stress.

As people are discovering the use of their secondary metabolites they are potentially using it in many industries like nutraceuticals, food additives, drugs, biofuels, biomaterial, dye, cosmetics, perfumes, waxes and in agriculture for protecting the crops as they are a rich source of nutrients. Due to high demand in industry, it is important to produce in a large amount which can be done with the help of biotechnology methods rather than depending on traditional methods and chemicals. Synthesis of secondary metabolites using chemicals is not feasible due to its highly complex structures, specific stereo chemical characteristics and consumers prefer using natural products over artificially made.

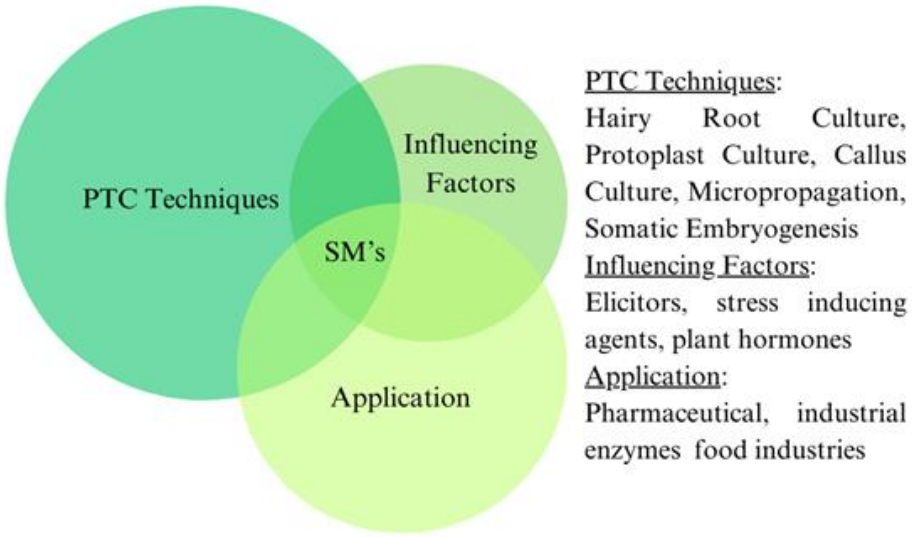

Figure 1: Representing inter-relationship between production of secondary metabolites (SMs) using plant tissue culture, influencing factors and its application

In plant biotechnology, plant tissue cell lines are being used in laboratories under a controlled environment like by alternating in components of nutrients, supplying precursors and elicitors which helps in inducing gene expression, grow faster, minimise damage from pests and give high productivity. The genetically modified plants which are genetically engineered have more effective production of secondary metabolites. There are many tissue culture techniques like molecular pharming, elicitation, hairy root culture, biotransformation, protoplast culture, callus culture, micropropagation, somatic hybridisation and somatic embryogenesis which are utilised to enhance the production. The representation in Figure1 is showing the interconnecting

link between the enhanced production of secondary metabolites with the help of hairy root culture, elicitors and their potential application is various industries.

Classification: Secondary metabolites are majorly classified into three classes based on their biosynthesis pathway: terpenes, phenolics and nitrogen-containing compounds.

1. Terpenes are lipophilic in nature, products of 5-carbon isoprene units and present in hormones, essential oils, steroids and pigments. Terpenes help in photoprotection (carotenoids), transfer of electrons (plastoquinone), contribute in giving flavours and pigments (vitamin A).
2. Phenolics contain one or more phenolic groups and are water soluble. Phenolics help in the formation of lignin which gives strength to the cell wall and rigidity. Flavonoids are one of the phenolics which protects the tissues of plants from UV damage by absorbing ultraviolet light from sunlight.
3. Alkaloids is one of the nitrogen-containing compounds which are physiologically active in humans and used as chemotherapeutics agents like cocaine, nicotine, morphine, vincristine and vinblastine. L-DOPA, L-3,4-dihydroxyphenylalanine is the precursor of catecholamines and is potentially being used as a potent drug for the treatment of Parkinson's disease.

Function: They have diverse functions in biological activities like antiviral, anti-inflammatory, anti-mutagenic, anti-microbial, anti-oxidants, anti-allergic, anti-tumor and growth regulatory properties.

Hairy root culture

Hairy root culture is in-vitro tissue culture technique where SMs are produced associated with roots by providing optimised and controlled aseptic conditions like temperature, light, stress induction, nutrient composition, genetic manipulations, growth hormones. When the abiotic or biotic stress is being induced, it enhances the synthesis of SMs as a defence response. Hairy roots are induced by infecting the explant with the bacteria like *Agrobacterium rhizogenes* which is gram negative in nature by the process of genetic transformation and mostly performed in dicotyledons.

Withania somnifera also known as ashwagandha belongs to the Solanaceae family which is one of the most important and highly considered as valuable medicinal plants. It is being used as anti-angiogenic, anti-metastatic, anti-inflammatory, antiarthritic, antidepressant and to treat diseases like Parkinson's disease, epilepsy, Alzheimer disease, arthritis, rheumatism. Withanolide is one of the secondary metabolites which is a steroidal lactone synthesis in the roots of *W. somnifera* plant. Figure2 shows the steps involved in the synthesis of withaferin-A at laboratory level. Some other metabolites that can be synthesised through hairy root culture are

withaferin-A, L-DOPA, shikonin, anthraquinone, berberine, quinine (malarial treatment), nicotine, monoterpenes.

Procedure: Withaferin-A is a type of withanolide and here is a brief protocol for the production of withaferin-A from the *W. somnifera* plant using hairy root culture.

1. Leaf is taken as an explant from the W. somnifera plant. It is sterilised and the wound is created by making a small cut on the leaf.
2. Bacterial suspension is prepared by transferring *A. rhizogenes* culture from solid to liquid YMB (Yeast Mannitol Broth) media.
3. Explant is infected by exposure with bacterial suspension and co-cultivated with different strains of *A. rhizogenes* on MS (Murashige and Skoog) basal media.
4. Hairy roots are then cultivated on liquid MS basal medium when the growth is initiated.
5. Biomass of roots were determined by separating the roots from media, washed with distilled water and kept at 60 °C. Dry weight is recorded.
6. Withaferin-A is extracted.

For a mass scale production of withaferin-A, hairy roots are isolated and transferred to bioreactors which consist of a proper growth environment for roots.

Advantages: Hairy roots are better than normal roots because-

- Growth rate is faster as liquid media is used
- Higher production yield
- Genetically more stable
- There is lack of geotropism in hairy roots lead to formation of branches
- Lateral branching is higher

Figure 2: Brief protocol for the production of withaferin-A using hairy root culture

Elicitor

Elicitors are molecules which induce the stress responses in plants and also activate the expression of genes which help for the synthesis of these metabolites. Elicitation is the process where the chemical or physical stress is being given to plant cell suspension culture and triggers the synthesis of secondary metabolites by triggering physiological, morphological responses and phytoalexins accumulation in plants. Ex: Stress inducing elicitors like chitosan, hormones, microbial and physical stress treatment along with proline and melatonin are usually used which activate stress inducing pathways that lead to enhanced production of secondary metabolites.

The production of metabolites via elicitors depends on several factors like concentration, exposure time of elicitor in plant cell culture and selectivity of elicitor based on type of secondary metabolites that need to be produced.

Classification:

On the basis of nature:

1. Abiotic elicitors- The elicitors which are non-biological and chemical in nature, mostly are inorganic salts and metal ions like Cu, Cd and Ca^{2+} ions to act as elicitors.
2. Biotic elicitors- The elicitors which are biological in nature such as polysaccharides of cell walls of plants like pectin or cellulose, of microorganisms like chitin and cellulose and glycoproteins.

On the basis of origin:

1. Exogenous elicitors- These elicitors originate from outside the cells. Endogenous elicitors are such as arachidonic acid, glucans and chitosan.
2. Endogenous elicitors- These elicitors are formed when secondary metabolites are induced by a signal of biotic or abiotic nature in the cell.

Molecular farming

Molecular farming is a branch of plant biotechnology where we genetically modify the plants to produce valuable compounds like pharmaceutical proteins, vaccines, enzymes and utilise the plants for agronomic significance to produce biomolecules on a large scale. Using plants to produce biopharmaceutical opens up new avenues and addresses challenges associated with the traditional expression system. Plant cells can synthesise proteins that are structurally and functionally identical to those made in mammalian cells, ensuring high quality products that are devoid of human pathogens. Plant can serve as a natural storage system when biomolecules are targeted to seeds or storage organs, allowing for preservation at room temperature. Moreover, the substance might be delivered orally through edible plant tissue, including as, fruits, leaves and roots. In this manner, the recombinant product extraction and purification are avoided. Additionally the biomolecules are protected by a cell wall allowing for longer activity on the mucosal surface. Lastly, the existing agronomic practices for crops provides significant

economic benefits, with the only negatives being production control and genetically modified plant containment.

Applications

Due to several biological activities of secondary metabolites it is widely used in many industries. Some of the metabolites are mentioned and their applications.

1. Cyanidin-3-rutinoside: It is an anthocyanin having antioxidant properties which help to eliminate or neutralise the free radicals in the body. It is used in medicine to reduce the risk of problems related to heart and cancer.
2. Flavonoids: They are phenolic compounds having anti-inflammatory and anti-microbial properties which are used as dietary supplements.
3. Coumarins: Coumarins act as the plant's defence mechanisms against disease and pests. They are used as anticoagulants in pharmaceuticals.
4. Terpenes: Terpenes are aromatic compounds used in fragrance and flavour industry. They have biological activities like antimicrobial and anti-inflammatory which have potential value in therapeutics effects.

Conclusion:

With the increase in population, it is important to advance in technology because traditional methods cannot be employed as it is a slow process and gives limited yield. Plant source is never stopping in exploiting their rich and valuable compounds present in them and will be having continuous growth in industry. The emerging technologies in the field of biotechnology have a potential application in plant biotechnology which enables us to perform various genetic engineering like deletions, insertion, transformation which has potential activity to give more productivity.

Plants have evolved themselves in such a way that it produces secondary metabolites however it might not be useful them as primary need but it has several benefits like antiviral, anti-inflammatory, anti-mutagenic, anti-microbial, anti-oxidants, anti-allergic, anti-tumor and growth regulatory properties in industries. Methods like hairy root culture and elicitation using elicitors have been utilised to enhance the productivity of secondary metabolites. We can gain more yield by optimising or modifying the conditions as per need of a plant to induce the secondary metabolite production. Secondary metabolites extraction has commercial importance and hold a valuable position in industry and market. Hence, molecules having complex structures like alkaloids, steroids, terpenes can be synthesised with the help of techniques based on plant cell cultures. Through this technique we can either produce a novel metabolite or have modifications in existing metabolites for effective and additional properties.

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SEED SURFACE CHARACTERISTICS AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *CUSCUTA REFLEXA* ROXB. SEEDS OF FAMILY CONVOLVULACEAE

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Abstract:

In Ayurveda medicinal plants have great value. It gives remedies on different diseases. The *Cuscuta reflexa* Roxb. plant is highly medicinal belongs to family Convolvulaceae. It is also known as 'Amarvel' or 'Giant dodder'. It is parasitic plant species. Amarvel seeds are used for drug preparation in pharmaceutical industry. Phytochemical constituents present in seeds. The morphological and anatomical variations is very helpful for identification of seed. The micromorphological characters of seed coat through scanning electron microscopy (SEM) were well studied. The seed surface study shows that seed coat not smooth. It contains polygonal to hexagonal cellular structure seen on seed coat; cell wall thicker. The hilar region shows agreegated cellular mass. Seed coat study shows structural, cellular variations. It shows cuticular epidermis, aleurone layer, palisade layer, endodermis etc. The qualitative analysis helps for detection of various chemical constituents which are helpful for drug preparation. The seeds of *Cuscuta reflexa* Roxb. having medicinal properties. The preliminary phytochemical analysis is used for drug preparations. The plant is parasitic leafless climber having theruptic value. Above observations is very important for seedcoat study which is useful for taxonomic study and identification of seed.

Keywords: Seed Morphology, Scanning Electron Microscopy (SEM), Seed Anatomy, Biochemical, Phytochemical Analysis, Convolvulaceae.

Introduction:

The plant is an annual parasitic herb. It is also known as "Dodder Plant". It gives 170 different types of plants. Flowering period is July to September. The fruiting period between August and October. It is parasitic plant. It occures in many tropical and temperate regions. Occure in many countries. The plant shows no true roots. The leaves are like scales on the stems. The plant interwine around other plants for survival period (Fig: 1, 2). The *Cuscuta reflexa* Roxb. plant belongs to family Convolvulaceae. The local name is amarvel. Inflorescence shows Cyme or raceme axillary. The plant has high medicinal value.

Figure 1: Habit *Cuscuta reflexa* Roxb. Yellow colour interwine with other plants

Figure 2: *Cuscuta reflexa* Roxb. Spreading of yellowish interwine on other plant. (Parasitic plant / Dodder plant, leaves scaly or leafless.)

Materials and Methods:

Sample collection: Seeds of family Convolvulaceae like *Cuscuta reflexa* Roxb. were collected from local area. For seed coat study, all the seeds parameters were studied using discecting and binocular microscope. Digital weighing balance was used for weighing the seeds in mg. The morphological observations of seeds were done followed by their photography, using 1 cm. scale.

Seed coat morphology (SEM): To study the seed coat morphology scanning electron microscopy is most important. For this purpose, the individual seeds were dipped in alcohol for 5-10 min. to remove the dust from them. The seed mounted on pin type stubs using double sided adhesive tape or conductive silver paint to prevent charging of the surface during scanning and then coated with a very thin layer of gold in a polaron sputter coating unit. For spermoderm study of seed photomicrograph were taken in the scanning electron microscope (SEM) (LEO 430) at Birbal Sahani Institute of paleobotany, Lucknow.

Seed coat anatomy: For the anatomical observation of seed coat study take the transverse sections of seed coat. Using permanent slide preparation method or double staining method place the section on various alcohol grades like 30%, 50%,70%,90% absolute alcohol, xylene, DPX etc. The staining like safranine and light green stain used for staining.

Preliminary phytochemical tests: The preliminary phytochemical analysis is most important for detection of various chemical constituents. Trease and Evans (1989) test were done. Qualitative phytochemical analysis of the crude powder of the seeds of the plant for the identification of phytochemicals like alkaloids, carbohydrates, reducing sugars, steroids, glycosides, flavonoides, terpenoides, saponine, protein, tannins, amino acids, volatile oil or essential oil. Preliminary phytochemical test was done using different extract.

Thin layer chromatography: Using BAW (Butanol 80ml: Acetic acid 20ml: Water 20ml) solvent, aqueous extract with seed powder, TLC plate (MERCK)silica with aluminium sheet, capillary tube, chromatography chamber, lid, wax for sealing, spray, etc. use for chromatography. All the above biochemical and phytochemical methods were done by using Trease and Evans (1989), Sadashivam and Manikam (2005), Thimmaiah S.R. (1999), Harborne J.B., (1994) method.

Observations:

Morphologically Seed oval or circular, brownish, 0.66 mg, radial, hilum sub-basal, linear, surface smooth, glabrous with shallow depression near hilar region. The seeds pale brown, whitish deposition on brownish seed surface. Seeds are radial or suborbicular (Fig. 3, 4, 5). It has been used traditionally for the treatment of various ailments throughout the world. Extracts and purified metabolites from plants exhibit a range of bioactivities which can be exploited for drug development.

Figure 3: *Cuscuta reflexa* Roxb. red, brown seeds small seeds

Figure 4: *Cuscuta reflexa* Roxb. small, minute seeds, rounded or suborbicular shape

Figure 5: *Cuscuta reflexa* Roxb. seeds brownish, prominent hilum, whitish coating on surface

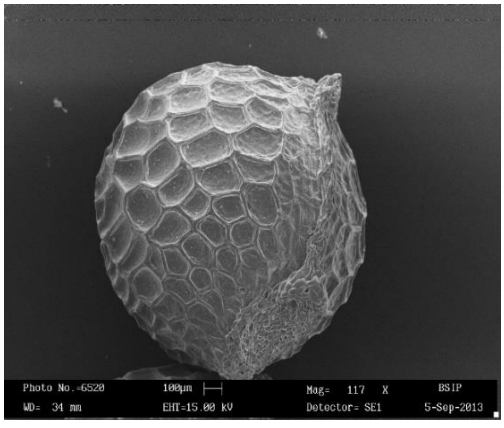

Figure 6: X 117 -Scanning electron microscopy of *Cuscuta reflexa* Roxb. seed. with suborbicular seed shape, pentagonal to hexagonal seed surface with prominent hilar region

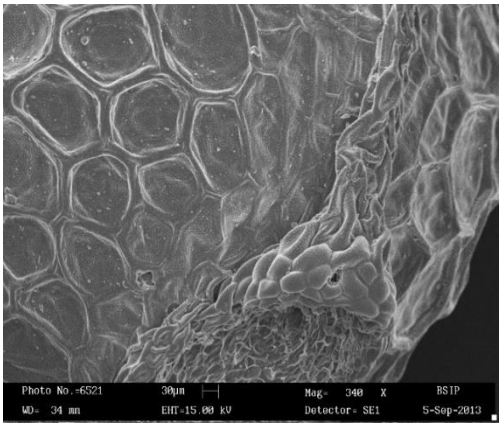

Figure 7: X 340 – Scanning electron microscopy of *Cuscuta reflexa* Roxb. seed. Surface with prominent cell wall, elongated thick mass near hilar region

Scanning electron microscopy (SEM) study shows particular pentagonal, hexagonal cellular network present on surface. The prominent hilar region with granulated, irregular thick mass deposited near hilum. Cell wall thick (Fig. 6, 7).

Figure 8: X 160 T.S. of *Cuscuta reflexa* Roxb. seeds. Seedcoat shows epidermis palisade endosperm tissue

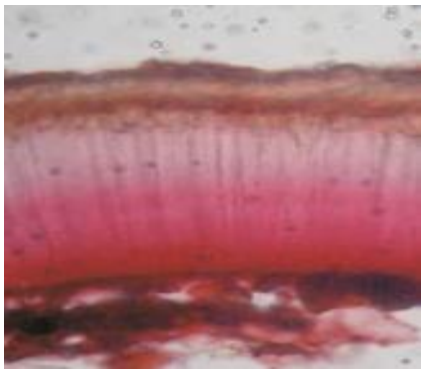

Figure 9: X 640 T.S. of *Cuscuta reflexa* Roxb. seeds

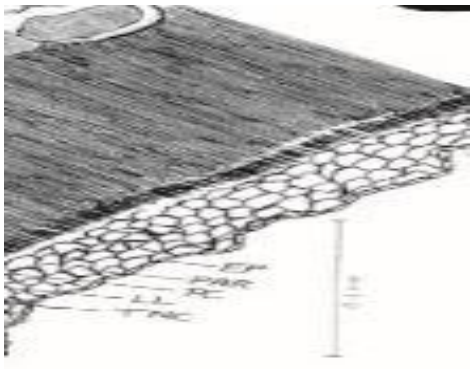

Figure 10: 400x -T.S. of *Cuscuta reflexa* Roxb. seeds coat shows Epidermis, parenchyma, palisade cell, aleurone grains, endosperm etc.

The internal structure of seedcoat (T.S) shows outer epidermis which is parenchymatous, below it palisades layer developed. The epidermal cell shows aleurone grains. Below the palisade tissue endosperm layer developed (Fig. 8, 9, 10). *Cuscuta reflexa* seeds are alterative, anthelmintic and carminative. Also used in the treatment of bilious disorder (Saini *et al.*, 2015).

Table 1: Priliminary phytochemical observation of various extracts of seeds of *Cuscuta reflexa* Roxb.

Sr no.	Test for active constituents	Ethyl acetate extracts	Ethanol extracts	Aqueous extracts	Methanol extracts
01	Alkaloids	-	+	+	-
02	Carbohydrates	-	+	+	+
03	Reducing sugars	-	-	-	-
04	Steroids	-	-	+	+
05	Glycosides	-	+	+	+
06	Flavonoids	+	+	+	-
07	Terpenoids	-	+	-	+
08	Saponine	+	-	+	-
09	Protein	-	+	-	-
10	Tannins	-	+	-	-
11	Amino acids	-	+	+	-
12	Volatile oil or essential oil	-	+	+	-

Present (+), Absent (-)

Preliminary phytochemical observations when seed powder treated with various chemicals like ethyl acetate, ethanol, aqueous, methanol it shows detection of various constituents. Carbohydrates detect in ethanol, aqueous, methanol extract, Glycosides detect in ethanol, aqueous, methanol extract, Flavonoids detect in ethyl acetate, ethanol, aqueous extract, Alkaloid detect in ethanol, aqueous extract, Steroid detect in aqueous,methanol, Terpenoid detect in ethanol, methanol, Saponin detect in ethyl acetate, aqueous extract, Amino acids detect in ethanol, aqueous extract, Volatile oil or essential oil detect in ethanol and aqueous extract, Protein**,** Tannin detect in ethanol, Reducing sugar not detected.

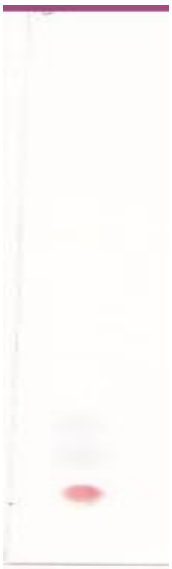

Figure 11: *Cuscuta reflexa* Roxb. seeds showing Thin layer chromatography for detection of 03 amino acids like DL-Alanine, DL-Ornithine monohydrochloride, DL-Valine

Thin Layer Chromatography done for detection of various amino acids present in it. *Cuscuta reflexa* Roxb. seeds showing Thin layer chromatography detect 03 amino acids like DL-Alanine, DL-Ornithine monohydrochloride, DL-Valine. The test shows presence of amino acids in *Cuscuta reflexa* Roxb. seeds (Fig. 11).

Discussion:

From the above observation it is seen that the seed of *Cuscuta reflexa* Roxb. seeds are highly useful for drug preparation. Various chemical constituents present in it. The micromorphological character were studied by scanning electron microscopy which magnify the seed surface view. The seed anatomy also focusses on cellular variation in seed also. Various extract detected alkaloids, carbohydrates, steroids, glycosides, flavonoids, terpenoids, saponine, protein, tannins, amino acids, volatile oil or essential oil etc. Plants used traditionally for treatment of various ailment in all over world. Extract and purified bioactive compounds exploited for drug development. The study gives identification of seeds, detection of various components in it for threptic efficacy of seeds. The thin layer chromatography also shows identification, detection of amino acids like DL-Alanine, DL-Ornithine monohydrochloride, DL-

Valine & analysis are most important for better research and various purposes, economic use also. Medicinal uses of a seeds were tested in using different formulations in research laboretories, pharmaceutical industries for their scientific, economic and beneficial use. Above study shows that the morphological, anatomical and phytochemical analysis of seeds of *Cuscuta reflexa* Roxb. is highly informative for taxanomical study and identification of seeds. Detection of various types of chemical constituents helps for drug preparation which is useful on various diseases.

Acknowledgement:

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STRATEGIES FOR PLANT DISEASE RESISTANCE

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In the intricate landscape of agricultural systems, the management of plant diseases has consistently posed significant challenges, impacting global food security and economic stability (Pieterse *et al.*, 2012). Over time, both farmers and scientists have worked diligently to develop effective strategies for combating crop diseases and mitigating their adverse effects on yields. Among these strategies, breeding plants for disease resistance has emerged as a fundamental aspect of sustainable agriculture, offering a proactive and enduring solution to the threats posed by pathogens (Van der Biezen and Jones, 2004).

A plant disease is defined by abnormalities in the structure or function of plant cells resulting from persistent irritation by pathogens or environmental factors (Agrios, 2005). These pathogens encompassing fungi, bacteria, viruses, and nematodes can severely impact crops, leading to considerable reductions in both yield and quality (Dean *et al.*, 2012).

Historically, the management of plant diseases predominantly relied on chemical pesticides and fungicides. However, the extensive use of these chemicals has introduced several concerns, including risks to human health, environmental degradation, and harm to non-target organisms (Mauch and Staehelin, 1989). Furthermore, the overuse of chemical treatments has facilitated the development of pathogen resistance, which diminishes the effectiveness of these methods over time (Liu *et al.*, 2014).

In response to these issues, there has been a notable shift towards employing advanced breeding techniques to cultivate crops with inherent disease resistance. By utilizing advances in genetics and molecular biology, researchers can identify and modify genes associated with disease resistance. This innovative approach has led to the development of resilient crop varieties capable of withstanding pathogen attacks more effectively (Haas and Lück, 2017)

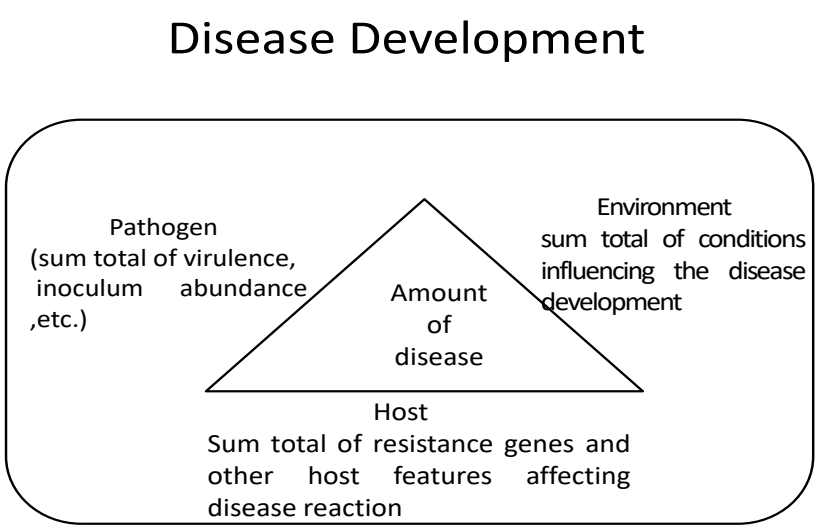
Disease development:

Understanding the development of plant diseases is essential for effective breeding strategies aimed at disease resistance. Plant diseases result from complex interactions between the host plant, the pathogen, and environmental factors. Each stage of disease progression offers opportunities for intervention through breeding techniques designed to enhance resistance (Jones and Dangl, 2006).

1. **Pathogen introduction:** The initial stage of disease development involves the entry of pathogens into the plant's environment. Pathogens can infiltrate plants via various routes such as soil, air, water, insects, and human activities (Agrios, 2005). To establish infection,

pathogens must bypass the plant's physical and chemical defenses (Mauch and Staehelin, 1989).

2. **Infection:** Successful infection occurs when the pathogen breaches the plant's defenses and enters its tissues. Pathogens utilize diverse mechanisms to overcome plant barriers, including physical penetration, secretion of cell wall-degrading enzymes, or exploiting natural openings like stomata (Dean *et al.*, 2012). Once inside, pathogens proliferate and cause cellular damage, disrupting normal physiological processes (Liu *et al.*, 2014).
3. **Disease establishment:** Following entry, pathogens establish themselves and spread within the plant, leading to visible symptoms such as leaf spots, wilting, necrosis, and growth stunting. The severity of the disease depends on factors including environmental conditions, host susceptibility, and pathogen virulence (Pieterse *et al.*, 2012).
4. **Systemic spread:** Some pathogens can disseminate systemically through the plant's vascular tissues, moving from the initial infection site to other parts of the plant. This systemic spread can exacerbate damage, affecting vital functions like water and nutrient uptake, and photosynthesis (Haas and Lück, 2017).
5. **Host response:** Plants have evolved sophisticated defense mechanisms to counter pathogen invasion. These include physical barriers like cell walls and cuticles, chemical defenses such as antimicrobial compounds, and specific immune responses like the hypersensitive response (HR) and systemic acquired resistance (SAR) (Ausubel *et al.*, 2006).
6. **Pathogen adaptation:** Over time, pathogens may evolve to overcome host defenses and adapt to changing environmental conditions. This evolutionary arms race requires ongoing monitoring of pathogen populations and adaptations in breeding strategies to effectively address emerging threats (Fenton *et al.*, 2011)



Disease resistance

Disease resistance in crop plants refers to their inherent ability to withstand or overcome the effects of pathogens or other damaging factors. Plant responses to pathogens can be categorized into several types, each reflecting different levels of resistance or susceptibility (Agrios, 2005).

1. **Susceptible:** Susceptible plants are highly vulnerable to specific pathogens and often exhibit severe disease symptoms upon exposure. These plants lack effective defense mechanisms against the pathogen, leading to significant disease development and damage (Dean *et al.*, 2012).
2. **Immune:** Immunity occurs when a host plant does not display symptoms of disease despite pathogen presence. This immunity can result from physical barriers, such as closed flower structures preventing spore entry, or from a hypersensitive reaction where a localized group of plant cells dies to limit pathogen establishment (Jones and Dangl, 2006). In hypersensitive reactions, the pathogen's reproduction rate within the plant is effectively zero ($r = 0$), preventing disease progression (Mauch and Staehelin, 1989).
3. **Resistant:** Resistant plants exhibit limited or no symptoms when infected by a pathogen. Resistance can be complete, where the pathogen fails to establish itself, or partial, where the plant shows reduced symptoms or delayed disease progression (Pieterse *et al.*, 2012). Resistance is often controlled by specific genes and is a key trait in breeding programs aimed at developing disease-resistant varieties (Van der Biezen and Jones, 2004).
4. **Tolerant:** Tolerant plants can become infected by a pathogen but display minimal damage or symptoms compared to susceptible plants. While they may still experience infection, they can endure the disease's effects without significant loss in yield or quality (Liu *et al.*, 2014).
5. **Moderately Resistant (MR)/Moderately Susceptible (MS):** Plants classified as moderately resistant or susceptible show an intermediate reaction to pathogen infection. They exhibit some level of resistance, which limits disease severity, but not as effectively as highly resistant plants. Conversely, their symptoms are less severe compared to susceptible plants (Fenton *et al.*, 2011).
6. **Asymptomatic Carrier (AC):** Asymptomatic carriers are plants that harbor pathogens without showing visible symptoms. Despite the absence of symptoms, these plants can still spread the pathogen to other susceptible hosts, serving as potential sources of disease dissemination (Haas and Lück, 2017).
7. **Necrotic (N):** Necrotic reactions involve tissue death at the infection site, resulting in necrotic lesions or overall tissue death. This response can be localized or systemic, depending on the pathogen and plant interaction (Agrios, 2005).

8. **Chlorotic (C):** Chlorotic reactions are characterized by yellowing of plant tissues due to disruptions in chlorophyll synthesis or function. Chlorosis can be caused by various pathogens, including viruses, bacteria, or nematodes, and can negatively impact plant growth and productivity (Dean *et al.,* 2012).

Types of disease resistance

Disease resistance in plants can be categorized into several types, each with distinct mechanisms and genetic controls. Understanding these categories is crucial for developing effective disease management strategies and breeding plants with improved resistance (Agrios, 2005). The primary types of disease resistance include:

1. **Non-host resistance:** Non-host plants are those that do not become infected by pathogens that typically affect other plant species. This resistance occurs because non-host plants lack the necessary conditions for pathogen growth and reproduction. Their resistance is a result of a combination of physical barriers, chemical defenses, and genetic factors that prevent pathogen establishment (Heath, 2000). Non-host resistance is highly effective against a wide range of pathogens, making it valuable for maintaining plant health in agricultural and natural ecosystems (Stahl *et al.,* 2009).
2. **True resistance:** True resistance, often conferred by specific resistance (R) genes, involves several key categories:

   (a) **Vertical (Qualitative) resistance:** Vertical resistance, also known as qualitative resistance, is controlled by one or a few major genes, commonly referred to as R genes. These genes provide strong, race-specific resistance to particular pathogens or strains. This resistance is characterized by a binary response: plants either exhibit complete resistance or full susceptibility. The interaction between R genes in the plant and corresponding avirulence (Avr) genes in the pathogen triggers defense mechanisms such as the hypersensitive response (HR) and localized cell death, leading to pathogen elimination (Jones and Dangl, 2006). However, pathogens can overcome vertical resistance through the evolution of new virulence traits (Bent and Mackey, 2007).

   (2) **Horizontal (Quantitative) resistance:** Horizontal resistance, or quantitative resistance, is controlled by multiple genes with small individual effects. This type of resistance provides broad-spectrum protection against a range of pathogens and is generally non-race-specific. Unlike vertical resistance, horizontal resistance is characterized by a continuous range of resistance levels, from highly susceptible to highly resistant. It is often more durable due to its polygenic nature and may involve various defense mechanisms, including enhanced cell wall fortification, production of antimicrobial compounds, and activation of systemic defense responses (Pilet *et al.,* 1990; Brueggeman *et al.,* 2015)

3. **Apparent resistance:** Apparent resistance refers to the plant's ability to avoid or tolerate infection by pathogens, often through mechanisms that do not directly involve pathogen exclusion or destruction.

   (1) **Disease escape:** Disease escape is a form of apparent resistance where plants avoid infection by escaping exposure to pathogens or their conducive environments. This can occur through several mechanisms:

   (i) Early maturity: Plants that mature quickly may avoid disease by completing their life cycle before pathogen pressure increases (Moss, 1975).

   (ii) Seasonal growth patterns: Plants that grow during periods of low pathogen activity or unfavorable environmental conditions for pathogen proliferation can reduce infection risk (Agrios, 2005).

   (iii) Spatial separation: Growing in locations with low pathogen populations or in microenvironments that are less conducive to pathogen survival can minimize infection (Heath, 2000).

   (iv) Diurnal or circadian rhythms: Some plants have growth rhythms that align with reduced pathogen activity, enhancing their resistance during specific times (Kempken *et al.,* 2003).

   (2) **Tolerance:** Tolerance is another form of apparent resistance where plants withstand pathogen infection without significant loss in growth or yield. Tolerance mechanisms include:

   (i) **Compensatory growth:** Tolerant plants may show increased branching, tillering, or root growth to compensate for pathogen-induced damage (Khan *et al.,* 2014).

   (ii) **Enhanced nutrient uptake:** Some tolerant plants have improved nutrient uptake or utilization to maintain growth in the presence of pathogens (McDonald and Linde, 2002).

   (iii) **Stress tolerance:** Tolerant plants may exhibit increased resilience to environmental stresses associated with disease, such as drought or salinity (Bertin *et al.,* 2015).

   (iv) **Reduced sensitivity to toxins:** Mechanisms to detoxify or sequester pathogen-produced toxins can reduce their impact on plant health (Cohen and Glogauer, 2016).

4. **Partial resistance:** Partial resistance, or incomplete resistance, provides an intermediate level of protection against pathogens, reducing disease severity and slowing disease progression compared to susceptible plants. Governed by multiple genes with moderate effects, partial resistance is effective against a broad range of pathogens. This type of resistance often involves a combination of pre-existing and inducible defense mechanisms, including physical barriers, antimicrobial compounds, and systemic defense responses (Nelson *et al.,* 2018).

5. **Induced resistance:** Induced resistance occurs when plants activate defense responses after exposure to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). This heightened state of readiness enhances the plant's ability to recognize and respond to subsequent pathogen attacks. Induced resistance can be triggered by various stimuli, such as beneficial microbes, chemical elicitors, and environmental factors (Bostock, 2005). It involves both local and systemic defense responses, providing enhanced protection against future infections.

Understanding these types of resistance is essential for developing effective disease management strategies and breeding programs aimed at improving plant health and productivity (Van der Biezen and Jones, 2004)

Steps in breeding for disease resistance

1. **Source of resistance**

Identifying and obtaining sources of resistance is the first critical step in developing disease-resistant plant varieties. Various sources of resistance can be utilized, including:

- **Known varieties:** Resistance can be sourced from existing commercial varieties that exhibit natural resistance to specific pathogens. Examples include:
  - **Cabbage yellows resistance:** Isolated from commercial cabbage varieties resistant to *Fusarium oxysporum* f. sp. *conglutinans*.
  - **Curly-top resistance in sugarbeets:** Identified in certain sugarbeet varieties.
  - **Mildew and leaf spot resistance in alfalfa:** Found in specific alfalfa cultivars.
- **Germplasm collections:** When resistance to new diseases or pathotypes is not available in cultivated varieties, screening germplasm collections becomes essential. Examples include:
  - **Net-blotch resistance in barley:** Sourced from germplasm collections.
  - **Wilt resistance in watermelon:** Identified in diverse germplasm pools.
- **Related species:** Resistance genes may be transferred from related species if the resistance is not present in the crop itself. For instance:
  - **Grassy stunt virus resistance in rice:** Transferred from *Oryza nivara*, a wild rice relative.
- **Mutations:** Resistance can be developed through spontaneous or induced mutations. Examples include:
  - **Victoria blight resistance in oats:** Induced by irradiation with X-rays or thermal neutrons.
- **Somaclonal variation:** Genetic variation occurring in plants regenerated from in vitro cultured cells can lead to new sources of resistance. For example:
  - **Ono variety of sugarcane:** A somaclone of variety Pindar resistant to Fiji disease.

- **Unrelated organisms:** Innovative approaches involve utilizing genes from unrelated organisms. Examples include:
  - **Coat protein gene of pathogenic viruses:** Used for virus resistance.
  - **Genes for novel phytoalexins:** Sourced from other plants for improved disease resistance.

2. Methods of breeding for disease resistance

Several breeding methods are employed to incorporate disease resistance into crop varieties:

- **Conventional breeding:** Involves crossing resistant varieties with susceptible ones and selecting progeny with enhanced resistance traits. This method relies on traditional selection techniques to integrate resistance genes into new cultivars.
- **Marker-Assisted Selection (MAS):** Utilizes molecular markers linked to resistance genes to identify and select resistant individuals more efficiently. MAS accelerate the breeding process by targeting specific genes associated with resistance (Ribaut and Ragot, 2007).
- **Genetic engineering:** Involves the direct manipulation of plant genomes to introduce or enhance resistance traits. Techniques include:
  - **Gene cloning:** Insertion of specific resistance genes into the plant genome.
  - **CRISPR/Cas9:** Gene editing to create plants with improved resistance by targeting specific genetic sequences (Voytas, 2013).
- **Biotechnological approaches:** Employs methods such as transformation with genes from other organisms to impart resistance. For example:
  - **Agrobacterium-mediated transformation:** Introduces foreign genes into plant genomes.

3. Testing for disease resistance

Testing is crucial to evaluate the effectiveness of resistance in breeding lines. This involves:

- **Field trials:** Conducting trials in natural environments to assess the performance of resistant varieties under realistic conditions.
- **Controlled environment testing:** Using growth chambers or greenhouses to expose plants to specific pathogens under controlled conditions to evaluate resistance.
- **Pathogen challenge tests:** Inoculating plants with pathogens to assess their resistance levels and identify effective resistance mechanisms.

4. Selection for resistant lines

The selection process focuses on identifying and advancing lines with desirable resistance traits. Steps include:

- **Screening:** Evaluating a large number of plant lines for resistance to specific pathogens.
- **Evaluation:** Assessing the severity of disease symptoms and pathogen impact on growth and yield.
- **Selection:** Choosing the best-performing lines with the highest levels of resistance for further breeding and development.
- **Reevaluation:** Continuously testing selected lines under varying conditions to ensure stability and durability of resistance.

By following these steps, breeders can develop new crop varieties with enhanced disease resistance, contributing to improved agricultural productivity and sustainability.

Methods of breeding for disease resistance

1. Plant introduction Plant introduction involves acquiring and testing resistant varieties from different regions or sources. This method is used to incorporate disease-resistant traits into local crop varieties.

- **Examples:**
  - **Kalyan sona and sonalika wheat:** These varieties, introduced from CIMMYT, Mexico, are known for their resistance to rust diseases. Their development illustrates the effectiveness of international collaborations in breeding programs (Ginkel *et al.*, 2012).
  - **Ridley wheat:** Introduced from Australia, this variety has been used for its rust resistance traits, showcasing how genetic resources from different regions can enhance crop resilience (McIntosh, 1992).
  - **Early groundnut varieties:** Varieties introduced from the USA demonstrated resistance to leaf spot or tikka disease, highlighting the utility of plant introductions for disease management (Amin, 1990).
  - **African pearl millet:** Introduced to develop downy mildew-resistant male sterile lines (Tift23A cytoplasm), crucial for hybrid seed production in India. This example illustrates the use of international germplasm for addressing specific regional challenges (Rai *et al.*, 2000).

2. Selection Selection involves isolating and propagating plants with desirable disease-resistant traits from existing varieties or sources. The method used depends on the plant's pollination system and propagation method.

- **Self-pollinated crops:**
  - **Mass selection:** Utilized for crops where individuals are selected based on resistance and then grown together.
  - **Pure line selection:** Involves selecting resistant individuals to establish a pure line with consistent resistance.

- **Example: Cotton (*Gossypium hirsutum*) variety MCU1:** Selected from Coimbatore 4 for its resistance to black-arm disease (Rao *et al.,* 1998).
- **Example: Kufri red potato:** Selected from Darjeeling Red Round for its resistance to diseases (Singh *et al.,* 2000).

- **Cross-pollinated species:**
  - **Mass selection:** Used for crops with cross-pollination, involving selecting resistant plants from a population.
  - **Recurrent selection:** Involves repeated cycles of selection and cross-breeding to enhance resistance.
    - **Example: Resistance to curly-top in sugarbeets:** Developed through selection from a diverse pool of genetic material (Miller *et al.,* 1995).
    - **Example: Resistance to mildew in alfalfa:** Achieved through recurrent selection to enhance resistance across generations (Miller *et al.,* 1995).
- **Vegetatively propagated crops:**
  - **Clonal selection:** Involves selecting and propagating disease-resistant clones from a population.
    - **Example: Pusa swani bhindi:** Selected from a collection in Bihar for resistance to yellow mosaic disease (Kumar *et al.,* 2010).

3. Hybridization Hybridization involves crossing plants with desirable resistance traits. The choice between methods (pedigree or backcross) depends on the genetic nature of resistance and the adaptation of the resistant variety.

- **Pedigree method:** Used when resistance is controlled by multiple genes and the resistant variety also has desirable agronomic traits.
  - **Examples: Kalyan sona, sonalika, and malviya wheat varieties:** Developed using pedigree methods from diverse sources to enhance rust resistance (Joshi, 2003).
  - **Example: *G. hirsutum* variety laxmi:** Developed for red leaf blight resistance from a cross between susceptible Gadag 1 and resistant Coimbatore Combodia 2 (Rao *et al.,* 1998).
- **Backcross method:** Used when resistance is governed by a few genes, often from related species or less agronomically desirable varieties.
  - **Example: Resistance to Grassy Stunt Virus (GSV) in rice:** Transferred from wild species Oryza nivara using backcross techniques (Harlan and de Wet, 1971).

4. Mutation Mutation techniques involve using physical or chemical agents to induce genetic changes, leading to disease resistance.

- **Induced mutations:** Achieved using mutagens such as X-rays or thermal neutrons.

- **Example: Victoria blight resistance in oats:** Induced by irradiation with X-rays or thermal neutrons, leading to new resistant varieties (Wicks and Jones, 1991).

- **Spontaneous mutants:** Rare mutations occurring naturally.
  - **Example: Resistance isolated from spontaneous mutants:** Found in various crops, demonstrating natural variation that can be exploited for breeding (Cox *et al.*, 2002).

5. Somaclonal variation Somaclonal variation involves genetic variation in plants regenerated from cultured cells, which can be screened for disease resistance.

- **Screening:** Plants regenerated from cell cultures are tested for disease resistance.
  - **Example: Ono sugarcane:** A somaclone of the variety Pindar, showing resistance to Fiji disease (Larkin and Scowcroft, 1981).
- **Cell selection:** Involves selecting cultured cells for resistance to toxins or pathogen filtrates and regenerating plants from these cells.
  - **Example: Cell selection in various crops:** Used to develop resistant varieties through targeted cell culture techniques (Oono *et al.*, 1996).

6. Genetic engineering Genetic engineering involves isolating, cloning, and transferring genes that confer disease resistance into crops.

- **Viral pathogens:** Transgenes such as virus coat protein genes and ribozymes.
  - **Example: Transgenic plants for viral resistance:** Incorporation of virus coat protein genes has been effective in enhancing resistance to various viral diseases (Leke *et al.*, 2008).
- **Bacterial and fungal pathogens:** Transgenes encoding enzymes, peptides, or phytoalexins.
  - **Example: Transgenic plants for bacterial and fungal resistance:** Includes genes for antibacterial peptides and lysozymes, demonstrating enhanced resistance in genetically engineered plants (Kumar *et al.*, 2008).
  - **Example: Resistance to bacterial blight in rice:** Achieved through genetic engineering of resistance genes (James, 2011).

Testing for disease resistance

Testing for disease resistance involves creating controlled conditions to distinguish resistant plants from susceptible ones. This process can be conducted in the field or in glasshouses, with glasshouse tests being more reliable due to the controlled environment (Ginkel *et al.*, 2012).

1. Soil-borne diseases

For soil-borne diseases, sick plots are created to test resistance. These plots are infected with pathogens by various methods:

- **Mixing soil from sick plots:** Soil from previously infected areas is mixed into the test plot.
- **Adding diseased plant remains:** Remains of diseased plants are incorporated into the soil to introduce the pathogen.
- **Laboratory-grown inoculum:** Pathogen inoculum produced on host seeds, seedlings, or nutrient media in a laboratory is added to the soil.
- **Growing susceptible varieties:** A susceptible variety is grown in the plot for one or more years to naturally increase the pathogen population (Rai *et al.*, 2000).

Glasshouse tests for soil-borne diseases use soil taken from these sick plots to maintain controlled conditions for disease development (Harlan and de Wet, 1971).

2. Air-borne diseases

Air-borne diseases, such as rusts, smuts, mildews, blights, and leaf spots, are caused by fungi that spread through the air. Testing methods include:

- **Dusting spores:** Spores from infected plants are dusted onto test plants.
- **Spraying spore suspensions:** A suspension of spores or mycelium is sprayed onto plants.
- **Injecting spore suspension:** A spore suspension is injected into individual plants or leaves.
- **Using infector rows:** Rows of highly susceptible varieties, known as infectors, are planted to produce large inoculum spread by wind or other natural forces (McIntosh, 1992).
    - **Example:** In wheat rust testing, infector rows are commonly used to create uniform disease pressure.

For fungi infecting ovaries, such as loose smut of wheat and barley, spores are introduced into the flowers during anthesis using forceps or a hypodermic needle (James, 2011).

3. Seed-borne diseases

Seed-borne diseases, such as certain smuts and wheat bunt, require specific testing methods:

- **Dusting dry spores:** Dry spores are dusted onto seeds before planting.
    - **Example:** For sorghum smut, this method is commonly used (Amin, 1990).
- **Soaking seeds in spore suspension:** Seeds are soaked in a suspension of pathogen spores under vacuum conditions to facilitate spore entry under the seed husk.
    - **Example:** This method is used for oat smut and covered smut of barley (Wicks and Jones, 1991).

4. Insect-transmitted diseases

Many viral diseases are transmitted by insect vectors, such as aphids and leafhoppers. Testing for these diseases involves:

- **Transferring infected insects:** Insect vectors feeding on diseased plants are collected and transferred to healthy test plants (Leke *et al.*, 2008).
- **Mechanical inoculation:** Juice extracted from diseased plant tissues is rubbed onto healthy plant tissues after inflicting mechanical injury to facilitate pathogen entry (Singh *et al.*, 2000).

Economic and environmental impact of disease-resistant crops

- Breeding disease-resistant crops offers significant economic benefits for farmers. Reduced reliance on chemical pesticides lowers production costs and minimizes financial risks associated with crop failures due to diseases. Moreover, higher yields from disease-resistant crops can lead to increased income for farmers, contributing to the economic stability of agricultural communities.
- Environmentally, disease-resistant crops play a crucial role in reducing pesticide use, which helps protect non-target organisms, including beneficial insects, birds, and soil microorganisms. This reduction in chemical inputs also contributes to soil health and reduces the risk of water contamination from pesticide runoff

Challenges and Limitations

- Breeding for disease resistance is not without challenges. Pathogens can evolve and develop new virulence traits, potentially overcoming plant resistance. Additionally, breeding for polygenic resistance, which involves multiple genes, can be complex and time-consuming. Ensuring the durability and stability of resistance in different environmental conditions also presents significant challenges.

Future directions in disease resistance research

Emerging technologies are revolutionizing the field of plant disease resistance:

- **Gene editing**: Techniques such as CRISPR/Cas9 enable precise modifications to plant genomes, enhancing resistance traits more efficiently (Voytas, 2013).
- **Genomic selection**: This approach uses genome-wide markers to predict and select for disease resistance traits, accelerating the breeding process (Ribaut and Ragot, 2007).
- **Big data and artificial intelligence**: Advanced data analytics and AI can help predict disease outbreaks and optimize breeding strategies by analyzing large datasets of plant health and environmental conditions.

Conclusion:

The management of plant diseases is crucial for global food security and economic stability. It has shifted from chemical pesticides to advanced breeding strategies for disease-

resistant crops. This proactive approach uses genetic and molecular advancements to create resilient varieties. Understanding disease development and resistance categories immune, resistant, tolerant, and asymptomatic guides effective breeding. Integration of conventional techniques, marker-assisted selection, genetic engineering, and biotechnological methods enhances resistance. Despite benefits like reduced pesticide use and environmental protection, challenges persist, including pathogen evolution and polygenic resistance. Future research in gene editing, genomic selection and AI promises further advancements, ensuring sustainable agricultural practices.

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ADVANCEMENTS AND CHALLENGES IN BREEDING SELF-POLLINATED CROPS: MECHANISMS, METHODS, AND FUTURE PROSPECTS

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Self-pollination, where a plant's flowers are fertilized by its own pollen, is a crucial reproductive strategy in many crops. This process promotes genetic uniformity and stability by ensuring the transfer of genetic material within the same plant. For agriculture, self-pollination is advantageous as it allows for the consistent production of uniform crops, which is vital for food security (Crow, 1998; Simmonds and Smartt, 1999).

Key self-pollinated crops include staples like wheat, rice, barley, peas, and beans. These crops are essential for human consumption, animal feed, and industrial uses. For instance, wheat is a primary food source globally, while rice is a staple for over half of the world's population, especially in Asia. Their self-pollinating nature ensures reliable yields, crucial for food security and economic stability (Duvick, 2005; Gupta and Varshney, 2020).

Moreover, self-pollinated crops are valuable in genetic and breeding research due to their stable genetic makeup. Studies on these crops have led to advancements in plant genetics, breeding techniques, and the development of new varieties with improved traits like disease resistance and drought tolerance (Brummer *et al.*, 2011; Gupta *et al.*, 2018). This chapter will review the breeding strategies for self-pollinated crops, covering both traditional methods and modern innovations that have impacted agriculture (Heffner *et al.*, 2009; Charpentier and Libermann, 2016)

1. Mechanisms of self-pollination

Self-pollination is a key reproductive strategy for many crops, characterized by the transfer of pollen from the anther to the stigma within the same flower or between flowers on the same plant. This process ensures fertilization without the need for external pollen vectors such as wind, insects, or animals. Self-pollination involves several biological processes and has significant genetic implications for the crops that utilize this method. Understanding these mechanisms is crucial for developing effective breeding strategies aimed at improving crop yield, quality, and resilience.

2. Biological processes involved in self-pollination

Self-pollination begins with pollen production in the anthers. Pollen grains, which contain male gametes, are formed through meiosis and mitosis, producing haploid cells with genetic variation. When the anther dehisces, it releases pollen grains that are transferred to the stigma via gravity, mechanical contact, or environmental factors like wind. Self-pollinated crops often have flower structures that facilitate this process, such as cleistogamy (non-opening flowers) and homogamy (synchronous maturation of anthers and stigmas). Environmental conditions, including temperature and humidity, can affect the efficiency of self-pollination (Simmonds and Smartt, 1999).

Upon landing on the stigma, pollen germinates and forms a pollen tube that grows down the style toward the ovule. This growth is directed by chemical signals and physical structures within the pistil. Sperm cells travel through the pollen tube to the ovule, where one sperm cell fertilizes the egg cell to form a zygote, while another sperm cell may fuse with additional cells to create endosperm, which nourishes the developing embryo. This process of double fertilization, unique to angiosperms, synchronizes seed development with fertilization.

3. Genetic implications of self-pollination

Self-pollination leads to high levels of homozygosity, resulting in genetically uniform populations. Each generation of self-pollination reduces progeny heterozygosity by half, rapidly fixing alleles. This genetic uniformity can preserve desirable traits but also limits genetic diversity, increasing susceptibility to diseases and environmental stresses (Crow, 1998). However, it also allows for the rapid stabilization of beneficial traits, which is advantageous in commercial agriculture for achieving consistent crop characteristics (Heffner *et al.*, 2009).

To counteract the limitations of reduced genetic diversity, breeding programs for self-pollinated crops use techniques such as controlled cross-pollination, mutation breeding, and advanced molecular methods like marker-assisted selection and genomic selection. These approaches introduce genetic variation while leveraging the stability and uniformity of self-pollination, accelerating the development of improved crop varieties (Gupta and Varshney, 2020).

4. Traditional breeding methods for self-pollinated crops

Breeding self-pollinated crops involves several traditional methods that have been refined over time to improve crop yield, quality, and resilience. The key methods include pure-line selection, mass selection, pedigree breeding, the bulk method, and single-seed descent. Each of these methods offers unique advantages and is suited to different breeding objectives and crop types.

4.1 Pure-line selection

Pure-line selection is one of the oldest and most straightforward breeding methods used for self-pollinated crops. It involves selecting the best-performing plants from a genetically heterogeneous population and propagating them to produce a homogeneous line.

1. **Identification of superior plants**: Initially, a diverse population of plants is grown, and individual plants exhibiting desirable traits such as high yield, disease resistance, and quality are identified.
2. **Isolation and propagation**: Seeds from these superior plants are collected and grown separately to maintain their genetic purity. This process is repeated for several generations until a stable, pure line is established.
3. **Advantages**: This method ensures uniformity and stability in the resulting crop, which is particularly beneficial for commercial farming where consistency is crucial.
4. **Limitations**: The primary limitation is the time required to develop a pure line, as it involves multiple generations of selection and propagation (Simmonds and Smartt, 1999).

4.2 Mass selection

Mass selection involves selecting a large number of superior plants from a population and using their seeds to form the next generation.

1. **Selection process**: The best-performing plants are identified based on phenotypic traits such as size, yield, and resistance to diseases and pests.
2. **Seed mixing and sowing**: Seeds from the selected plants are mixed and sown together to produce the next generation, retaining a broad genetic base.
3. **Advantages**: This method is simple, cost-effective, and maintains genetic diversity within the population, which can be beneficial for adapting to changing environmental conditions.
4. **Limitations**: The genetic improvement achieved through mass selection is gradual and may not result in as uniform a crop as pure-line selection (Johnson, 1987).

4.3 Pedigree breeding

Pedigree breeding involves the detailed tracking of the ancestry of selected plants over multiple generations to combine desirable traits from different parent plants.

1. **Initial crosses**: Parent plants with complementary desirable traits are cross-pollinated to produce hybrid seeds.
2. **Progeny selection**: The progeny from these crosses are evaluated, and plants exhibiting the best combination of traits are selected.

3. **Detailed record keeping**: The pedigree of each selected plant is meticulously recorded, allowing breeders to track the inheritance of traits and make informed selections in subsequent generations.
4. **Advantages**: Pedigree breeding allows for the combination of multiple desirable traits and provides a clear understanding of trait inheritance.
5. **Limitations**: It is labor-intensive and requires significant time and resources to manage and evaluate multiple generations (Crow, 1998).

4.4 Bulk method

The bulk method, also known as the population method, involves growing a large, genetically diverse population and allowing natural selection to occur within this population.

1. **Initial crosses**: Diverse parent plants are crossed to produce a heterogeneous population.
2. **Bulk propagation**: The population is grown together without selection for several generations, allowing natural selection to eliminate weak or less-adapted plants.
3. **Final selection**: After several generations, selection is applied to identify and propagate the best-performing plants.
4. **Advantages**: This method is less labor-intensive and allows for natural selection to contribute to the breeding process, which can enhance adaptability.
5. **Limitations**: The genetic improvement process is slow, and the resulting population may still be quite heterogeneous (Duvick, 2005).

Single-Seed Descent (SSD)

Single-seed descent is a method designed to accelerate the breeding process by advancing generations quickly without selection until later stages.

1. **Initial crosses**: Diverse parent plants are crossed to produce hybrid seeds.
2. **Rapid generation advance**: A single seed from each plant is selected and grown to produce the next generation, repeating this process for several generations without selection.
3. **Final selection**: After reaching the desired number of generations, plants are grown and evaluated, and selection is applied to identify the best-performing lines.
4. **Advantages**: SSD significantly reduces the time required to achieve homozygosity and allows breeders to quickly advance generations.
5. **Limitations**: The initial lack of selection means that undesirable traits may persist until the final selection stage, requiring careful evaluation and selection at that point (Gupta *et al.*, 2018).

5. Genetic basis of self-pollination

Self-pollination, a mechanism where pollen from a flower fertilizes the same flower or another flower on the same plant, is an essential trait in many crop species. This reproductive

strategy has profound implications for the genetic structure of populations and their evolution. Understanding the genetic basis of self-pollination involves examining the concepts of homozygosity, inbreeding depression, and genetic diversity.

5.1 Homozygosity and its advantages

Homozygosity refers to the presence of identical alleles at a given locus on both chromosomes. In self-pollinated crops, homozygosity arises due to the repeated self-fertilization over generations. This genetic uniformity can offer several advantages:

1. **Genetic stability**: Homozygosity ensures that the traits of a plant are consistent and stable, which is beneficial for maintaining desirable characteristics such as high yield or disease resistance. This stability is crucial for commercial crop production, where uniformity in crop performance is often a key requirement (Simmonds and Smartt, 1999).
2. **Predictable inheritance**: In homozygous lines, the inheritance of traits is predictable. This predictability simplifies breeding programs as it allows for accurate forecasting of trait expression in subsequent generations. For example, in wheat and rice breeding, homozygous lines can be developed to ensure uniform grain quality and yield (Gupta and Varshney, 2020).
3. **Ease of breeding**: Homozygous lines can be used directly in breeding programs to develop new varieties with fixed traits. This reduces the need for extensive selection processes and accelerates the breeding cycle (Duvick, 2005).

5.2 Inbreeding depression and genetic load

Despite its advantages, self-pollination can also lead to inbreeding depression and increased genetic load:

1. **Inbreeding depression**: Inbreeding depression occurs when self-pollination leads to the expression of deleterious recessive alleles, reducing the overall fitness of the plant. This can manifest as reduced vigor, lower yield, increased susceptibility to diseases, and other negative traits. Inbreeding depression is a common concern in self-pollinated crops and can limit the benefits of homozygosity (Heffner *et al.*, 2009).
2. **Genetic load**: The genetic load refers to the presence of deleterious alleles in the population. In self-pollinated crops, the accumulation of these alleles can be more pronounced due to the lack of genetic recombination that typically occurs in outcrossing species. Over time, this genetic load can reduce the overall fitness and adaptability of the population (White *et al.,* 2012).
3. **Mitigation strategies**: To mitigate inbreeding depression, breeders may use techniques such as cross-pollination with other lines or introduce genetic diversity through hybridization with related species. These strategies can help maintain or enhance the overall fitness and adaptability of the crop (Gupta *et al.*, 2018).

5.3 Genetic diversity in self-pollinated populations

Genetic diversity in self-pollinated populations is typically lower than in outcrossed populations. However, several factors influence the level of genetic diversity:

1. **Population size**: Larger populations tend to retain higher levels of genetic diversity compared to smaller populations. This is because larger populations are less susceptible to genetic drift, which can lead to the loss of genetic variation over time (Fiorani and Schurr, 2013).
2. **Mutation and selection**: While self-pollination reduces genetic variation through homozygosity, new genetic variations can still be introduced through mutations. Additionally, natural and artificial selection can act on these variations to maintain or increase genetic diversity within a self-pollinated population (Charpentier and Libermann, 2016).
3. **Breeding practices**: Breeding practices, such as the use of diverse parent lines or the incorporation of wild relatives, can help maintain or enhance genetic diversity in self-pollinated crops. By introducing new alleles and maintaining genetic variability, breeders can help ensure that crops remain adaptable to changing environmental conditions and evolving pests and diseases (Makarova *et al.,* 2011)

6. Contemporary breeding techniques

Modern advancements in plant breeding have significantly transformed the approach to improving crop varieties. Key contemporary techniques include Marker-Assisted Selection (MAS), Genomic Selection (GS), CRISPR/Cas9 and other gene-editing technologies, and High-Throughput Phenotyping. These methods enhance precision, efficiency, and speed in developing new crop varieties.

6.1 Marker-Assisted Selection (MAS)

Definition and process: Marker-Assisted Selection (MAS) involves using molecular markers linked to desirable traits to aid in the selection of plants. These markers are specific DNA sequences associated with genes that control traits such as disease resistance, yield, or stress tolerance (Gupta and Varshney, 2020). MAS enables breeders to identify and select plants with the desired genetic attributes early in the breeding process, even before the traits are physically expressed.

Advantages:

1. **Increased efficiency**: MAS accelerates the breeding process by allowing early selection of plants carrying desirable traits, reducing the time required to develop new varieties (Heffner *et al.,* 2009).

2. **Enhanced precision**: The use of molecular markers provides more accurate and reliable selection compared to traditional phenotypic selection, which can be influenced by environmental factors (Duvick, 2005).

Applications: MAS is widely used in breeding programs for crops such as wheat, rice, and maize, where it helps in selecting for traits like disease resistance, drought tolerance, and quality characteristics (Gupta *et al.*, 2018).

6.2 Genomic Selection (GS)

Definition and process: Genomic Selection (GS) uses genomic information to predict the breeding value of individuals. Unlike MAS, which focuses on specific markers, GS employs genome-wide marker data to estimate the genetic potential of a plant for multiple traits simultaneously (Fiorani and Schurr, 2013). This approach involves the use of statistical models to predict the performance of breeding candidates based on their entire genome.

Advantages:

1. **Comprehensive assessment**: GS provides a holistic view of the genetic potential of plants, considering all genetic variations rather than focusing on specific traits (Heffner *et al.*, 2009).
2. **Reduced breeding cycle time**: By predicting the performance of plants early in the breeding cycle, GS accelerates the development of new varieties and reduces the time required to achieve desired traits (Charpentier and Libermann, 2016).

Applications: GS is particularly valuable in crops with complex traits controlled by many genes, such as maize and barley. It helps in improving traits like yield, quality, and resilience by integrating data from entire genomes (Gupta *et al.*, 2018).

6.3 CRISPR/Cas9 and Other Gene-Editing Technologies

Definition and process: CRISPR/Cas9 is a revolutionary gene-editing technology that allows precise modification of DNA sequences. This method uses a guide RNA to target specific locations in the genome and the Cas9 enzyme to create double-strand breaks, which are then repaired in a way that introduces or removes genetic material (Makarova *et al.*, 2011). Other gene-editing technologies, such as TALENs and ZFNs, offer similar capabilities with varying degrees of precision and efficiency.

Advantages:

1. **Precision**: CRISPR/Cas9 allows for highly specific changes to the DNA sequence, minimizing unintended effects and off-target mutations (White *et al.*, 2012).
2. **Speed**: The technology enables rapid development of genetically modified plants with desired traits, reducing the time required for traditional breeding methods (Gupta and Varshney, 2020).

Applications: CRISPR/Cas9 has been used to create genetically modified crops with enhanced traits such as improved disease resistance, increased yield, and better nutritional quality. For instance, researchers have used CRISPR to develop rice varieties with enhanced resistance to bacterial blight and wheat varieties with improved resistance to fungal pathogens (Charpentier & Libermann, 2016).

6.4 High-throughput phenotyping

Definition and process: High-Throughput Phenotyping (HTP) involves the use of advanced technologies to rapidly and accurately measure phenotypic traits across large numbers of plants. Techniques such as imaging, robotics, and sensors are employed to collect data on traits such as growth, yield, and stress responses (Fiorani and Schurr, 2013). This data is then analyzed to evaluate plant performance and select the best candidates for breeding.

Advantages:

1. **Data richness**: HTP provides extensive and detailed phenotypic data that can be correlated with genotypic information to improve breeding decisions (Gupta *et al.,* 2018).
2. **Efficiency**: The automation and scale of HTP reduce the time and labor involved in measuring plant traits, allowing for more efficient breeding processes (Heffner *et al.,* 2009).

Applications: HTP is used in various crops to assess traits such as drought tolerance, disease resistance, and yield potential. It supports the identification of superior plants and accelerates the development of new varieties with enhanced performance (Simmonds and Smartt, 1999).

7. Challenges in breeding self-pollinated crops

Breeding self-pollinated crops presents unique challenges that stem from their genetic and reproductive characteristics. Addressing these challenges is critical for improving crop yield, quality, and resilience. Key challenges include maintaining genetic diversity, overcoming inbreeding depression, and enhancing disease resistance and environmental stress tolerance.

7.1 Maintaining genetic diversity

Challenge: One of the primary challenges in breeding self-pollinated crops is maintaining genetic diversity within the breeding population. Self-pollinated crops inherently tend to have a high degree of homozygosity due to their reproductive strategy, which can limit the introduction of new genetic variation (Crow, 1998). This low genetic diversity can reduce the ability of breeding programs to respond to changing environmental conditions or to develop new varieties with improved traits.

Strategies to address the challenge:

1. **Cross-pollination:** Introducing controlled cross-pollination through techniques such as hybridization or crossing with genetically diverse varieties can help increase genetic diversity (Johnson, 1987).

2. **Genetic resources utilization:** Using germplasm collections and wild relatives to introduce new alleles into the breeding population can enhance genetic diversity (Simmonds and Smartt, 1999).
3. **Gene banks:** Maintaining and utilizing gene banks that store diverse genetic material from various populations can provide valuable resources for breeding programs (Gupta and Varshney, 2020).

7.2 Overcoming inbreeding depression

Challenge: Inbreeding depression is a reduction in fitness and vigor that occurs when closely related plants breed, leading to the expression of deleterious recessive alleles. This phenomenon is particularly pronounced in self-pollinated crops, where the same genetic material is repeatedly passed down through generations (Duvick, 2005). Inbreeding depression can lead to decreased yield, poor quality, and reduced adaptability to stress.

Strategies to address the challenge:

1. **Pedigree and bulk selection:** Employing breeding methods such as pedigree and bulk selection can help reduce the effects of inbreeding depression by introducing new genetic material and selecting for overall plant health (Heffner *et al.,* 2009).
2. **Hybridization:** Using hybridization strategies, including the development of hybrid varieties, can introduce new genetic combinations and mitigate inbreeding depression (White *et al.,* 2012).
3. **Genomic tools:** Applying genomic selection and molecular markers to identify and select individuals with reduced inbreeding depression can help manage and mitigate its effects (Gupta *et al.,* 2018).

7.3 Disease resistance and environmental stress tolerance

Challenge: Self-pollinated crops must be resilient to various diseases and environmental stresses to maintain yield and quality. The challenge lies in breeding for these traits while ensuring that the genetic improvements do not lead to unintended negative consequences. Additionally, the reduced genetic diversity in self-pollinated crops can limit their ability to adapt to new or evolving stressors (Fiorani and Schurr, 2013).

Strategies to address the challenge:

1. **Trait mapping and marker-assisted selection:** Identifying and mapping genes associated with disease resistance and stress tolerance, followed by using marker-assisted selection to incorporate these traits into new varieties, is a crucial strategy (Gupta *et al.,* 2018).
2. **Functional genomics:** Employing functional genomics to understand the mechanisms underlying stress responses and resistance can aid in developing more resilient varieties (Charpentier and Libermann, 2016).

3. **Integrated pest and stress management:** Combining breeding efforts with integrated pest and stress management practices can improve the overall resilience of crops (Simmonds and Smartt, 1999).

Addressing these challenges requires a multifaceted approach involving advanced breeding techniques, strategic use of genetic resources, and the integration of new technologies. By maintaining genetic diversity, managing inbreeding depression, and enhancing disease resistance and stress tolerance, breeders can develop self-pollinated crop varieties that are more productive, resilient, and adaptable to changing environmental conditions.

8. Innovations and future prospects

As agriculture evolves to meet the demands of a growing global population and the challenges posed by climate change, innovations in breeding self-pollinated crops are increasingly important. Key advancements include integrating omics technologies, precision breeding and digital agriculture, and developing climate-resilient breeding strategies. These innovations promise to enhance crop productivity, adaptability, and sustainability.

8.1 Integrating omics technologies

Genomics: Genomics involves the comprehensive study of an organism's entire genome. In self-pollinated crops, genomic tools facilitate the identification of genes associated with desirable traits such as yield, disease resistance, and stress tolerance. Techniques such as genome-wide association studies (GWAS) and whole-genome sequencing provide insights into the genetic architecture of crops, enabling breeders to select for beneficial traits more efficiently (Gupta and Varshney, 2020).

Transcriptomics: Transcriptomics focuses on the study of RNA transcripts produced by the genome. By analyzing gene expression patterns, researchers can identify key genes involved in various biological processes and stress responses. This information is crucial for understanding how self-pollinated crops react to different environmental conditions and for developing varieties with improved performance under stress (Fiorani and Schurr, 2013).

Proteomics: Proteomics is the large-scale study of proteins, which are the functional products of genes. In self-pollinated crops, proteomic analyses can uncover the role of specific proteins in growth, development, and stress responses. This knowledge aids in identifying biomarkers for breeding and understanding the molecular mechanisms underlying crop traits (Charpentier and Libermann, 2016).

Metabolomics: Metabolomics involves the study of metabolites, which are small molecules produced during metabolic processes. By profiling metabolites, researchers can gain insights into the biochemical pathways active in self-pollinated crops and identify key metabolites associated with stress resistance, quality, and yield (Gupta *et al.,* 2018). This approach complements

genomic and proteomic studies by providing a comprehensive view of the crop's physiological state.

8.2 Precision breeding and digital agriculture

Precision breeding: Precision breeding utilizes advanced technologies to enhance the efficiency and accuracy of breeding programs. Techniques such as marker-assisted selection (MAS) and genomic selection (GS) allow for the precise identification of desirable traits and their incorporation into breeding lines. These methods reduce the time and resources required to develop new varieties and increase the precision of trait selection (Heffner *et al.,* 2009).

Digital agriculture: Digital agriculture integrates technologies such as sensors, drones, and satellite imagery to collect and analyze data on crop performance and environmental conditions. This data-driven approach enables breeders to make informed decisions about crop management and breeding strategies. By combining digital tools with breeding techniques, researchers can optimize the selection process and enhance the overall efficiency of breeding programs (Fiorani and Schurr, 2013)

8.3 Climate-resilient breeding strategies

Climate-resilient breeding: As climate change impacts agriculture, developing climate-resilient crop varieties becomes increasingly critical. Breeding for climate resilience involves selecting for traits that enhance a crop's ability to withstand extreme weather conditions, such as drought, heat, and flooding. This includes identifying and incorporating genetic variations that confer tolerance to these stresses into breeding programs (Gupta *et al.,* 2018).

Strategies:

1. **Adaptive trait selection:** Selecting for traits such as deep rooting, efficient water use, and heat tolerance can improve crop resilience to climate variability (Duvick, 2005).
2. **Genetic engineering:** Utilizing genetic engineering techniques to introduce genes associated with stress tolerance can accelerate the development of climate-resilient varieties (Makarova *et al.,* 2011).
3. **Breeding for diversity:** Incorporating genetic diversity from wild relatives and landraces into breeding programs can enhance the adaptive capacity of self-pollinated crops to changing environmental conditions (White *et al.,* 2012).

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ASSESSING THE IMPACT OF NOISE POLLUTION IN NANDED CITY: SOURCES, EFFECTS, AND SOLUTIONS

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Abstract:

The study examines the problem of noise pollution in the wake of its ill effects on people's lives. A cross-sectional survey of the population in Nanded city reveals that the main sources of noise pollution are loudspeakers and automobiles. Interestingly, the female population is slightly more affected by religious noise than the male population. Major effects of noise pollution include interference with communication, sleeplessness, and reduced efficiency. Extreme effects, such as deafness and mental breakdown, cannot be ruled out. Generally, the aggrieved parties make requests to reduce or stop the noise. However, complaints to the administration and police have also been considered as a way of addressing this issue. Public education appears to be the most effective method for combating noise pollution, as suggested by the respondents. Additionally, the government and NGOs can play a significant role in this process.

Keywords: Pollution. Human Health. Noise Standards. Social and Religious Ceremonies. Noise Effects Noise Reduction. Public Eduducation

Introduction:

Noise is derived from the Latin word “nau-sea,” implying 'unwanted sound' or 'sound that is loud, unpleasant, or unexpected.' It originates from human activities, especially urbanization, and the development of transport and industry. Although the urban population is more affected by such pollution, small towns and villages alongside roads or industries are also victims of this problem. Noise is becoming an increasingly omnipresent, yet unnoticed, form of pollution, even in developed countries.

According to Birgitta and Lindvall (1995), road traffic, jet planes, garbage trucks, construction equipment, manufacturing processes, and lawn mowers are some of the major sources of this unwanted sound routinely broadcast into the air. Though noise pollution is a slow and subtle killer, very little effort has been made to ameliorate it. Along with other types of pollution, noise has become a hazard to the quality of life. Kiernan (1997) finds that even relatively low levels of noise adversely affect human health. It may cause hypertension, disrupt sleep, and hinder cognitive development in children. The effects of excessive noise could be so

severe that it might lead to permanent memory loss or psychiatric disorders (Bond, 1996). Thus, there are many adverse effects of excessive noise or sudden exposure to noise.

In India, the problem of noise pollution is widespread. Several studies report that noise levels in metropolitan cities exceed specified standard limits, contributing to the rising incidence of deafness among the inhabitants (Bhargawa, 2001). A study by Singh and Mahajan (1990) conducted in Delhi and Calcutta found that the noise level is 95dB, far above the ambient limit of 45dB. Even in "calm" places, it does not fall below 60dB. Murli and Murthy (1983) also found that traffic noise in Visakhapatnam exceeds 90dB even in the morning hours, acting as a source of nuisance.

Noise pollution is not a unique problem for developing countries like India. In China, until the third century B.C., noise was used to torture criminals instead of hanging them for dangerous crimes. The effects of noise are worrisome enough that it is considered second only to crime by certain countries (Kapoor and Singh, 1995). Bond (1996) reports that 16% of people in Europe are exposed to 40dB or more of traffic noise in their bedrooms at night, compared to W.H.O.'s average estimates of 30 to 35dB for undisturbed sleep.

Several initiatives have been taken by various countries to check noise levels. For example, the USA has created sites where human-caused noise pollution will not be tolerated (Geary, 1996). Similarly, the European Union requires that 'noise maps' of big cities with more than 250,000 inhabitants be drawn up by 2002 (New Scientist, 1998). To safeguard against the ill effects of noise, the laws of the Netherlands do not permit the building of houses in areas where 24-hour average noise levels exceed 50dB. In Great Britain, the Noise Act empowers local authorities to confiscate noisy equipment and fine people who create excess noise at night. Recently, several countries have also invested in 'porous asphalt' technology, which can curtail traffic noise by up to 5dB.

The movement against noise pollution is weak in India. Most people do not consider it a pollutant and accept it as a part of routine life. However, it has recently been recognized as a pollutant (Negi *et al.*, 1999). In India, the Noise Pollution (Regulation and Control) Rules, 2000, have been framed under the Environment (Protection) Act, 1986. These rules provide guidelines for the regulation and control of noise, with specified ambient noise levels for different areas/zones, as indicated in the chart.

Chart 1: Ambient noise standards

Area Code	Category of Area/ Zone	Day Time Limits in dB	Night Time Limits in dB
(A)	Industrial Area	75	70
(B)	Commercial Area	65	55
(C)	Residential Area	55	45
(D)	Silence Zone	50	40

The limit in decibels (dB) denotes the time-weighted average of the sound level on Scale A, which is relatable to human hearing. Source: Environment (Protection) Act, 1986, as amended in 2002.

A survey by the Central Pollution Control Board (CPCB) shows that in Delhi, noise levels in most places exceed the permissible limits (The Times of India, New Delhi). Similarly, a study by the National Environmental Engineering Research Institute (NEERI) revealed that noise levels in residential, commercial, and industrial areas, as well as silent zones in Delhi and the National Capital Region (NCR), far exceed the prescribed standards. The average noise level in Delhi is 80 dB, while the ambient limit is 55 dB (The Business Line, New Delhi). Bombay also suffers from high levels of noise pollution. For example, Shetye *et al.,* (1980) estimated that noise levels in crowded locations in Bombay were almost double the residential standards adopted by most countries (45 dB during the day and 35 dB at night).

Evidently, noise pollution has reached alarming proportions, adversely affecting the efficiency of various populations, mental health, and overall quality of life. It is also becoming a law and order issue, with an increasing number of complaints to the police and administration. Unless measures are taken to control noise levels, ongoing urbanization and industrialization may exacerbate the problem to the point of becoming incurable.

Several methods can be utilized to control noise levels:

1. **Design and technology:** Altering the design and technology of machines and equipment to reduce noise emission.
2. **Noise Barriers:** Installing barriers to control noise spread.
3. **Soundproofing:** Protecting sound receptors with shields, such as insulating buildings and soundproofing body and window panes.

In addition to technological solutions, regulating the behavior of machine and equipment users can also help. While a legal framework could be enforced to regulate users of vehicles and equipment, it requires substantial resources and effective governance. Public education appears to be a promising option, as sheer ignorance about the adverse effects of noise pollution is a key factor in the inadequate stress on controlling or reducing noise levels.

To make India a world-class destination for tourism, industry, and healthy living, there is an urgent need for the development and implementation of a comprehensive noise control program. This study identifies the sources of noise pollution, explores its effects on the public and their reactions, and contemplates various measures to control the pollution. The empirical evidence gathered through this study can be employed to develop appropriate legal and public action programs.

Methodology:

This empirical study is based on a sample survey conducted in Nanded. A total of 150 respondents were interviewed personally. The sample represents a cross-section of different age groups, genders, geographic locations, educational levels, and income levels of respondents, making it a representative sample for this exploratory study. Nanded was selected for the study because it is one of the most populous cities in India, reflecting both modern and traditional infrastructure (roads, localities, buildings, etc.). Moreover, its inhabitants represent a cross-section of Indian culture.

Data was collected using a structured questionnaire that included both closed and open-ended questions. The analysis was carried out using percentages and cross-classifications, focusing on sources of noise, effects of noise, reactions to noise, and suggestions for controlling noise, categorized by age and gender.

Results:

Sources of noise:

Sources of noise pollution include, inter alia, vehicular traffic, neighborhood disturbances, electrical appliances, TV and music systems, public address systems, railway and air traffic, and generating sets. Even household equipment contributes to the noise. Most people living in metropolitan cities or big towns and those working in factories are susceptible to the adverse effects of noise. This issue affects both the rich and the poor alike. Noise pollution is less severe in small towns and villages, but those residing in towns or villages along national/state highways or close to railway tracks experience higher levels of excessive noise. Indiscriminate use of vehicle horns and the widespread use of loudspeakers in Indian social and religious ceremonies cause several health hazards to urban inhabitants. These include deafness, nervous breakdown, mental disorders, heart troubles, high blood pressure, headaches, dizziness, inefficiency, and insomnia (Bhargawa, 2001).

The noise level and exposure area depend on its source and strength. Road noise, especially at some distance from the road, can be described as steady-state noise that does not fluctuate much. In contrast, rail and aircraft noise are characterized by high noise levels of relatively short duration. Noise from industrial installations, construction sites, and fixed recreation facilities radiates from a point source, generally forming a circular exposure area. Noise from various sources may either be steady for a long period or fluctuate significantly over time.

Road traffic is a key source of noise in big cities. The speed and exhaust system of vehicles determine the noise released by road traffic. The contact between tires and the road surface is a dominant source of noise at speeds above 60 km/h for light vehicles. In the future, tire-to-surface noise is likely to become an important issue in noise abatement strategies. In

urban areas, fast acceleration and restarting engines in traffic can result in emissions up to 15 dB higher than normal levels from smooth driving.

Another major source of noise is public address systems used in temples, mosques, etc. The Indian Constitution under Article 25-28 guarantees freedom of religion, but this freedom is not absolute. It is subject to public order, health, and morality. In a recent decision, the Supreme Court held that no religion prescribes the use of loudspeakers or drums for prayers. It was further held that if religious groups use such equipment, it should not affect others' rights. The High Court of Tamil Nadu allowed a petition filed by the Welfare Association of KKR Nagar (Chennai) against a church and directed that the noise level should not exceed permissible decibels. Thus, the state can impose restrictions on institutions to maintain public health.

Since noise disturbs living conditions and negatively impacts health, restrictions imposed by a state on noise levels do not amount to a violation of fundamental rights. The analysis (Table 1) indicates that a very large proportion of respondents in each age group is affected by noise from loudspeakers, ranging from 71% to 86%, with an overall percentage of 83%. However, the percentage of affected individuals in the 20-40 year age group is marginally lower.

Table 1: Sources of noise in terms of age groups

Source of Noise	Up to 20 Age groups	20-40 Age groups	40-60 Age groups	Above 60 Age groups	Total
Loud speaker	30	34	35	22	121
Automobiles	24	30	33	17	104
Neighborhoods	20	20	21	12	73
Religious functions	22	28	24	15	89
Total respondents	34	49	43	24	150

A similar situation is observed with automobiles. The majority of respondents across different age groups feel that automobile noise affects their activities. A relatively small proportion of respondents (54% across various age groups) acknowledge the adverse effects of noise generated by neighborhoods. An almost equal proportion of respondents (58%) across different age groups claim that noise originating from religious functions affects them.

In general, apart from loudspeakers and automobiles, religious functions and neighborhood disturbances act as significant sources of noise pollution. Thus, metropolitan cities are becoming victims of a new class of pollution—noise. Further, we examine whether sources of noise pollution affect male and female populations differently.

Table 2 presents figures and percentages of male and female respondents affected by different sources of noise. There are marked differences in the population affected by noise from religious functions, with women being more affected than men. For other sources, such as loudspeakers, automobiles, and neighborhood noise, there is no significant difference in the

percentages of male and female respondents affected. This means that other sources of noise affect both male and female populations equally.

Effects of noise:

There is no doubt that noise adversely affects human health. It can result in hearing loss, stress, high blood pressure, loss of sleep, distraction affecting productivity, and a general reduction in the quality of life. The effects of noise are difficult to quantify because tolerance levels among different populations and types of noise vary considerably. Scientific literature extensively assesses the effects of noise on humans. Indiscriminate use of vehicle horns and widespread use of loudspeakers in Indian social and religious ceremonies have caused several health hazards to urban inhabitants, including deafness, nervous breakdowns, mental disorders, heart troubles, high blood pressure, dizziness, and insomnia (Bhargawa, 2001).

Exposure to noise pollution exceeding 75 decibels for more than eight hours daily over a long period can cause hearing loss. The hazards increase with the intensity of the noise and the duration of exposure. For instance, the sound from a bursting firecracker exceeding 150 dB can cause a ringing sensation called 'tinnitus' and may impair hearing permanently. Approximately 1% of the population suffers from noise-induced hearing loss. Nagi *et al.,* (1993) found that noise levels from household equipment and appliances sometimes reach up to 97 dB, which is more than double the acceptable noise level of 45 dB. This excessive noise can cause several ill effects, including annoyance, speech interference, sleep disturbance, mental stress, headaches, and lack of concentration.

Similarly, Singh (1984) noted that workers exposed to high noise levels have a higher incidence of circulatory problems, cardiac diseases, hypertension, peptic ulcers, and neurosensory and motor impairment. The adverse effects of noise also extend to birds (e.g., robins, sparrows, wrens, and blackbirds). Birds living near busy roads cannot communicate effectively for propagation (Deutche Presse-Agentur, 2003).

Table 2 shows that noise interferes with communication, disturbs sleep, and reduces individual efficiency. The majority of respondents exposed to noise pollution report annoyance and hearing problems. As many as 35% reported deafness, and almost an equal number reported mental breakdowns. The survey data indicates that the effects of noise are not uniform across various age groups. Generally, older individuals bear the brunt of excessive noise pollution. For example, a rising proportion of respondents in higher age groups acknowledge issues such as depression, sleeplessness, and hearing impairment. A large proportion of respondents feel that noise interferes with interpersonal communication and causes annoyance. Extreme effects, such as mental breakdown and deafness, are acknowledged by one-third of the survey population. However, there is a much higher incidence of deafness among older people (above 60 years of

age). Additionally, psychosomatic disorders (e.g., depression, sleep issues) and physiological disorders (e.g., deafness) are acknowledged by a smaller proportion of respondents.

Table 2: Effect of noise on different age groups

Effect of noise	Age Groups 0-20	Age Groups 20-40	Age Groups 40-60	Above 60	Total
Effect on hearing	20	31	32	24	107
Interfere with communication	36	44	40	21	141
Cause annoyance	33	30	35	18	116
Disturb sleep	37	43	40	12	132
Result in deafness	10	14	18	10	52
Mental breakdown	9	16	18	7	50
Total	34	49	43	24	150

Conclusion:

This research paper explores the sources, effects, reactions, and suggestions for controlling excessive noise pollution. The major sources identified are automobiles and public address systems (loudspeakers). Notably, loudspeakers are frequently used for religious functions, including temple prayers. Disturbances caused by loudspeakers and automobiles are somewhat less felt by the 20-40 years age group compared to other age groups. Across various age groups, there is an almost equal proportion of respondents reporting neighborhood noise, music, and religious functions as sources of noise. There are no significant variations between male and female populations; the proportions affected by each source of noise are similar for both genders.

The survey indicates that noise affects individuals in multiple ways, including impaired communication, sleeplessness, and reduced efficiency. Psychosomatic effects, such as annoyance and depression, are common; however, extreme effects like deafness and mental breakdown are also observed. In most cases, the affected parties request that the noise be stopped. A substantial proportion of respondents from various age groups complain to the administration. Interestingly, about one-third of young people (below 20 years) prefer to quarrel with the offending party.

Public education emerges as the most favored method for addressing noise pollution, as suggested by the respondents. However, government and NGOs can also play a significant role in the process.

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PHARMACOLOGICAL ACTIVITIES OF LEAVES OF *PHASEOLUS VULGARIS* LINN. A COMPRESSIVE REVIEW

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Abstract:

The French bean (*Phaseolus vulgaris* Linn.), which is widely cultivated and commonly accessible, is a vegetable plant that is commonly encountered. The seeds from this plant contain bioactive components, including essential oils, alkaloids, flavonoids, and polyphenols, which are responsible for its medicinal properties. The French bean is a member of the Fabaceae family, and its seeds have been found to possess antioxidant properties, as well as anti-inflammatory, anticancer, and antidiabetic effects, among other pharmacological properties. Moreover, the seeds are a rich source of nutritional value. The present review aims to highlight the medicinal relevance of the leaves of *Phaseolus vulgaris* Linn. in light of the limited research conducted in this area. This review offers a comprehensive overview of the pharmacological activities of the leaves of *Phaseolus vulgaris* Linn., as it is a noteworthy herbal remedy with a plethora of pharmacological properties that warrant further exploration.

Keywords: *Phaseolus vulgaris* Linn, Leaves, Antidiabetic, Anti-microbial

Introduction:

Plants have been utilized for medicinal purposes since ancient times. The French bean, or *Phaseolus vulgaris* Linn., is a commonly grown vegetable plant that belongs to the Fabaceae family. Originating from America, it is now found in warm countries such as England, India, and Pakistan. The leaves and seeds of the plant have been used in various dishes, including salads, daal, and sabji, for centuries. (Padmavathi *et al.,* 2021) The seeds of the French bean are rich in nutrients and contain bioactive components, such as flavonoids, alkaloids, phenols, and coumarin derivatives, which are responsible for the plant's pharmacological activity. *Phaseolus vulgaris* is a plant belonging to the Plantae kingdom and the Fabaceae family. It is a significant crop globally due to its agricultural importance. This species belongs to the angiosperm division (Magnoliophyta) and the dicotyledon class (Magnoliopsida) and is characterized by flowering plants with two seed leaves. It is included in the order Fabales, which comprises various leguminous, nitrogen-fixing plants. The beans of the common bean plant have been found to possess a range of biological activities, including antioxidant, anticancer, antidiabetic, anti-inflammatory, anti-depressant, and antihypertensive properties. (Devi *et al.,* 2020)

The current state of research on the medicinal value of the leaves of this particular plant is limited due to a lack of extensive study. However, research is ongoing to investigate the bioactivity of the plant leaves. The study of the bioactivity of widely available plants has the potential to yield valuable bioactive compounds that are readily accessible, sustainable, and economically beneficial. This not only holds the potential to advance scientific knowledge and industry, but also to provide more affordable and effective healthcare solutions. Therefore, the exploration of the plant leaves is not only beneficial for the advancement of scientific knowledge, but also for the economy and healthcare.

Synonyms for botanical name:

The botanical name for the plant is *Phaseolus vulgaris* Linn, and it has a number of synonyms, including *Phaseolus aborigineus Burkart, Phaseolus nanus L., Phaseolus esculentus Salisb., Phaseolus compressus DC, and Phaseolus communis Pritz.* (Devi *et al.,* 2020)The following text provides synonyms for the common bean, which is scientifically known as *Phaseolus vulgaris*. This species has been referred to by various historical or classificatory names, which reflect its widespread cultivation and importance.

Vernacular name:

Phaseolus vulgaris, commonly known as the common bean, is a versatile plant with various names across different languages and regions, reflecting its widespread cultivation and importance. Commonly known as the common bean, this versatile plant has been given different names in English, including Kidney bean, Snap bean, Green bean, Dry bean, and String bean. In India, it is called Rajma in Hindi, Lal lobia in Urdu, Barbati Beej in Bengali, Chikkuduginjalu in Telugu, and Sigappu Kaaramani in Tamil. In Europe, it is known as Fagiolo in Italian, Haricot commun in French, and Gartenbohne in German. In Spanish-speaking regions like Venezuela, it is called Caraota, while in Portuguese, it is referred to as Feijão for the dry variety and Feijão-vagem for the green variety. In Malayalam, it is simply called Beans. These diverse names underscore the bean's global significance as a staple food. (Abdulrahman *et al.,* 2020) (Saleem *et al.,* 2016)

Plant Morphology:

The French bean (*Phaseolus vulgaris*), which is highly regarded for its culinary versatility, derives its productivity and adaptability from certain physiological traits. Its taproot system contributes to soil fertility by producing lateral roots and nitrogen-fixing nodules. This herbaceous stem can grow in bushy or climbing forms, with the latter requiring support. The trifoliate leaves are made up of three ovate to lanceolate leaflets with a slightly glossy texture. The plant bears papilionaceous blooms in axillary racemes, typically in shades of pink, purple, or

white. The fruit is an elongated, cylindrical pod with several seeds arranged in a row, which can be green, yellow, or purple. The kidney-shaped seeds vary in color from white to black. The stipules are lanceolate to triangular in shape and approximately 4 mm in length. Both the rachis and petiole exhibit grooves. The leaves are oblong to elliptical in shape, broadly ovate to entire, acuminate, thin, glabrous to pubescent, and measure 4–16 cm in length and 2.5–11 cm in width. Each lateral leaflet has a 1-3 mm long stipel at its base, while the terminal leaflet has only one stipel. (Saleem *et al.,* 2016)

Pharmacological activity of leaves of *Phaseolus vulgaris* Linn.

Antimicrobial activity

Phaseolus vulgaris Linn. is a medicinal plant. The crude pet ether extract of the leaves of *Phaseolus vulgaris* Linn. shows remarkable antimicrobial activity. The plant leaves are extracted in various solvents like pet ether (60–80 °C), 95% ethanol, and aqueous extract. The extract shows antibacterial *(Salmondlae typhi, Klebsiella pneumoniae, and Escherichia coli)* and anti-fungicidal activity (Aspergillus fuinigatus, Rhizopus stolonifer, and Mucor mucedo). The inhibitory effect does not observe at very low concentrations of extract. All extracts had a minimum inhibitory concentration of 20%.(Olajide, 2020)

Antidiabetic Activity.

Freshly prepared aqueous leaves extract of *Phaseolus vulgaris* Linn. were first subjected to phytochemical screening for a variety of constituents. Alkaloids, balsams, cyanogenic glycosides, flavonoids, saponin, tannins, terpenes, steroids, and other active ingredients were found in the samples. The *in vivo* antidiabetic activity was studied on group of wit star rats. A single intraperitoneal dose of 150 mg/kg body weight of alloxan monohydrate solution was given. After administering alloxan for 48 hours, the blood glucose level was measured to confirm diabetes. For the study, rats with blood glucose levels below 7.0 mmol/L were chosen. The extract was able to lower the levels of Tri glyceraldehyde, which was statistically significant when compared to the normal control, and it was also able to improve the total cholesterol level in diabetes rats at the dosage that was administered (Cd *et al.,* 2013).

Result and Conclusion:

Alkaloids, flavonoids, glycosides, and tannins were discovered in leaf extracts of *Phaseolus vulgaris* Linn. Made with three different solvents (petroleum ether, ethanol, and water). The test bacteria (*Salmonella typhi, Klebsiella pneumonia,* and *Escherichia coli*) and fungi (*Aspergillus fumigatus, Rhizopus stolonifera,* and *Mucor Macedo*) showed that the extracts had an inhibitory effect on their growth. Another study with aqueous extract shows the antidiabetic activity of *Phaseolus vulgaris* Linn. leaves. In support of its hypoglycaemic and anti-diabetic potential, *Phaseolus vulgaris* Linn. suggests that it can be used in complementary medicine to treat people with diabetes. The pharmaceutical industry greatly benefits from natural medicine. Beans play a significant role in terms of wholesome and safe food. This crop is a

popular legume both in developed and developing nations, the latter of which aim to make healthy, primarily low-fat diets a priority.

The leaves of the same plant contain important chemical constituents. Leaves are also showing pharmacological activities; hence, they should be explored more in the future. To fully grasp the potential of these natural compounds for their clinical utility, however, more research on these substances is required. Investigating the use of various solvents, ranging from polar to non-polar, in the extraction of crude plant extracts is crucial for maximizing the discovery of bioactive compounds and understanding their therapeutic potential. Additionally, this approach allows for the optimization of extraction processes for further research and development.

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METABOLOMICS AND PLANT SECONDARY METABOLITES

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Introduction:

Definition of metabolomics

Metabolomics is the comprehensive study and analysis of the small molecule metabolites within a biological system. These metabolites, which include amino acids, sugars, lipids, and other organic molecules, represent the final products of cellular processes and reflect the physiological state of a cell, tissue, or organism.

Metabolomics is often defined as the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind," particularly the study of their small-molecule metabolite profiles.

The field encompasses both primary metabolites, which are directly involved in normal growth, development, and reproduction, and secondary metabolites, which are not essential for basic survival but are important for interaction with the environment. Key technologies used in metabolomics include mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and chromatography techniques (GC-MS, LC-MS).

Latest advancements:

- Integration with Other Omics: Modern metabolomics is increasingly integrated with genomics, transcriptomics, and proteomics to provide a more comprehensive understanding of biological systems.
- Single-Cell Metabolomics: Emerging technologies now allow for metabolomic analysis at the single-cell level, offering unprecedented insights into cellular heterogeneity.
- AI and Machine Learning: The application of artificial intelligence (AI) and machine learning algorithms is enhancing data analysis, enabling more accurate predictions and insights from complex metabolomic data.

Overview of plant secondary metabolites

Plant secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of plants. Instead, they play key roles in interactions with the environment, including defense against herbivores and pathogens, attraction of pollinators, and adaptation to abiotic stresses.

Types of secondary metabolites:

- **Alkaloids:** Nitrogen-containing compounds with significant pharmacological effects (e.g., morphine, caffeine).

- **Terpenoids:** Largest group of plant secondary metabolites, involved in the synthesis of essential oils and resins (e.g., menthol, rubber).
- **Phenolics:** Compounds with antioxidant properties, including flavonoids, tannins, and lignins.
- **Glycosides:** Compounds that consist of a sugar moiety bound to another functional group (e.g., cardiac glycosides like digoxin).

There Functions in Plants:

- **Defense mechanisms:** Secondary metabolites often serve as toxins or repellents against herbivores and pathogens.
- **Communication and attraction:** Some secondary metabolites are involved in signaling, attracting pollinators, or deterring competitors.
- **Stress response:** These compounds also help plants adapt to environmental stresses such as UV radiation, drought, and salinity.

Importance in plant biology and human applications

Plant secondary metabolites are of immense importance not only in plant biology but also in various human industries, including medicine, agriculture, and cosmetics.

1. **Ecological significance:** Secondary metabolites play critical roles in plant survival by mediating interactions with other organisms and protecting against abiotic stress.
2. **Biochemical pathways:** Understanding the biosynthesis and regulation of these metabolites provides insights into plant physiology and development.

Human applications:

a) **Pharmaceuticals:** Many plant secondary metabolites have therapeutic properties and are used in modern medicine (e.g., taxol, quinine).

b) **Agriculture:** Natural plant-derived compounds are increasingly used as biopesticides and growth promoters.

c) **Nutraceuticals and functional foods:** Certain secondary metabolites, such as flavonoids and carotenoids, are incorporated into foods for their health benefits.

Fundamentals of metabolomics

1. Metabolome: The complete set of metabolites in an organism

The metabolome represents the full complement of metabolites, which are the small molecules produced during metabolism within a biological organism. It reflects the organism's physiological state, environmental interactions, and genetic makeup. The metabolome includes all endogenous and exogenous metabolites found in a biological sample, such as tissues, cells, or biofluids. These metabolites are involved in various cellular processes, including energy production, signaling, and structural maintenance.

1. Complexity of the metabolome:

- The metabolome is highly dynamic, with metabolite concentrations fluctuating in response to internal and external factors like diet, stress, disease, and environmental changes.
- The metabolome is considered to be more complex than the genome or proteome due to the vast number of metabolites and their diverse chemical structures.

2. Analytical techniques for metabolomics:

- Advanced analytical technologies such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are essential for the detection, identification, and quantification of metabolites.
- The use of high-resolution MS, such as Fourier Transform Ion Cyclotron Resonance (FT-ICR) and Orbitrap, allows for precise metabolomic profiling.

Primary vs. Secondary metabolites

Metabolites are generally categorized into two main groups: primary and secondary metabolites. Both play crucial roles in the survival and adaptation of organisms.

1) Primary metabolites:

a) These are directly involved in the growth, development, and reproduction of an organism.
b) Examples include amino acids, nucleotides, and carbohydrates.
c) Primary metabolites are essential for life and are universally present in all living organisms.

2) Secondary metabolites:

a) These are not directly involved in the primary life-sustaining processes but play crucial roles in the organism's interaction with its environment.
b) Examples include alkaloids, flavonoids, and terpenoids.
c) Secondary metabolites often have species-specific functions, such as defense mechanisms, signaling, or pigmentation.

3) Comparison and interconnections:

a) Primary metabolites are the precursors for the biosynthesis of secondary metabolites.
b) The production of secondary metabolites is often regulated by environmental factors, such as light, temperature, and stress conditions.

Some latest insights:

1. Advances in metabolomics have led to the identification of complex regulatory networks that control the biosynthesis of secondary metabolites.
2. Industrial Applications: Secondary metabolites are widely used in pharmaceuticals, agriculture, and cosmetics due to their bioactive properties.
3. Biotechnological advancements have enabled the large-scale production of valuable secondary metabolites through microbial fermentation and plant cell cultures.

Metabolic pathways involved in secondary metabolite production

The biosynthesis of secondary metabolites involves complex metabolic pathways that are intricately linked to primary metabolism. These pathways often vary between species and can be highly specialized.

Key metabolic pathways:

- Shikimate Pathway: Responsible for the synthesis of aromatic amino acids, which serve as precursors for phenolics, alkaloids, and other secondary metabolites.
- Mevalonate (MVA) and Methylerythritol Phosphate (MEP) Pathways: These pathways are crucial for the biosynthesis of terpenoids, a large class of secondary metabolites.
- Polyketide Pathway: Involved in the production of polyketides, which include antibiotics, pigments, and anticancer agents.

Plant secondary metabolites

1. Classes of secondary metabolites

Plant secondary metabolites are diverse organic compounds that are not directly involved in the basic metabolic processes of plants but play crucial roles in ecological interactions, defense mechanisms, and human applications. These metabolites are classified into several major classes based on their chemical structure and biosynthetic origin.

A. Phenolics

Phenolics are a large and diverse group of secondary metabolites characterized by the presence of one or more hydroxyl groups attached to an aromatic ring. They are widely distributed in the plant kingdom and are involved in various physiological and ecological functions.

Phenolics include a wide range of compounds such as flavonoids, tannins, lignins, and phenolic acids. Flavonoids are further divided into subclasses like flavones, flavonols, flavanones, and anthocyanins, each with distinct roles and properties. Phenolics are involved in plant defense against pathogens, herbivores, and UV radiation. They also contribute to the structural integrity of plants (e.g., lignins in cell walls) and play a role in plant pigmentation (e.g., anthocyanins).

Recent studies have highlighted the potent antioxidant properties of phenolics, which contribute to their protective effects in plants and their health benefits when consumed by humans.

B. Terpenes

Terpenes are the largest class of plant secondary metabolites, comprising a vast array of structurally diverse compounds. They are derived from five-carbon isoprene units and play a variety of roles in plant biology.

Terpenes are categorized based on the number of isoprene units they contain, ranging from monoterpenes (C10) to polyterpenes.

Key subclasses include monoterpenes (e.g., limonene), sesquiterpenes (e.g., farnesene), diterpenes (e.g., gibberellins), and triterpenes (e.g., saponins).

Terpenes serve multiple ecological functions, including deterring herbivores, attracting pollinators, and protecting plants against microbial pathogens. They are also involved in plant growth regulation (e.g., gibberellins) and are major components of essential oils.Terpenes are of significant interest in biotechnology for their potential uses in pharmaceuticals, biofuels, and as natural pesticides.

C. Alkaloids

Alkaloids are nitrogen-containing secondary metabolites that have potent biological activities. They are well known for their use in medicine, owing to their wide range of pharmacological effects. Alkaloids are a structurally diverse group of compounds that typically contain one or more nitrogen atoms in a heterocyclic ring. Common subclasses include pyrrolidine alkaloids (e.g., nicotine), indole alkaloids (e.g., strychnine), tropane alkaloids (e.g., atropine), and isoquinoline alkaloids (e.g., morphine).

In plants, alkaloids primarily function as defense compounds against herbivores and pathogens due to their toxicity and deterrent properties. They also play roles in plant growth regulation and symbiotic relationships with microorganisms.Advances in synthetic biology are enabling the production of alkaloids in engineered microorganisms, offering a sustainable alternative to traditional extraction from plants.

D. Glucosinolates

Glucosinolates are sulfur-containing secondary metabolites predominantly found in the Brassicaceae family (e.g., cabbage, broccoli, mustard). They are known for their role in plant defense and their potential health benefits.

Glucosinolates are characterized by a core structure consisting of a β-thioglucose moiety, a sulfonated oxime, and a variable side chain derived from amino acids. They are classified into aliphatic, aromatic, and indole glucosinolates based on their side-chain structure.

Glucosinolates and their hydrolysis products (e.g., isothiocyanates, thiocyanates) play a key role in plant defense against herbivores and pathogens. They are also responsible for the characteristic pungent flavors of mustard and horseradish. Recent research has focused on the anticancer properties of glucosinolates and their breakdown products, particularly in reducing the risk of certain cancers.

Advanced analytical techniques in metabolomics

Advanced analytical techniques are essential in metabolomics to capture the complexity and diversity of metabolites in biological systems. These techniques offer high sensitivity, accuracy, and throughput, enabling comprehensive profiling and characterization of the metabolome.

1. Mass Spectrometry (MS)

Mass spectrometry (MS) is a cornerstone of metabolomics, used for the identification, quantification, and structural elucidation of metabolites. MS measures the mass-to-charge ratio (m/z) of ions, providing detailed information about the molecular composition of samples.

1.1. GC-MS (Gas Chromatography-Mass Spectrometry)

GC-MS is a widely used technique that combines gas chromatography (GC) with mass spectrometry (MS) for the analysis of volatile and semi-volatile compounds.

- ✓ **Principle**: In GC-MS, samples are first vaporized and then separated based on their volatility and interaction with the chromatographic column. The separated compounds are ionized and their mass-to-charge ratios are measured by the mass spectrometer, generating mass spectra for identification.

Applications: GC-MS is highly effective for the analysis of small, volatile metabolites, such as fatty acids, alcohols, and organic acids.

It is commonly used in metabolomics for profiling primary metabolites and assessing metabolic responses to environmental or genetic changes.

- ➢ Innovations in ionization methods, such as chemical ionization (CI) and electron impact ionization (EI), have improved the sensitivity and accuracy of GC-MS analyses. Coupling with high-resolution MS (HRMS) enhances the identification of metabolites with greater specificity.

1.2. LC-MS (Liquid Chromatography-Mass Spectrometry)

LC-MS combines liquid chromatography (LC) with mass spectrometry, offering a versatile and powerful approach for analyzing a wide range of metabolites, including polar, non-volatile, and thermally labile compounds.

- ✓ **Principle:** In LC-MS, metabolites are separated based on their polarity and interaction with the chromatographic column's stationary phase. The eluted compounds are ionized, typically by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), and detected by the mass spectrometer.

Applications: LC-MS is widely used in metabolomics for the analysis of complex biological samples, including biofluids, tissues, and cell extracts. It is particularly useful for profiling lipids, amino acids, nucleotides, and other polar metabolites.

2. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy is a non-destructive analytical technique that provides detailed information about the structure, dynamics, and environment of metabolites in solution or solid-state.

- ✓ **Principle:** NMR spectroscopy detects the magnetic properties of atomic nuclei (typically hydrogen or carbon) in a magnetic field. The chemical environment of these nuclei influences the resonance frequency, providing structural information about the metabolite.

Applications: NMR is used in metabolomics for the comprehensive profiling of biofluids (e.g., urine, plasma) and tissue extracts, allowing for the simultaneous quantification of multiple metabolites. It is particularly useful for identifying unknown compounds and studying metabolite-protein interactions.

3. Capillary Electrophoresis (CE)

Capillary electrophoresis (CE) is a separation technique that separates metabolites based on their charge-to-size ratio in a capillary filled with an electrolyte solution.

- ✓ **Principle:** In CE, an electric field is applied across a capillary, causing metabolites to migrate at different rates based on their size, charge, and shape. This technique is particularly effective for analyzing small, charged metabolites, such as amino acids, organic acids, and nucleotides.

Applications: CE is used in metabolomics for high-resolution separation and quantification of polar and charged metabolites, especially in complex biological samples.It is also employed for the analysis of post-translational modifications of peptides and proteins.

4. Ion Mobility Spectrometry (IMS)

Ion Mobility Spectrometry (IMS) is a technique that separates ions based on their mobility in a gas phase under the influence of an electric field. IMS is often coupled with MS for enhanced separation and structural elucidation of metabolites.

- ✓ **Principle:** In IMS, ions are separated based on their size, shape, and charge as they travel through a drift tube filled with a neutral gas. This separation occurs on a millisecond timescale, providing an additional dimension of separation prior to mass spectrometry analysis.

Applications: IMS-MS is used in metabolomics for resolving isomeric and conformationally similar metabolites that are difficult to separate using traditional chromatography. It is also employed for the structural characterization of complex metabolites, including lipids and glycans.

Cutting-edge technologies in metabolomics

Recent advancements in metabolomics have been driven by the development of cutting-edge technologies that enhance the resolution, sensitivity, and scope of metabolic analyses. These innovations are enabling deeper insights into the complexity of the metabolome, with applications ranging from basic biological research to clinical diagnostics.

1. High-Resolution MS Techniques (e.g., Orbitrap, FT-ICR)

High-resolution mass spectrometry (HRMS) techniques, such as Orbitrap and Fourier-transform ion cyclotron resonance (FT-ICR), provide unparalleled mass accuracy and resolution, enabling precise identification and quantification of metabolites.

a) **Orbitrap Mass Spectrometry:** The Orbitrap analyzer measures ions' frequencies as they oscillate around a central spindle, providing high mass resolution (up to 1,000,000 FWHM) and accurate mass determination. It is widely used in metabolomics for comprehensive metabolite profiling, particularly in complex biological matrices.

b) **Fourier-Transform Ion Cyclotron Resonance (FT-ICR) MS:** FT-ICR MS uses a magnetic field to trap ions, with their cyclotron frequency measured to determine the mass-to-charge ratio. It offers the highest mass resolution and accuracy available in mass spectrometry. FT-ICR is ideal for analyzing complex mixtures, such as in environmental metabolomics and lipidomics, where high mass accuracy is crucial for resolving isobaric species.

2. Imaging Mass Spectrometry (IMS) for spatial metabolomics

Imaging Mass Spectrometry (IMS) is a technique that combines the molecular specificity of MS with spatial resolution, enabling the visualization of metabolite distributions within tissues and cells.

- ✓ **Principle:** In IMS, a biological sample is ionized, typically by MALDI or DESI (Desorption Electrospray Ionization), and the resulting ions are analyzed by MS. By scanning across the sample, a spatial map of metabolite distribution is created. IMS allows researchers to visualize the spatial heterogeneity of the metabolome, providing insights into metabolic processes in different tissue regions.

Applications: IMS is widely used in cancer research to identify metabolomic signatures associated with tumor regions, offering potential biomarkers for diagnosis and treatment.It is also employed in plant science to study the distribution of secondary metabolites within tissues, helping to understand plant defense mechanisms and interactions with the environment.

3. Single-cell metabolomics: Single-cell metabolomics is an emerging field that aims to analyze the metabolome at the level of individual cells, providing insights into cellular heterogeneity and the role of metabolism in cell function and disease.

- ✓ **Principle:** Single-cell metabolomics involves the isolation and analysis of metabolites from individual cells, often using advanced MS techniques combined with microfluidics, laser capture microdissection, or capillary electrophoresis.

Applications: Single-cell metabolomics is being applied in cancer research to investigate the metabolic differences between cancerous and non-cancerous cells, potentially leading to new therapeutic targets. It is also used in stem cell research to study metabolic changes during differentiation and in neuroscience to explore the metabolism of individual neurons.

4. Real-time metabolomics using ambient ionization techniques

Real-time metabolomics using ambient ionization techniques allows for the rapid and direct analysis of metabolites in their native environment, without the need for extensive sample preparation.

Real-time metabolomics is being used in surgical settings to provide immediate feedback on tissue composition, helping surgeons distinguish between healthy and cancerous tissues during operations.

Integration with artificial intelligence: The combination of real-time metabolomics with AI-driven data analysis is enhancing the ability to rapidly interpret complex datasets, leading to more accurate and timely decision-making in clinical and industrial applications.

Applications of metabolomics in studying plant secondary metabolites

Metabolomics provides powerful tools for exploring the complex biochemical networks of plants, particularly in understanding secondary metabolites. These compounds play crucial roles in stress responses, interactions with microbes, chemical ecology, and more. This chapter discusses how metabolomics is applied to these areas and highlights recent advancements.

1. Stress responses and environmental adaptations

Stress responses: Plants produce a variety of secondary metabolites in response to abiotic stresses (e.g., drought, salinity) and biotic stresses (e.g., pathogen attacks). Metabolomic profiling helps identify stress-responsive metabolites and pathways, providing insights into plant resilience mechanisms.

Environmental adaptations: Plants adapt to changing environments by altering their metabolite profiles. Metabolomics can reveal how plants adjust their secondary metabolism to cope with environmental challenges.

Latest developments: Recent studies use high-throughput metabolomics to profile stress-induced metabolite changes across multiple plant species and conditions. For example, work by Zhang *et al.,* (2023) investigated drought stress responses in crops using integrated metabolomic and transcriptomic analyses.

Advances in functional metabolomics allow researchers to link specific metabolites with stress tolerance traits. For instance, the discovery of flavonoids and phenolic compounds that enhance drought resistance in wheat (Khan *et al.,* 2022).

2. Plant-microbe interactions

Microbial interactions: Plants and microbes engage in complex interactions, influencing secondary metabolite production. Metabolomics helps in understanding how plants alter their metabolome in response to beneficial or pathogenic microbes.

Symbiosis and pathogenesis: Metabolomics can differentiate between beneficial symbiotic interactions and pathogenic infections by profiling metabolite changes.

Metabolomic profiling of plant-microbe interactions: Recent research uses metabolomics to map the metabolic exchanges between plants and microbes. For example, Yang *et al*. (2024) used metabolomics to study the impact of arbuscular mycorrhizal fungi on the secondary metabolism of maize.

Microbiome metabolomics: The application of metabolomics to study plant-associated microbiomes is expanding. Advances include using metabolomic tools to analyze the impact of root-associated microbiomes on plant metabolite profiles -Smith *et al.,* (2024).

3. Chemical ecology and plant defense mechanisms

Chemical ecology: Secondary metabolites play critical roles in plant interactions with other organisms, including herbivores and pollinators. Metabolomics provides insights into how plants produce and regulate these compounds for ecological interactions.

Plant defense mechanisms: Metabolomics can uncover the mechanisms of plant defense against herbivores and pathogens, revealing how secondary metabolites contribute to resistance and defense. New research focuses on the role of specialized metabolites in plant defense. Instance, the discovery of novel defense compounds in response to insect feeding (Huang *et al.*, 2023) showcases the dynamic nature of plant chemical defenses.

Ecological metabolomics: Studies like those by He *et al.*, (2024) use metabolomics to investigate how plants modify their metabolite profiles in response to herbivory and environmental stressors.

4. Quality control and authentication of plant-based products

Quality control: Metabolomics is used for the quality control and authentication of plant-based products by profiling their metabolite composition. This ensures product authenticity and consistency.

Metabolomic fingerprinting: Advances in metabolomic fingerprinting techniques are improving the ability to authenticate and verify plant-based products. Studies such as those by Zhang *et al.,* (2023) focus on the use of metabolomic data to detect adulteration and ensure product quality.

Regulatory and Standards Development: New guidelines and standards for metabolomic analysis in quality control are being developed to ensure reliable and reproducible results in the authentication of plant-based products (Yang *et al.,* 2024).

Conclusion:

Metabolomics, the comprehensive study of metabolites within an organism, offers profound insights into plant biology, particularly concerning secondary metabolites. This emerging field leverages advanced analytical techniques and technologies to explore complex metabolic networks and their roles in plant physiology and ecology. The metabolome encompasses all metabolites in an organism. Metabolomics distinguishes between primary metabolites, essential for growth and development, and secondary metabolites, which contribute to ecological interactions and stress responses. Metabolic pathways involved in secondary metabolite production are complex and diverse, involving various enzymatic processes. Plant secondary metabolites are classified into several categories, including phenolics, terpenes, alkaloids, and glucosinolates. Recent advancements in analytical techniques have greatly enhanced our ability to analyze metabolites. Mass spectrometry (MS), including GC-MS, LC-MS, and MALDI-TOF MS, provides high sensitivity and resolution. Nuclear Magnetic Resonance (NMR) spectroscopy offers detailed structural information, while Capillary

Electrophoresis (CE) and Ion Mobility Spectrometry (IMS) contribute to separation and identification of metabolites.

Metabolomics has various applications, including studying stress responses, plant-microbe interactions, chemical ecology, and metabolic engineering. It helps elucidate plant adaptations to stress, understand plant-microbe interactions, enhance production of valuable compounds, and ensure the quality and authenticity of plant-based products.

The future of metabolomics is bright, with emerging technologies and multi-omics integration driving progress. Precision agriculture and plant breeding will benefit from metabolomic advancements, offering new tools for optimizing crop production and quality. However, ethical considerations and regulatory frameworks must evolve to address privacy, data handling, and standardization challenges in metabolomics research.

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