Department of Agriculture Development & Farmers' Welfare
Farm Information Bureau

Government of Kerala

JANUARY 2023
VOLUME - 10
ISSUE - 07

KERALA KARSHAKAN

English journal

The First English farm journal from the house of Kerala Karshakan

ICAR-IARI

THE PRIDE OF INDIAN AGRICULTURE

INSIDE

KERALA KARSHAKAN
English journal

JANUARY 2023 Volume - 10 Issue - 07

Mail: editorejournalkkfib@gmail.com Log on to http://www.fibkerala.gov.in
Phone: 0471-2314358

The First English farm journal from the house of Kerala Karshakan

ATTENTION AUTHORS

Articles for Kerala Karshakan e-journal should be certified by head of the institution concerned stating that the article is original and has not been published anywhere. Reference should also be included wherever relevant.

41

Chief Editor
Padmam S

Editor
Sreekala S

Asst. Editor
AL Unais A J

Editorial Assistant
Anoop R J

Design & Layout
Rajesh V

Articles/ Features appearing in this e-journal are either commissioned or assigned neverthless, other articles of farm relevance are also welcome. A maximum of 750 wordage is appreciated. Such items should be addressed to The Editor, Kerala Karshakan e-journal, Farm Information Bureau, Kowdiar PO, Thiruvananthapuram, Pin: 695003
These may also be mailed to editorejournalkkfib@gmail.com in word format. Responses can be also sent to this mail. Authors are requested to provide the following details along with the articles, for quick processing of the remuneration, after the articles are published: Account Number, Name of Bank, Branch (Place), IFSC Code.

1. Introduction

Enhancing agricultural production with environmental sustainability is the major goal of agricultural research. To meet the food demand by 2050, the growth rate of yield gain must be doubled. This needs the efficient use of available genetic diversity and use of modern biotechnology to genetically enhance the resource (water & nutrient mainly nitrogen) use efficiency and crop productivity. Phenotyping or characterization of plants is one of the earliest agricultural activities of humans started with domestication of crops plants. Later the phenotypic diversity within each species was exploited by the earliest crop improvement methods. Once the concepts of genetics were understood, it was established that the phenotype is the product of genotype x environment. Establishment of genotype-phenotype relationship is the key for modern genetic improvement methods of both plants and animals. The two pillars of analytical breeding are genotyping and phenotyping. Efficient use genomic information for crop breeding is potential solution to develop high yielding, resource use efficient and climate resilient crop varieties. Genomic technologies such as Next Generation Sequencing (NGS) and SNP arrays have enabled the plant scientist to obtain genotypic information of breeding material with relatively low cost and shorter time. However, the principal goal of identifying specific genotypes that are associated with phenotypes

"PHENOMICS"

Next Generation Phenotyping Technologies For Trait Dissection And Breeding Climate Resilient Crops

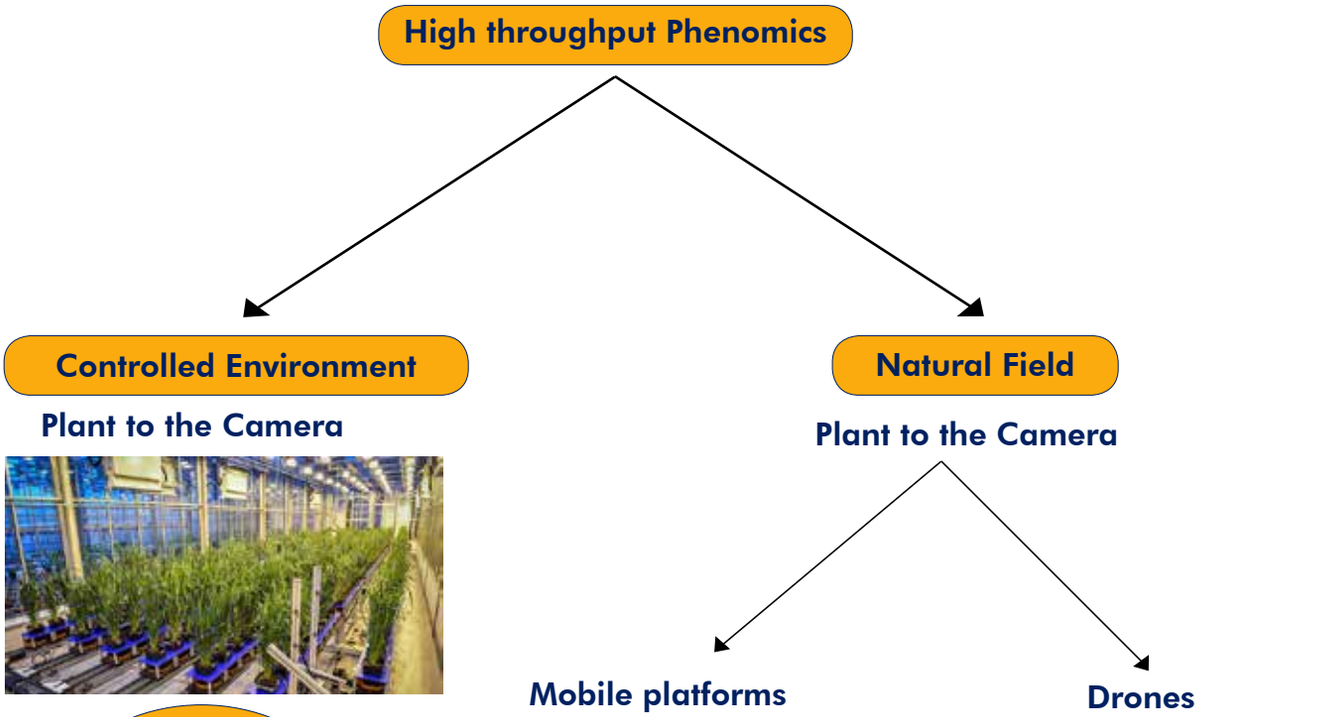


Figure 1. Phenomics approach used in controlled environment and field condition

progressed only slowly as development in phenotyping has not kept pace with genomics. The wet chemistry and other actual measurement of growth and physiological processes-based phenotyping is inherently low throughput, labor-intensive, costly, time consuming and often destructive due to organism-wide phenotypic data for same plant cannot be obtained, which lead to genotype-phenotype gap (Furbank and Tester 2011).

The "Plant Science Decadal Vision for the decade 2015 to 2025" on food, energy, environment, and health (Plant Science Research Summit 2013), as well as "nine big ideas" proposed by National Science Foundation (NSF), USA for solving pressing societal problems emphasizes the necessity of "understanding the rules of life: predicting phenotype and assemble plant traits in different ways to solve problems" (Mervis 2016). Thus, accurate phenotyping to obtain physiological information necessary for crop improvement is the key for further genetic improvement of crops. To address these necessities, the multi-disciplinary science of phenomics emerged recently. Phenome is defined as expression of the genome as traits in a given environment. The human phenome project initiated in 1997 (Freimer and Sabatti 2003) led to the birth of phenomics (Bilder et al. 2009). Phenomics is multidisciplinary science of sensor aided non-destructive high throughput automated acquisition and analysis of high-dimensional phenotypic data on an organism-wide scale. Phenomics, the Next Generation Phenotyping (NGP), offers solution to discover the inner workings of living plants and thus bridge the phenotype-genotype gap (Cobb et al. 2013; Fiorani and Schurr 2013; Fahlgren et. al. 2015b; Großkinsky et al. 2015).

Phenomics involves

1) non-invasive sensors,
2) automated data processing to obtain phenotypic traits,
3) robotized delivery of plants to sensors or vice versa,
4) robotized plant culturing, and

Table 1. High throughput non-invasive sensors for phenomics (Kumar et al. 2016)

Sensor	Principle of trait capture	Traits measured	References
Visible light imaging camera	Reflectance in the visible (400-700 nm) range depend upon plant growth, morphology, pigments, wax, health, etc. Image processing and segmentation in binary image, Hue Saturation Intensity (HSI) color model and L*a*b* color space are used to obtain phenotypic data.	Shoot/root growth, architecture, greenness, Leaf area, leaf rolling, senescence, growth rates, tillering, early vigor, plant height, phenology, biomass, convex hull, compactness, eccentricity	Golzarian et al. (2011) Das et al. (2016)
Fluorescence imaging camera	Light absorbed by short wave length is emitted as long wave fluorescence depending upon the composition of plant tissues with molecules with innate (auto) fluorescence characters. Chlorophyll molecules absorb light at shortwave length and emit fluorescence at red/ far-red wavelength (680 & 735 nm). Nicotinamide (NAD) and flavin (FMN, FAD) coenzymes, pyridoxal phosphate, folic acid and secondary metabolites (phenolics, alkaloids and terpenoids) emit blue-green fluorescence when excited with UV light (340 to 360 nm).	Maximum quantum efficiency of PSII, photochemical quenching and non-photochemical quenching, which are highly sensitive to resource availability and stresses; Secondary metabolite mapping; Fluorescence can be used to detect metabolites and gene expression when tagged with non-native fluorophores such as transgenic plants expressing GFP/ YFP fusion proteins.	Mishra et al. (2016)
Thermal imaging camera	The infrared energy (8 to 13 μm) emitted from object is converted into an electrical signal by the imaging (microbolometer) sensor. Tissue temperature is determined mainly by evapotranspiration.	Thermal images are used to infer stomatal conductance and plant health (biotic and abiotic stress).	Möller et al. (2007) Ludovisi et al. (2017)

Sensor	Principle of trait capture	Traits measured	References
Bioluminescence imaging camera	Emission of visible light from an enzymatic reaction inside the plan is captured using low-light imaging CCD cameras in darkness.	Transgenic plants expressing firefly LUCIFERASE or free calcium sensor AEQUORIN are useful to identify mutants and to assess physiological processes and stress responses.	Grant et al. (2000) Chinnusamy et al. (2002)
Near infrared imaging (NIR) and multispectral line scanning cameras	The reflectance of plants in the range of 900 to 1700 nm depends upon water content. Plants reflects large amount of 800 to 1400 nm light while soil reflectance is negligible.	Water content, leaf thickness, leaf area index; root soil moisture extraction pattern	Neilson et al. (2015)
Hyperspectral Reflectance imaging camera (indium gallium arsenide sensors)	Spectral reflectance is imaged at nm resolution by VIS-NIR (visible-near infrared, 400–1000 nm) and SWIR (short wavelength infrared, 1000–2500 nm) cameras.	Several spectral indices are available to assess chlorophyll content, relative water content, nutrient status, chemical composition, plant health, photochemical reflectance index, genotype bar-coding	Romer et al. (2012) Sahoo et al. (2015) Wahabzada et. al. 2016
Stereo camera	Two RGB (red, green, blue) cameras to capture three-dimensional images	Shoot biomass and structure, leaf angle distributions, canopy structure	Biskup et. al. 2007
Light detection and ranging (LIDAR) and laser triangulation sensors	A laser light beam is projected onto plants and the energy scattered from the plant is captured for the computation of depth maps and 3D point clouds.	Shoot biomass and structure, leaf angle distributions, canopy structure	Kjaer and Ottosen (2015) Vadez et. al. (2015)
NIR and Fourier transform infrared spectroscopy (FTIR) spectroscopy	NIR and FTIR measure chemical composition, respectively, from the NIR and long wave IR absorption and emission properties of plant tissues	Quantification of water, protein, oil, sugars, starch, cell wall composition, lignin, and other larger molecules in seeds & other plant tissues	Chaerle et. al. (2009) Bağcıoğlu et. al. (2017) Legner et. al. (2018)

5) automated analysis of processed data in a data management pipeline.

Robotized delivery of plants to the imaging sensors is commonly used in controlled environment phenomics platform, while sensors are delivered to the plants in field phenomics platforms. Non-invasive sensors commonly used in non-destructive automated plant phenomics facility consists of various imaging cameras namely visual imaging, Hyperspectral imaging, IR thermography, NIR image analysis, Chlorophyll fluorescence imaging, bioluminescence imaging, fluorescence imaging, etc. Wide-range of phenotypic data on whole-plant during its entire life cycle can be acquired by using phenomics technologies that are not possible through conventional phenotyping methods (Kumar et al. 2016). In addition, Light detection and ranging (LIDAR) and laser triangulation sensors are used for assessment of plant growth, shoot biomass, leaf angle distributions and canopy structure, while magnetic resonance imaging (MRI) is used for three-dimensional imaging of roots to obtain spatial information on the root system architecture of plants (van Dusschoten et al. 2016). Phenomics is being employed in both controlled environment as well as in the natural field conditions (Figure 1). Different imaging methods, sensors used and phenotype data acquired are summarized in Table 1.

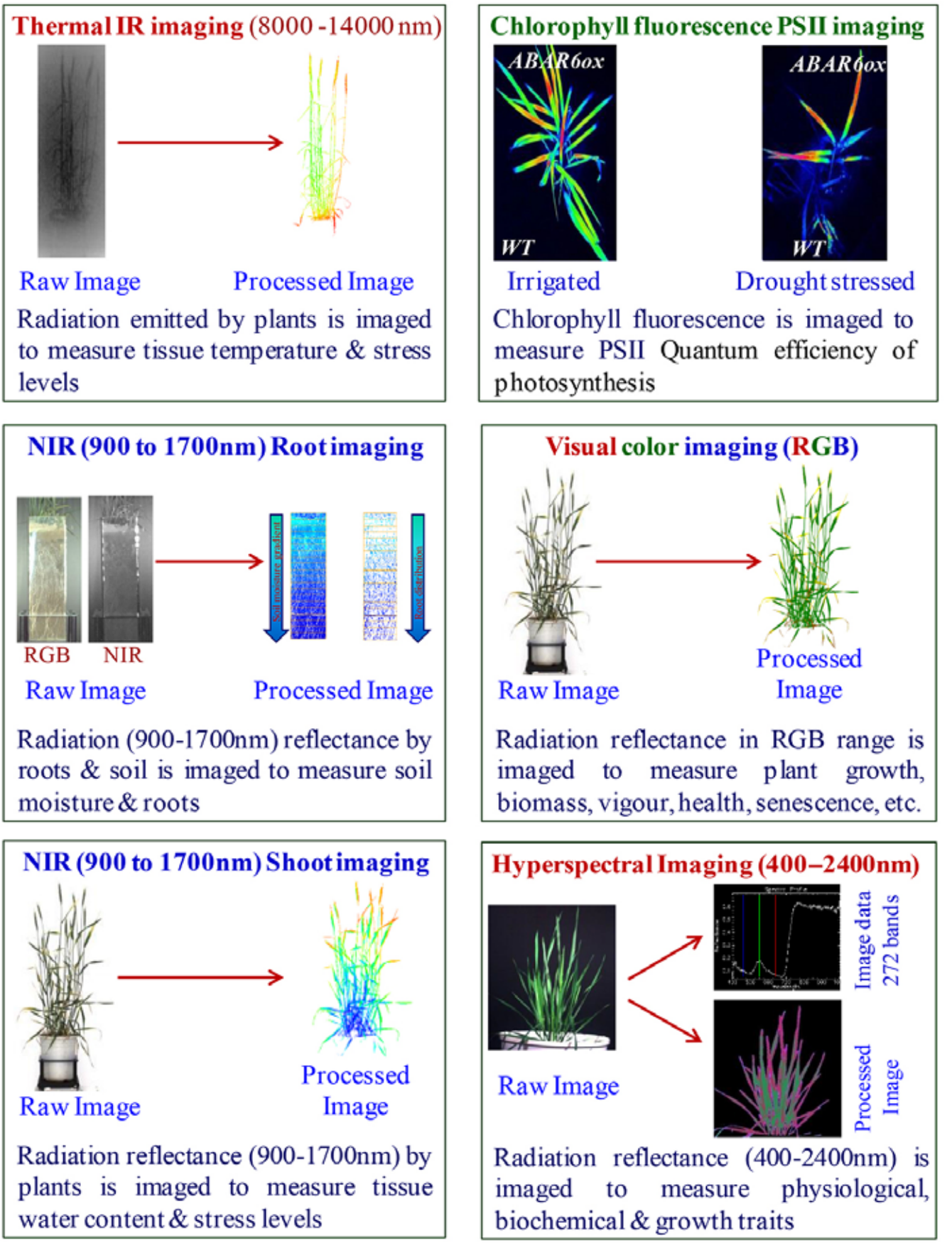
2. Phenomics Initiatives by Indian Council of Agricultural Research, New Delhi

Realizing the potential of phenomics, Australian government invested $51 million in 2007 and established the Australian Plant Phenomics Facility (APPF) in January 2010 (http://www.plantphenomics.org.au/about/). Since then, several government Institutes have established automated high throughput phenomics facility for crop plants. Soon, the Indian Council of Agricultural Research, New Delhi also initiated the establishment of phenomics facilities in India recently at ICAR-Indian Agricultural Research Institute, New Delhi; ICAR- Central Research Institute for Dryland Agriculture, Hyderabad; ICAR-Indian Institute of Horticultural Research, Bengaluru and National Institute of Abiotic Stress Management, Baramati, India. ICAR-IARI, New Delhi has established a state-of-the art automated high throughput plant phenomics facility for non-destructive and accurate characterization of a large number of germplasm and recombinant inbred lines under defined environmental treatment conditions (Funded by NASF, ICAR, New Delhi-110012). The phenomics facility has four hi-tech climate-controlled greenhouses for cultivation of plants in defined environmental conditions. For plant cultivation, the facility is equipped with 1200 plant carriers with RFID chip tag. The plant carrier on moving field conveyer system randomizes plants within the greenhouse and carries plants for automated weighing and watering, and imaging at various imaging platforms. The facility has 5 automated weighing and watering stations for precise imposition of drought stress to plants and to measure transpiration and water use efficiency of plants. The facility has the following non-invasive image-based sensor platforms for measuring various plant traits (Figure 2):

1) Visual high-resolution imaging: Reflectance in the visible (400-700 nm) range is captured by using high resolution camera from the top and side of the plants. Visual imaging is used to measure shoot/root growth, architecture, greenness, Leaf area, leaf rolling, senescence, growth rates, tillering, early vigor, plant height, phenology, biomass, convex hull, compactness, eccentricity, etc.

2) IR thermal imaging: The infrared energy (8 to 13 μm) emitted from plant is converted into an electrical signal by the imaging (microbolometer) sensor to measure tissue temperature. As tissue temperature is determined mainly by evapotranspiration, IR thermal images are used to infer stomatal conductance and plant health (biotic and abiotic stress).

3) Chlorophyll fluorescence imaging: Light absorbed by short wave length is emitted as long wave fluorescence depending upon the composition of plant tissues with molecules with innate (auto) fluorescence characters. Chlorophyll molecules absorb light at shortwave length and emit fluorescence at red/far-red wavelength (680 & 735 nm). This imaging system can measure chlorophyll fluorescence to calculate maximum quantum efficiency of PSII, photochemical quenching and non-photochemical quenching, which are highly sensitive to resource availability



Examples of image analysis from different sensors and the physiological traits measured Figure 2

and stresses.

4) Near infrared (NIR) imaging: The reflectance of plants in the range of 900 to 1700 nm depends upon water content. Plants reflects large amount of 800 to 1400 nm light while soil reflectance is negligible. NIR shoot imaging system is used to measure water content and distribution in plants, leaf thickness and leaf area index, while NIR root imaging system is used to phenotype root soil moisture extraction pattern and root growth.

5) Visual-Near Infrared (VNIR) & Short wave Infrared (SWIR) - hyperspectral imaging systems: Spectral reflectance is imaged at nm resolution by VIS-NIR (400–1000 nm) and SWIR (1000–2500 nm) cameras. Several spectral indices are available to assess chlorophyll content, relative water content, nutrient status, chemical composition, plant health, photochemical reflectance index, genotype bar-coding.

The automated weighing and watering stations will quantify the weight of pots before and after watering, in order to impose various drought/ waterlogging/ nutrient deficiency stresses, and to assess input use efficiency. Thus, critical physiological traits contributing to the yield and stress tolerance can be measured by phenomics platforms with high throughput for a large set of plants at defined intervals during crop growth. The depth of component phenotypic traits and the spatio-temporal dynamic phenotypic data generated in phenomics are enormous and unparallel to the conventional phenotyping. Some of the utilities of phenomics facility are (Kumar et al. 2016):

1. Dissection of complex traits into component traits
2. Germplasm screening to identify donors
3. Phenotyping of biparental population for Linkage mapping
4. Phenotyping of minicores for genome-wide association studies (GWAS)
5. Functional genomics, Forward & reverse phenomics
6. Gene function validation & selection of better transgenic events
7. Trait pyramiding in analytical breeding
8. Phenome-wide association studies (PheWAS)
9. Phenomic selection
10. Training of Genomic Selection models with deep phenotyping data
11. Development of ecophysiological crop simulation models for in silico phenotyping & ideotype design

Shri Narendra Modi, Hon'ble Prime Minister of India inaugurated and dedicated the "Nanaji Deshmukh Plant Phenomics Centre" to the Nation on 11th October 2017, on the event of the birth centenary celebration of Nanaji Deshmukh at IARI, Pusa, New Delhi. The major goals of this centre are:

1. To identify superior genotypes and novel genes useful for development climate resilient crop varieties.
2. To unravel the interaction of genes and the environment using big data analytics, the next step in expanding the boundaries of our knowledge in crop improvement and management.
3. To identify image features from different sensors that will be useful for UAV- and/or remote sensing-aided applications for resource and crop management in precision agriculture.
4. To develop globally competent scientific human resources in cutting edge research area of digital phenotyping, predicting plant behaviour in different environment and big data science useful for crop improvement and management.

3. Potential of phenomics for trait dissection and gene mapping

Phenomics is being extensively used for establishing phenotype genotype relationship and QTL mapping. Some examples of biparental population based QTL mapping and genome-wide association (GWA) mapping using data from NGP phenomics are given in the Table 2. The relationship between QTL mapping under field conditions and controlled environment phenomics facility where plants were grown in pots were studied. Phenomics approach was used to map QTLs in barley for growth under drought stress including growth rate and water use efficiency at seedling stage. Several QTLs showed co-localization with previously mapped QTLs under field conditions. A novel QTL that significantly increased biomass by about 36% was identified (Honsdorf et al. 2014). Further, in wheat by using phenomics approach, about 20 QTLs with strong effects, accounting for between 26 and 43% of the variation were in a controlled environment showing that the G×E interaction could be reduced. Comparative

Table 1. Examples of phenomics aided QTL mapping

Crop/ Model plant	Population	Phenomics platform	QTLs mapped	Remarks	Reference
Arabidopsis	162 RILs and 92 NILs derived from a Cvi) × Ler cross	Visual image every 2 min for 8 hr; Controlled environment	QTLs for mean tip angle at each of the 241 time points	Time-dependent QTL were detected on chromosomes 1, 3, and 4	Moore et al. 2013
Triticale	647 doubled haploid lines derived from four families; GWA mapping	A tractor pulled trailer equipped with two light curtains, three laser distance sensors, two 3D-Time-of-Flight cameras; Field conditions	23, 25 and 17 QTLs at 3 developmental stages; Two major QTLs	One major QTL on chromosome 5R is active throughout plant development while the other major QTL on chromosome 5A contributes strongly to biomass at the early stage	Busemeyer et al. 2013
Rice	171 RIL and parental plants Bala × Azucena	Visual imaging of root systems growing in nutrient-enriched gellan gum at days 12, 14, and 16 Post planting	89 univariate QTLs across all days of imaging for various RSA traits	One major QTL on chromosome 5R is active throughout plant development while the other major QTL on chromosome 5A contributes strongly to biomass at the early stage	Topp et al. 2013
Barley	47 wild barley ILs of the S42IL library and the recipient parent Scarlett	RGB images; gravimetric measurement of water use, end point phenotypic data; Controlled environment	44 QTL for 11 traits;	Three QTL were identified for Absolute Growth Rate Integral (AGRI); Two QTL were detected for WUE	Honsdorf et al. 2014

Crop/ Model plant	Population	Phenomics platform	QTLs mapped	Remarks	Reference
Triticale	647 DH lines derived from four families	phenomics data of biomass yield generated at three developmental stages; Controlled environment	10, 10, 9 QTLs were mapped for biomass at 3 stages respectively	Of the several QTLs mapped, only 4 were common in all three stages, while 5, 4, and 4 were specific for biomass at satge1, 2 & 3 respectively	Liu et al. 2014
Triticale	647 triticale DHs derived from 4 families	Visual image based plant height (PH) measurement at 3 developmental stages (PH1, PH2 & PH3); Field conditions	15 QTL for PH1, 18 for PH2 and 8 for PH3	Only 3 QTLs common for all three stages suggesting that the genetic control of plant height undergoes rapid temporal changes	Würschum et al. 2014
Wheat	5000 RILs from a cross between Drysdale and Gladius	Conventional phenotyping in the field; Image based phenotyping in controlled environment	84 QTLs in Field; 21 QTLs for plant growth using the imaging platform	7 co-located QTLs were found for traits from the phenomics platform with that from the field	Parent et al. 2015
Arabidopsis	324 accessions; GWA mapping	Visual top-view imaging and end-point fresh weight determination; Controlled environment	22 QTLs for fresh weight (endpoint), projected leaf area (at 12 different growth stages) and modelled parameters	Many of the growth QTLs would not have been identified with only endpoint fresh weight data	Bac-Molenaar et al. 2015
Sorghum	97 RILs and the two parental lines BTx623, IS3620C)	RGB time-of-flight depth camera; Controlled environment	Five QTLs were mapped; alleles closely linked with the sorghum Dwarf3 gene, an auxin transporter, was found to play important role in shoot architecture.	Many of the QTLs identified via image-based phenotyping overlapped with QTLs for comparable traits discovered in prior field experiments	McCormick et al. 2016

Crop/ Model plant	Population	Phenomics platform	QTLs mapped	Remarks	Reference
Maize	167 RILs with its parents (B73 and BY804)	106 traits across 16 developmental stages using the automatic phenotyping platform in controlled environment; also phenotyped under field conditions	988 QTLs were identified for 42 phenotypic traits across 16 time points; 42 to 82 QTLs at each time point	Several dynamic development QTLs were identified	Zhang et al. 2017
Maize	252 diverse inbred lines; GWA mapping	Automated non-invasive phenotyping at 11 different developmental time points	Several QTLs mapped for different growth stages	Main effect loci detected show complex developmental phase-specific patterns of expression	Muraya et al. 2017

analysis of QTLs mapped using phenomics approach with that are previously mapped under field conditions showed co-localization (Parent et al. 2015). Combination of phenomics and genome-wide association studies (GWAS) in rice, 141 associated loci for 15 traits, 25 of which are previously known genes (Yang et al. 2014). These performance evaluation studies demonstrated that phenomics approach is a suitable alternative to replace traditional laborious field-phenotyping for QTL mapping and positional cloning

Superiority of non-destructive phenomics over conventional field phenotyping

Conventional phenotyping is often destructive and phenotypic data is obtained at few crop growth stages or at the end of the crop cycle. Automated NGP using phenomics technologies captures multiple phenotypic data throughout the crop growth stages and thus adds time-scale to the phenotypic data which is not available in the conventional phenotyping. Time-scale phenotypic data during different growth and development of crop is necessary for mapping the QTLs for component traits that contributes to crop development during specific growth stages. Plant growth models quantify 1) absolute growth rate (AGR), 2) relative growth rate (RGR), and Net Assimilation Rate (NAR), which require measuring biomass/leaf area at successive time points. However, raking these destructive measurements in field is limited due to space, time and cost limitations, and thus often only two point measurements are taken and fitted into simple logistic models. However, the results do not often fit with observations (Paine et al. 2012). Phenomics is highly useful in measurement of plant growth and development on the organism-wide scale, and thus it is highly useful to measure dynamics of various component physiological traits that contribute to yield and stress adaptation. Automated phenomics enables the plant scientist to quantify traits that are difficult to measure under field conditions such as relative growth rate, transpiration, and water-use efficiency (WUE). Direct quantification of WUE requires gravimetric measurements of amount of water used for evapotranspiration and plant biomass. It is difficult to directly measure WUE for large number of germplasm lines and mapping populations. Hence only limited

success has been achieved in identification of donors and QTLs for this important trait. Further, the physiological causes of the genotypic differences are not understood. Temporal measurements of water use and biomass in automated phenomics facility using *S. viridis* and domesticated *S. italica* revealed that both have similar biomass production, but *S viridis* maintained the water-use efficiency, while *S. italica* become less efficient growth under water-deficit. Conventional end point measurement could not have detected this temporal physiological response of genotypes in WUE as the soil available water changes (Fahlgren et. al. 2015a). The dissection approach uses model-assisted methods to dissect complex phenotypes such as yield and drought tolerance into more simple and heritable traits. In barley, phenomics approach was used to identify novel traits, such as maximum growth rate and stress elasticity, associated with plant growth and drought tolerance. These traits are not measurable via traditional phenotyping approaches. In addition several image-based traits and model-derived parameters were identified which have potential for subsequent dissection of the genetic basis of complex agronomic traits (Chen et. al. 2014).

The genetic dynamics of plant traits were revealed by the introduction of time-axis by the use of automated phenomics to dissect the genetics of complex traits over the time-scale. Generally, heritability for a specific trait in a crop is considered stable, and traits with moderate to high heritability are given preference for genetic improvement. Automated image acquisition after every 2 min for 8 h of imposition of gravitropism and QTL mapping in Arabidopsis led to the mapping of time-dependent QTLs (Moore et al. 2013).Leaf growth and development, a major determinant of photosynthetic capacity, is highly regulated by moisture and nitrogen availability. Genetic dissection of this trait was difficult as it needs measurement of this trait throughout a growing season. Using a time-lapse image analysis approach of phenomics, this complex trait was dissected and found to be highly heritable in Arabidopsis (Zhang et al. 2012). The complexicity and plasticity of traits such as biomass and yield in triticale was studied with image-based phenotyping at three developmental stages. QTLs mapping identified some stage-specific QTLs and some QTLs common for two or more developmental stages, demonstrating a temporal contribution of these QTLs to the trait (Liu et al. 2014). Phenomics of rosette growth in 324 accessions of Arabidopsis was compared with end-point weight measurement for GWAS. Use of temporal growth data detected time-specific QTLs which were undetected by endpoint measurement. Eleven of these time-specific candidate genes identified were annotated to be involved in the determination of cell number and size, seed germination, embryo development, developmental phase transition, or senescence. Of these eight genes have been previously demonstrate role with mutants and overexpression studies, suggesting the time-specific QTLs are true regulators of growth and development (Bac-Molenaar et al. 2015). A recent study with non-destructive

high throughput phenome of Arabidopsis accessions over spatial and temporal scale revealed that heritability for some traits is dynamic. The heritability of ΦPSII (Fq'/Fm', a useful proxy for the light use efficiency for CO_2 fixation) showed recurrent daily rise which was unaffected by the difference in light intensity, while that of chlorophyll reflectance index and projected leaf area (PLA, an indirect estimate of above ground biomass) gradually changed through time and responded strongly to light intensity. The heritability of PLA showed significant temporal flexibility ranging from 0.04 to 0.83 within the course of 6 h. This suggests the necessity of organism-wide spatial and temporal phenotyping in phenomics to understand the heritability of traits of agricultural importance (Flood et al. 2016). Thus, spatial and temporal phenotyping of crops in phenomics facility will help understand and improve these important traits under water and nitrogen limited conditions.

5. In silico Phenotyping

Phenomics is highly useful for GWAS and linkage mapping of complex traits such as biomass and height in triticale (Busemeyer et. al. 2013; Würschum et. al. 2014), root architecture in rice (Topp et al. 2013), yield component in rice (Yang et al. 2014), root gravitropism in Arabidopsis (Moore et. al. 2013), etc. Phenomics was employed to map salinity tolerance using 378 diverse rice genotypes. Visual image based growth analysis led to the identification of a genomic region on chromosome 3 for the early growth response, while chlorophyll fluorescence imaging identified a region on chromosome 1 that regulates both the early growth rate and long term ionic stress effects under salinity stress (Campbell et. al. 2015). Rice genome is predicted to encode 37,544 genes. Functions of the some of the genes have been elucidated at molecular level, and their impacts on some phenotypes have been studied. However, effect of individual genes on whole plant phenome is critical ultimately to predict the plant traits from plant genome in different environmental conditions. An attempt has been made in yeast to study the phenotypes of essential gene mutations in yeast and PhenoM (Phenomics of yeast Mutants) database was developed (Jin et al. 2012). Loss function mutants, transcriptome and phenomics data was used to elucidate the differential functions two stress responsive genes AtRD22 and AtUSPL1belonging to BURP domain gene family in Arabidopsis (Harshavardhan et al. 2014). However, such efforts are limited in important food crops such as rice and wheat.

Development of a gene based crop model to prediction of complex traits under diverse environmental conditions is an important area of research. For instance, an ecophysiological model predicts pre-flowering duration as affected by temperature and photoperiod was developed using barley RILs. Along with this, QTLs were mapped for the model input trait and values of the model-input traits predicted for the RILs from the QTL were fed back into the ecophysiological model. This model could predict the flowering time for eight field trial environments, and thus ecophysiological model was capable of extrapolating QTL information from one environment to another (Yin et al. 2005).Similarly, wheat heading date could be predicted by using ecophysiological crop simulation model with QTL based parameter inputs (Bogard et al. 2014). Using the marker-based parameter trait values, marker-based values of ILs for seven yield component traits were estimated and were fed to the GECROS model. This model could simulate yields of the ILs under well-watered and drought conditions, and identify virtual ideotypes which had 10–36% more yield than those based on markers for yield per se (Gu et. al. 2014). Combining crop simulation models with genomic information and genetic modelling can accelerate delivery of future cereal cultivars suitable for different target environments. However, the robustness of model-aided ideotype design need to be further be enhanced through the inputs from phenomics and genomics and multi-model ensembles (Rötter et al. 2015). In India such efforts are totally missing now. We need to introduce gene/QTL and genomics information into existing ecophysiological models, and improve crop models based on information for lower organizational levels for complex traits (Kumar et al. 2016).

6. Image Processing, IAPs and Phenome Data Bank

Recently, significant progress has been made in development of image processing algorithms for quantification of

phenotypic parameters (Li et. al. 2014, Fahlgren et. al. 2015, Singh et. al. 2016). Thermal imaging which measures plant temperature, a surrogate for stomatal conductance, can be combined with visual images to differentiate shaded area from leaf area that is fully sunlit and a thermal index based on temperature differences between the canopy and reference surfaces was developed to calculate stomatal conductance and stress monitoring in *Vitis vinifera* (Leinonen and Jones 2004, Möller et. al. 2007). A method for the segmentation and the automated analysis of time-lapse plant images from phenotyping experiments was developed using a plant appearance model implemented with Gaussian mixture models, which utilizes incrementally information from previously segmented instances (Minervini et al. 2014). A fully automatic approach to image based 3D plant reconstruction was developed (Pound et al. 2016). Hyperspectral imaging containing reflectance values of continuous wavebands of the electromagnetic spectrum from Visual (400–700 nm), near-infrared (NIR, 700–1000 nm) and short wave-infrared (SWIR, 1000–2500 nm) are influenced by physiology and biochemical composition of plants. To uncover latent hyperspectral characteristics of diseased plants reliably, the hyperspectral images are converted into a corpus of text documents, and probabilistic topic models were applied to identify content and topics of documents. By this approach, intuitive tool for hyperspectral imaging has been developed to automatically track the development of three foliar diseases of barley (Wahabzada et al. 2016). Multimodal fusion methodology consisting of visual colour images and NIR reflectance images were used to study the dynamic phenotypic responses of a C4 cereal crop plant to nitrogen and water deficiency over time (Neilson et. al. 2015). Machine learning (ML) tools for extracting patterns and features from the image data and multimodal fusion of trait information from different kinds of images need to be developed to enable stress phenotyping (Singh et. al. 2016). Phenome database with curated and labelled phenome data will be useful to expedite both discovery and application in crop breeding (Krajewski et al. 2015).

Although image analysis software is provided by LemnaTec in the phenomics facility, significant research work is needed to develop models to estimate relevant phenotypic traits values from sensor measured digital images. Image acquisition in phenomics is relatively simple but data analysis to retrieve required parameters is not. Several methods are being developed for data analysis. Leaf area measurement is often complicated by overlap of leaves. For precisely measure the leaf area novel computational model called HPGA (High throughput Plant Growth Analysis) was developed to measure leaf area individually by using parameters such as leaf tips and the short curvature areas around them, and length to estimate the area in Arabidopsis (Paine et al. 2012). Since the leaf length-to-area model is genome specific, we need to develop such models for quantification of leaf area in rice and wheat. Some progress has been made in automated multimodal phenotyping using phenomics facility. The Plant Accelerator(®) at The University of Adelaide, Australia, analysed the phenome of sorghum under water-limited conditions with different levels of fertilizer. Using color RGB and NIR images, the study could identify accurately genotypic differences using R scripts based robust parsimonious models (Neilson et al. 2015). Combination of machine learning (ML) algorithms and computer vision appears to be a promising approach. Testing of three different algorithms: k-nearest neighbour (kNN), Naive Bayes Classifier (NBC), and Support Vector Machine revealed that different ML algorithms for segmentation are required for different kind of images (Navarro et al. 2016). A fully automated approach to image based 3D plan tre construction was developed based modelling of complex architecture of leaf surfaces in wheat and rice (Pound et al. 2016). Hyperspectral remote sensing is an automatic, quick and non-destructive method of assessing plant growth parameters, water and nutrient levels in crop plants. Team at IARI has employed different univariate and multivariate models to assess foliar N and relative water contents from captured crop spectra (Sahoo et al. 2015). They also explored physical radiative transfer models for retrieval of plant biophysical parameters like LAI, chlorophyll content and water from hyperspectral Bidirectional Reflectance factor (BRF) data on

Figure 3. Use of drones for phenotyping. Inset shows image obtained from the Drone clearly distinguishing crop with different irrigation and nitrogen treatments.

crops like maize, soybean, wheat and mustard both at controlled field condition and regional scale (Mridha et al. 2015, Tripathi et al. 2012). BRF data were further explored to characterize plant geometry which can further be used for genotypic discrimination and retrieval of morphometric parameters. Visible RGB image was analysed and indexed for assessing leaf area and chlorophyll content (Das et al, 2016). The hyperspectral reflectance data is used for genotypic discrimination, assessment of sugar levels in plants and water-deficit stress levels in rice and wheat crops identifying differential response of genotypes to water deficit stress (Das et al. 2016, 2017, 2018). Team also initiated drone based field phenotyping of rice and wheat genotypes. Recent trends in plant phenomics have been to explore stand alone use of hyperspectral imaging sensors for retrieval different biochemical and biophysical parameters. Fusion and processing of multimodal data acquired through sensor or sensors is many times preferred for assessing parameters with better accuracy which otherwise not possible.

The phenome data from plant phenomics is growing exponentially. One of the major issues the determination of the quality of phenotypic data, i.e. to remove system errors in the data collection process from the biological responses. The Integrated Analysis Platform (IAP) is highly useful for complete end-to-end pipeline for large-scale image based phenotyping, starting from the image capture to image analysis, extraction of relevant phenotypic component traits based on relational models, data management, to the automated generation of experiment reports (Klukas et al. 2014).A dynamic filter was developed to effectively identify the abnormalities in time-series quantum yield of photosystem II phenotype data (Xu et al. 2015). The Plant Genomics and Phenomics Research Data Repository (PGP) was initiated by the Leibniz Institute of Plant Genetics and Crop Plant Research and the German Plant Phenotyping Network. PGP is an infrastructure to comprehensively publish voluminous data on phenomics and genomics, and fulfils the FAIR data principles-findable, accessible, interoperable and reusable. PGP is registered as research data repository at BioSharing.org, re3data.org and OpenAIRE as valid EU Horizon 2020 open data archive (Arend et al. 2016). With a vision of transforming science through data-driven discovery, National Science Foundation, USA funded the establishment of CyVerse in 2015. It provides powerful computational infrastructure to handle huge datasets and complex analyses, thus enabling data-driven discovery in life sciences. CyVerse offers powerful extensible platforms, data storage, bioinformatics tools, image analyses, cloud services,

APIs, etc. (http://www.cyverse.org/). With large number of Institutes in India are involved in genomics and availability of automated phenomics platform in four of the ICAR Institutes viz, IARI, CRIDA, IIHR and NIASM warrant development of data repository similar to that of PGP in India.

7. Phenomics for Crop Management

Besides the tremendous potential of phenomics in basic sciences and crop breeding, it has huge potential in crop management and precision agriculture. The sensors used for non-invasive image acquisition can be loaded in drones and Unmanned Aerial Vehicles (UAVs). Drones and UAVs with different kinds of sensors (RGB Visual, IR Thermal, Multispectral and Hyperspectral) can be used to fly over a large area of crop field to obtain phenotypic information such as phenological stage, crop health, water status, and nitrogen status, etc. (Vergara-Díaz et al. 2016; Gracia-Romero et al. 2017) This information can be useful for variable rate application or precision application of water, nutrients and agrochemicals in the necessary areas of the crop field (Figure 3).

8. Conclusion and Perspectives

Recent advancements in use of NGP with phenomics platform enhanced the phenotyping capabilities as compared to few traits measured by conventional methods. Performance evaluation studies have shown that controlled environment as well as field phenomics is a suitable complementary approach, and in certain cases such as biotic stress, resource use efficiency and positional cloning phenomics can replace traditional laborious field-phenotyping. Besides GWA mapping, phenomics will be very useful in Phenome-wide Association Studies (PheWAS). Significant progress has been made in PheWAS to identify SNP-disease association in medical sciences. The availability of deep phenotypic data in spatial and temporal scale from NGP in phenomics is expected to accelerate PheWAS in plants. Besides, deep phenotypic data from phenomics will be very useful in training genomic selection models more accurately, and thus aid in genomic selection in crops. Further phenome features can also be used for phenomic selection (PS) in analogy with GS as complementary method (Kumar et al. 2016).

We need to develop human resource in the area of image analysis and big data science to effectively use the phenomics for accelerated analytical breeding for crop improvement.

Authorship of this paper should be cited as "Indian Plant Phenomics Network". Participants are arranged by affiliation.
ICAR-Indian Agricultural Research Institute, New Delhi: Viswanathan Chinnusamy, Rabi Narayan Sahoo, S. Naresh Kumar, Gopala Krishnan S, Lekshmy S, Dhandapani R, Sudhir Kumar, Harikrishna, Ranjith Kumar Ellur, Swati Goel, Krishna K Jangid, Rahul Tiwari;
ICAR-Indian Agricultural Statistics Research Institute, New Delhi: Anil Rai, A.R. Rao, Sudeep Marwaha, Sanjeev Kumar;
Indian Institute of Technology, New Delhi: Brejesh Lall, Santanu Chaudhury, Archana Chugh, Swati Bhugra; **ICAR-National Rice Research Institute, Cuttack:** Padmini Swain, Sushanta K Dash, Jitendriya Meher

Acknowledgement

The Nanaji Deshmukh Plant Phenomics Centre is funded by National Agricultural Science Fund, Indian Council of Agricultural Research, New Delhi (Grant No. Phen 2015/2011-12 and Grant No: NASF/CRISPR-Cas-7003/2017-18).

References

1. Al-Tamimi N, Brien C, Oakey H, Berger B, Saade S, Ho YS, Schmöckel SM, Tester M, Negrão S. 2016. Salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping. Nature Communications, 7, 13342, doi: 10.1038/ncomms13342.
2. Arend D, Junker A, Scholz U, Schüler D, Wylie J, Lange M. 2016. PGP repository: a plant phenomics and genomics data publication infrastructure. Database (Oxford). 2016. pii: baw033. doi: 10.1093/database/baw033.

Unlimiting the limited **CROP BREEDING** can save the future of Phosphorus in agriculture

Amrita Thomas
K. P. Abhijith
K. K. Vinod
Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012

Plants require several macro, micro, and trace nutrient elements for proper physiological functions and growth. Nitrogen, phosphorus, and potassium are recognized as the major nutrients besides elements like oxygen, hydrogen, and carbon. Phosphorus forms the com-

ponent of several biomolecules such as nucleic acids, structural compounds, and proteins that regulate physiological and biochemical functions in the plant. Although phosphorus is required in large quantities, its availability to plants is dependent on various soil factors. Despite being present in high quantities in the soil,plant availability of phosphorus often remains limited due to high fixation in the soil. The efficient uptake and utilization of phosphorus from the soil occur in a pH range of 6.5-7.0 in two ionic forms i.e.dihydrogen phosphate (H_2PO_4-) and hydrogen phosphate (HPO_4^{2-}) out of which the former is more prominent. Another important fact that makes phosphorus the most limited macronutrient is its limited mobility in the soil system contrary to the high mobility within the plant. This warrants a continuous supply of phosphorus from the soil because phosphorus gets assimilated within the plants quite rapidly. Since crop production is a continuous process, phosphorus reserves of the soil are continuously being depleted rendering them phosphorus deficient. Therefore, to sustain crop production the external application of phosphorus through fertilizers is essential to replenish the lost phosphorus in the soil.

Phosphate rocks are the only natural source of phosphatic fertilizers in the world. Predominant among the rock phosphate is apatite which is used for the manufacture of fertilizers. Apatites are mineral complexes that contain calcium phosphate along with other elements such as fluorine and chlorine. Fluorapatite is the most commonly mined rock phosphate globally. 95% of rock phosphate reserves of the world are found in the United States, Algeria, Australia, Brazil, China, Egypt, Finland, Morocco, Saudi Arabia, South Africa, and Syria. From the estimates for 2021, the global rock phosphate reserve is approximately 71 billion metric tonnes. It is assumed that at the current rates of consumption of natural rock phosphate the global reserves may last only for about the next 300-400 years.The constant demand for phosphorus fertilizer in agriculture and the continuous mining of phosphatic rock brings us to the reality that there will be a critical shortage in the supply of phosphorus fertilizer and the demand for the same is supposed to peak by 2030. Also, prices of phosphorus-based fertilizers are going to shoot up shortly. Realizing the future crisis of phosphatic rock depletion several countries have put in place an embar go on the export of phosphate

Figure: Low phosphorus tolerance breeding plots at the International Rice Research Institute, Los Banos, Philippines

fertilizers.

Beginning with the idea of Justus von Liebig in 1840, that insoluble phosphates could be converted to soluble forms by treating with sulfuric acid for the use of growing plants, John Bennett Lawes, in 1842 obtained a patent for producing superphosphate. This has led to the discovery of several mineral phosphatic sources such as rock phosphate and subsequently industries producing phosphate fertilizers. By the end of the 19th century industrial fertilizer production has flourished in America and subsequently spread to Europe by the 20th century. This fertilizer revolution has paved way for the easy availability of fertilizers to farmers at a lower cost. The industrial production of phosphate fertilizers begins with the conversion of rock phosphate into phosphoric acid and its conversion to different compounds such as single superphosphate, double superphosphate, triple superphosphate, diammonium phosphate, etc. These compounds can be directly used as fertilizers. Besides, the rock phosphate itself is used as a direct fertilizer in agriculture. The easy availability of phosphatic fertilizers and the green revolution subsequently happened has resulted in the indiscriminate use of fertilizers in the agriculture sector worldwide. It has become a practice to add more than what is necessary into the soil resulting in excess fertilization. Further, the excess addition has lowered the estimates of phosphorus use efficiency in cropping systems. Since the phosphorus use efficiency of crop plants is naturally low, excess fertilizers began to pollute the environment leading to hazards such as the eutrophication of water bodies. Eutrophication is a process that results from leaching off of the phosphorus source from the field into the water bodies resulting in algal blooms and thereby the reduction of the biological oxygen demand (BOD) of the water.

The continuous use of phosphorous from soil has also created another challenge. With the current production rates, approximately 7.0 million tonnes of phosphorus are exported from soil annually through major cereal grains such as rice, wheat and corn. A significant part of the grain exported P is permanently lost and never gets recycled back to agricultural systems. Although global soil phosphorus health maps show that 29 % of the cropland area is phosphorus-deficient and 71% has phosphorus surplus, in India, 49.3% of the districts fall in the category of low phosphorus having <10 kg phosphorus ha-1, and 48.8% of districts fall under the category of medium phosphorus having 10 to 40 kg phosphorus ha-1. The remaining 1.9% is classified under the high phosphorus category with > 40 kg phosphorus ha-1. This continuing shift from high phosphorus status to low phosphorus levels needs to be contained to safeguard future agriculture. For this, phosphorus resources are to be used more judiciously and there is a need to lower the fertilizer input in agricultural systems. However, this is not possible without improvement of crop use efficiency.

The outcome of phosphorus deficiency in crops is seen by the morphological effect of shoot stunting, reduced tillering ability, enhanced root growth and poor yield. The most sensitive parameter to phosphorus deficiency is shoot weight. Low phosphorus efficient genotypes are characterized by enhanced root growth, increased number of tillers, and ultimately higher yield potential under phosphorus-stressed conditions. Plants growing in the phosphorus starvation condition have adapted to the surrounding stresse nvironment by under going changes in the morphology of their root architecture in such a manner as to enhance the phosphorus foraging ability. Mechanism of low phosphorus tolerance in crops is identified to be governed by two independent systems, one associated directly with phosphorus metabolism, and the other related to uptake. Since uptake is primarily governed by the root system, low phosphorus-tolerant genotypes show extensive root adaptations. They include extensive root system to forage from a wider area, exudation of low molecular weight organic acids to facilitate P solubilisation, increased microflora association, increased aeration of rhizosphere matrix etc. Furthermore, roots act as sensors for nutrient deficiency and trigger the genetic response to counter stress.

The nutrient use efficiency of crop plants has two components, uptake efficiency, and assimilation efficiency. Uptake efficiency denotes the quantum of applied nutrients utilized for the building up of internal resources, while assimilation efficiency denotes the quantum of assimilated nutrients used towards crop productivity. Therefore, nutrient use efficiency is defined by the product of both uptake and assimilation efficien-

cies. The efficiency of crops to utilize available nutrients is governed by their genetic makeup. The semi-dwarf varieties that led to the green revolution were fertilizer responsive than the traditional tall cultivars.Crop breeding during the post-green revolution has initially become oriented towards improved yield, resulting in the replacement of landraces and traditional varieties to some extent. Since the selection of new varieties had happened under a nutrient-rich environment, there was a negative selection occurred for low nutrient tolerance in the breeding pool. Therefore, several of the modern high-yielding varieties lack genes for low nutrient stress. Now, efforts are being made to recruit nutrient starvation tolerant genes from traditional cultivars and landraces to improve the modern varieties for their nutrient use efficiency under low nutrient situations.

Several such genes have now been reported in various crops such as OsPSTOL1 in rice, Pht phosphate transporters in wheat and maize, phosphatases associated genes such as PAP genes in soybean, rice, maize and several vegetable species. Amongst these, a classical example is the identification of a quantitative trait locus (QTL) named Phosphorous uptake 1 (Pup1). Identified from anaus genotype, Kasalath that was found to grow well in a biogeographic region in Japan where there is a prevalence of volcanic ash and high phosphorus fixation in the soils, Pup1 is the only major QTL so far reported in rice.Kasalath is a variety of rice landrace from the northeastern parts of India which shows multiple abiotic stress tolerance. A cross was made between Kasalath and Nipponbare to map a region on chromosome 12 which showed sufficient phenotypic variance for low phosphorus tolerance. The sequencing of the Kasalath genome has led to the discovery of a 90kb Indel (insertion-deletion) region which was lacking in Nipponbare harbouring Pup1 QTL. The candidate gene involved in low phosphorus tolerance was later identified as OsPSTOL1 (Phosphorus starvation tolerance 1), coding for a serine-threonine protein kinase, driving the proliferation of the roots under low P conditions. Many post-green revolution mega rice varieties such as IR64, IR74, MTU1010, Samba Mahsuri, and Pusa 44,are found to be sensitive to low P conditions. Breeding efforts that are directed towards incorporating QTLs such as Pup1in the background of these varieties have provided promising results, showing improved phosphorus use efficiency and improved resilience under phosphorus-limited situations, making these varieties better suitable for marginal lands where limitation of phosphorus is common due to variety of reasons.

Availability of such improved varieties will prove to be a boon for Indian agriculture interms of extensive cultivation, conservation of resources and environmental security.

Suggested reading

Raghothama, K.G. and Karthikeyan, A.S., 2005. Phosphate acquisition. Plant and Soil, 274,37-49.

Fageria, N.K., He, Z. and Baligar, V.C., 2017. Phosphorus Management in Crop Production. CRC Press.

Cordell, D., Drangert, J.O. and White, S., 2009. The story of phosphorus: global food security and food for thought. Global Environmental Change, 19, 292-305.

Vinod, K.K. and Heuer, S., 2012. Approaches towards nitrogen-and phosphorus-efficient rice. AoB Plants, 2012.

Wissuwa, M., Kondo, K., Fukuda, T., Mori, A., Rose, M.T., Pariasca-Tanaka, J., Kretzschmar, T., Haefele, S.M. and Rose, T.J., 2015. Unmasking novel loci for internal phosphorus utilization efficiency in rice germplasm through genome-wide association analysis. PLoS One, 10, p.e0124215.

Wissuwa, M., Yano, M. and Ae, N., 1998. Mapping of QTLs for phosphorus-deficiency tolerance in rice (Oryza sativa L.). Theoretical and Applied Genetics, 97, 777-783.

Rose, T.J. and Wissuwa, M., 2012. Rethinking internal phosphorus utilization efficiency: a new approach is needed to improve PUE in grain crops. Advances in Agronomy, 116, 185-217.

Gamuyao, R., Chin, J.H., Pariasca-Tanaka, J., Pesaresi, phosphorus., Catausan, S., Dalid, C., Slamet-Loedin, I., Tecson-Mendoza, E.M., Wissuwa, M. and Heuer, S., 2012. The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. Nature, 488, 535-539.

López-Arredondo D.L., Leyva-González M.A., González-Morales S.I., López-BucioJ., Herrera-EstrellaL. (2014) Phosphate nutrition: Improving low-phosphate tolerance in crops. Annual Review of Plant Biology 65:1, 95-123

Dolamani Amat[*1]
Livleen Shukla[2*]
Sunil Pabbi[2*]
[*1.] Scientist (Senior Scale),
[*2.] Principal Scientist, Division of Microbiology, ICAR- Indian Agricultural Research Institute, New Delhi, India- 110012

XYLOOLIGOSACCHARIDES

An Emerging Prebiotic From Lignocellulosic Biomass

Diet is an important omponent for maintaining humans and other animals' health as it not only provides nutrition but also helps in prevention of many diseases by improving the immunity. Discovery of microorganisms in colon is a breakthrough as these have been found to exert beneficial effect. Prebiotics are the nutrients that help in proliferation of these colonic microfloras as these are used by them as food source. There are different types of prebiotics and among them xylooligosaccarides (XOs)that can be produced from lignocellulosic biomassesare considered as a fast emerging class. Lignocellulosic biomass includes, agricultural residues (sorghum strover, corn strover, paddy straw etc.) agro –industrial waste (wheat bran, rice bran,corn cobs etc.), forest biomasses, energy crops (switchgrass, alfalfa) and wastes from paper and pulp industries. Various techniques (chemical, autohydrolysis and enzymatic) have been employed for production of different XOs each having its own advantages and disadvantages.

Prebiotics

The prebiotics are generally non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve the host's health(Gibson &Roberfroid, 1995). The beneficial microbes in the colon carryout fermentation of the prebiotic compounds resulting in the production of short chain fatty acids which act as source of fuel for different cells in the body, regulates different cellular activities and also results in decreasing the pH in the colon.

Xylooligosaccharides as prebiotics

Stowell (2007) classified prebiotics as:

1. Established prebiotics: galacto-oligosaccharides (GOS), fructo-oligosaccharides(FOS), Inulin, polydextrose and lactulose

2. Emerging prebiotics: xylooligosaccahrides (XOs),

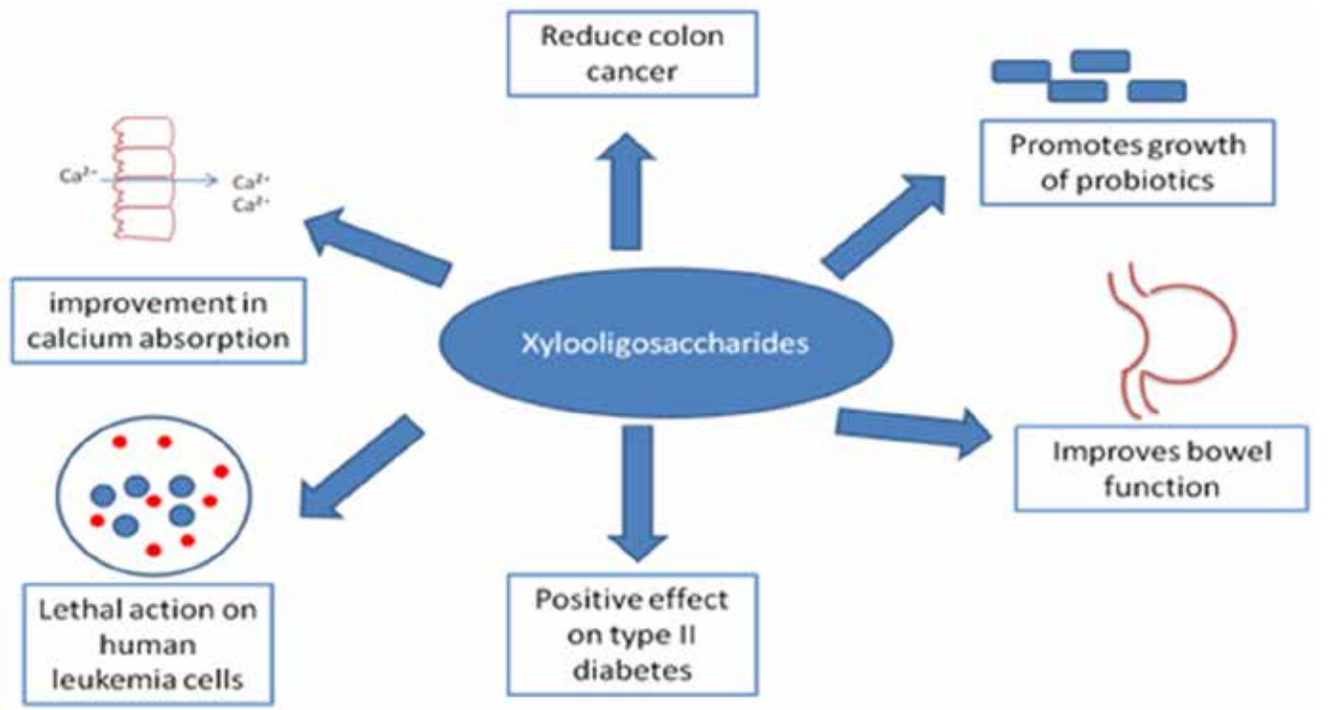


Fig. 1 Multifaceted benefits of xylooligosaccharides

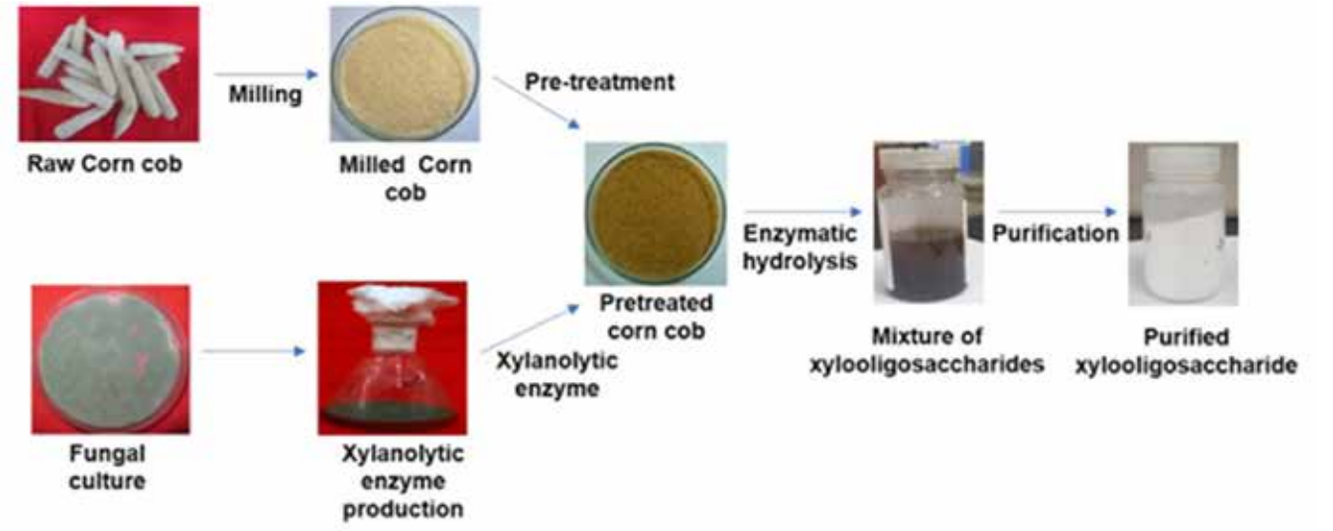
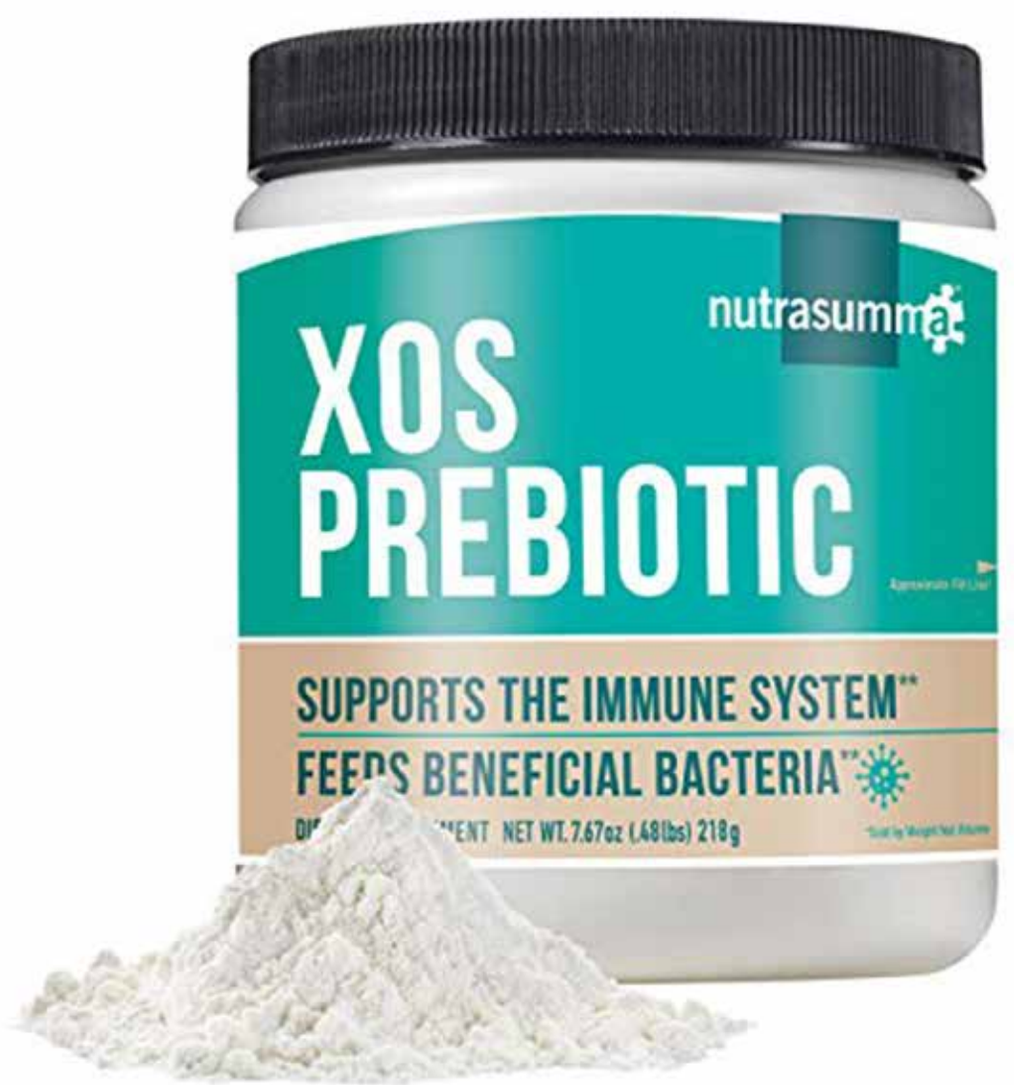


Fig.2. Xylooligosaccharides production process



isomalto-oligosaccharides (IMO), and lactitol Xylooligosaccharides (XOs) are sugar oligomers having 2-10 xylose molecules linked by β-1, 4 xylosidic linkage that form the backbone and may be substituted by arabino-furanose, glucuronic acid and acetyl group depending upon the source and method of production. XOs containing 2-5 xylose units (xylobiose, xylotriose, xylotetrose and xylopentose) exert prebiotic effects when taken with the diet. These are neither digested nor get absorbed in the upper portion of the gastrointesinal tract and cause selective stimulation of growth and activities of few bacteria in the colon region

and thus help in improvement of health of the host.Gastric juices don't cause breakage of the XOs and thus these are metabolized in the large intestines. Thus, xylooligosaccharides have remarkable potential of being novel prebiotics, having many exceptional benefits (Fig.1) apart from promoting growth of probiotics such as Lactobacillus spp., L. brevis, L. fermentum and L. acidophilus, Bifidobacteriumspp.

XOs have advantages over other common prebiotics like fructo-oligosaccharides and inulin in food processing industries with respect to acidity and heat resistance thus can be used in juices having low pH.They can also be incorporated in food items like cocoa drinks, soft drinks, tea, soy milk, nutritive preparations, dairy products like milk, yogurt, powdered milk, jam, jelly, candies and honey products for formulation of health foods for small children. These can also act as low calorie food for old age groupsas they exert considerable biological effects with low daily consumption. Lignocellulosic materials (LCMs) as source of Xylooligosaccharides

Xylooligosaccharides are naturally present in low quantity in fruits, vegetables, bamboo shoots, milk and honey but canalso be generated commercially by different methods like enzymatic (Fig. 2), chemical hydrolysis and chemo-enzymatic hydrolysis of xylan from various lignocellulosic biomasses like wheat bran, wheat straw, corn strover, corn cobs, rice hulls, barley hulls, brewery spent grains etc. The plant materials i.e. lignocellulosic biomasses are mainly made up of cellulose, hemicelluloses and lignins (LCM) and depending on the

source, the LCM composition varies, the average being : cellulose (30-50%), hemicellulose (20-40%) and lignin (15-25%) of the total dry matter. In agricultural residues, xylanis the most abundant of all the hemicellulosic componentswhich is a heteropolysaccharide having xylose units linked by β-1, 4 xylosidic linkage and forms the backbone. Corn cobs, an important by-products of corn industries are used as feed for the animals or used in the crop field after composting, possesses highest amount of xylan(~up to 35%). Corncobs have the potential for value addition by utilizing it for producing xylooligosaccharides(Amat and Shukla, 2021; Aachary and Prapulla, 2009), xylose and xylitol.Corncobs are first dried, milled to reduce the particle size followed by pretreated using alkali, acid or steam andfinally enzymatic hydrolysis of the pretreated substrate is carried out using xylanase from bacteria (Bacillus, Clostridium, Fibrobacter, Ruminococus)/actinomycetes (Streptomycetes)/fungi (Aspergillus, Trichoderma, Chytridiomycetes, Phanerochaetes, Thermoascus)under suitable conditions. The enzymatic hydrolysate contains mixture of xylooligosaccharides with different degree of polymerization (DP) ranging from 2-6. Adsorption on active solid surfaces is generally used in combination with solvent elution steps for fractionating oligomers from monomers along with the removal of other undesirable contaminants. Commonly used adsorbents for purifying XOs include activated charcoal, acid clay, bentonite, diatomaceous earth, aluminum hydroxide/oxide, titanium, silica, and porous synthetic materials. Chromatographic separation for XOs purification yields analytical grade high-purity fractions. The ultrafiltration and nanofiltration based technique is currently the most promising downstream processing strategy for preparation of high purity and concentrated oligosaccharides.Depending on different sources used for generating XOs, their structure and properties vary in terms of degree of polymerization and the type of linkages present.

Conclusion

Xylooligosaccharides as prebiotic has many health benefits but its wider use for human and animals need further studies. Of the different agri-residues, corn cobs is the best substrate as it contains highest amount of xylan.

Andenzymatic method is found to be best method for xylooligosaccharides productionas it doesnot results in undesirable by- products. However, since the reaction time is more, there is need to optimize the process. Lignocellulosic biomass is recalcitrant in nature; a suitable pretreatment method is required to expose the xylan to the endoxylanase. Further, for higher production of xylooligosaccharidesthrough enzymatic methods, requirement of novel microorganism which can produce higher titre of xylanase with lesser β-xylosidase. No doubt, XOs with their natural origin are considered as a very good prebiotic source, the optimization of process parameters is the need of hour. Finally, an economically viable technology is required for large scale production of xylooligosaccarides by enzymatic method.

References

1. Aachary, A. A. and Prapulla, S. G. (2009), Value addition to corncob: production and characterization of xylooligosaccharides from alkali pretreated lignin-saccharide complex using Aspergillus oryzae MTCC 5154. Bioresour. Technol., 100(2): 991-995.

2. Amat, D. and Shukla, L (2021). Evaluation of different pretreatment methods for xylooligosaccharides production. Int. J. Curr. Microbiol. Appl Sci., 10(1):517-527

3. Gibson, G.R. and Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J.Nutr. 125, 1401 -1412.

4. Stowell, J. (2007). Calorie control and weight management, In Mitchell, H. (Ed.), Sweeteners and sugar alternatives in food technology (pp. 54), Blackwell Publishing Ltd., Oxford, UK.

Dr. Rajeswari T
M.V.Sc. Ph: 9446424429

INFERTILITY IN DOMESTIC DOGS

The first domesticated animals were dogs. It is estimated that there are approximately 360 recognized dog breeds worldwide. These range from the smallest breed, the Chihuahua, to the largest breed, the Great Dane. Although some have formed through natural evolution over many years, all modern breeds have been derived from several breeds through careful breeding. The diversity among dog breeds is greater than among humans. Domesticated

dogs usually reach sexual maturity at the age of 6 to 12 months. Smaller breeds mature much earlier than larger ones. Larger ones take about 2 years to reach breeding age. Female dogs usually have estrus only twice a year. There are also breeds of dogs that have estrus only once a year.

A dog's estrus cycle has four stages

First stage: Proestrus; The time just before estrus. It lasts for about 9 days. At this time you will see blood-tinged vaginal discharge. The female will not voluntarily allow mating. The vulval lips are swollen and the mucosa appears rose-pink in color.

Second Stage: Estrus; Lasts for about 9 days. Vaginal secretions contain less blood. At this time the female will be willing to mate voluntarily.

Third stage: Metestrus; Vaginal secretions will be completely absent and the female will be reluctant to copulate. At the beginning of this stage, the cervix closes.

Fourth stage: Anestrus; The interval from one estrus cycle to the next.

Infertility in female dogs

Normal fertility in a dog, and the ability to reproduce puppies, requires a normal estrous cycle, with a healthy reproductive tract, normal ova, stable levels of reproductive hormones, fertilization by normal spermatozoa, implantation of embryos, normal placental placement, stable levels of progesterone. These conditions must be maintained throughout the two-month gestational period, or the process of the reproduction will be altered, with resultant infertility.

Infertility in female dogs is the condition in which the female is mated more than once near the time of ovulation with a fertile male dog and does not conceive, or does not give birth to healthy pups even after conception. Most major infertility problems are complex. Often, several factors, singly or in combination, can cause failure to produce offspring.

What needs to be taken care of before breeding?

Medical and surgical history: Before planning a mating the medical history and the bitch's age, physical condition, temperament, nutrition, supplements, medications, vaccination history, housing, and health testing should all be evaluated. what is acceptable will vary with each breed and with the area of the country where the dogs live. More specifically, significant prior medical or surgical conditions should be discussed to determine what if any impact they may have on pregnancy or lactation.

Body condition: It has a significant impact on fertility. More sedentary and overweight bitches will have more issues with fertility, ovulation rate, and ability to whelp normally.

Reproductive health history: Once we have confidence in the bitch's general health it is important to assess the reproductive health history like parity (the number of prior litters), cycle history including inter-estrus intervals, prior breeding history (when breedings occurred, types of breedings, what breeding management performed (includes looking at prior timing; just because progesterone tests were run doesn't mean they were correctly interpreted or followed through ovulation; male fertility; proven or not, semen evaluation and when performed); was the bitch pregnant (how was this determined;u/s, x-ray, appearance, whelping); the number of pups; ability to deliver naturally and the time it took to delivery; any stillbirths, mummies or SGA (small for gestational age) pups; mothering behavior; was lactation normal, etc.

General physical examination: It should be performed to assess all body systems, hematology (CBC), thyroid evaluation, pedigree analysis, assessment of mammary glands; assessment of perineal conformation, vulvar edema, and digital examination of the vulva, vestibule, and v/v junction, etc.

Major causes of infertility

Deficiencies in breeding management and infertility in male dogs are the major cause of infertility.

Infertility problems in female dogs can be broadly classified into four categories.

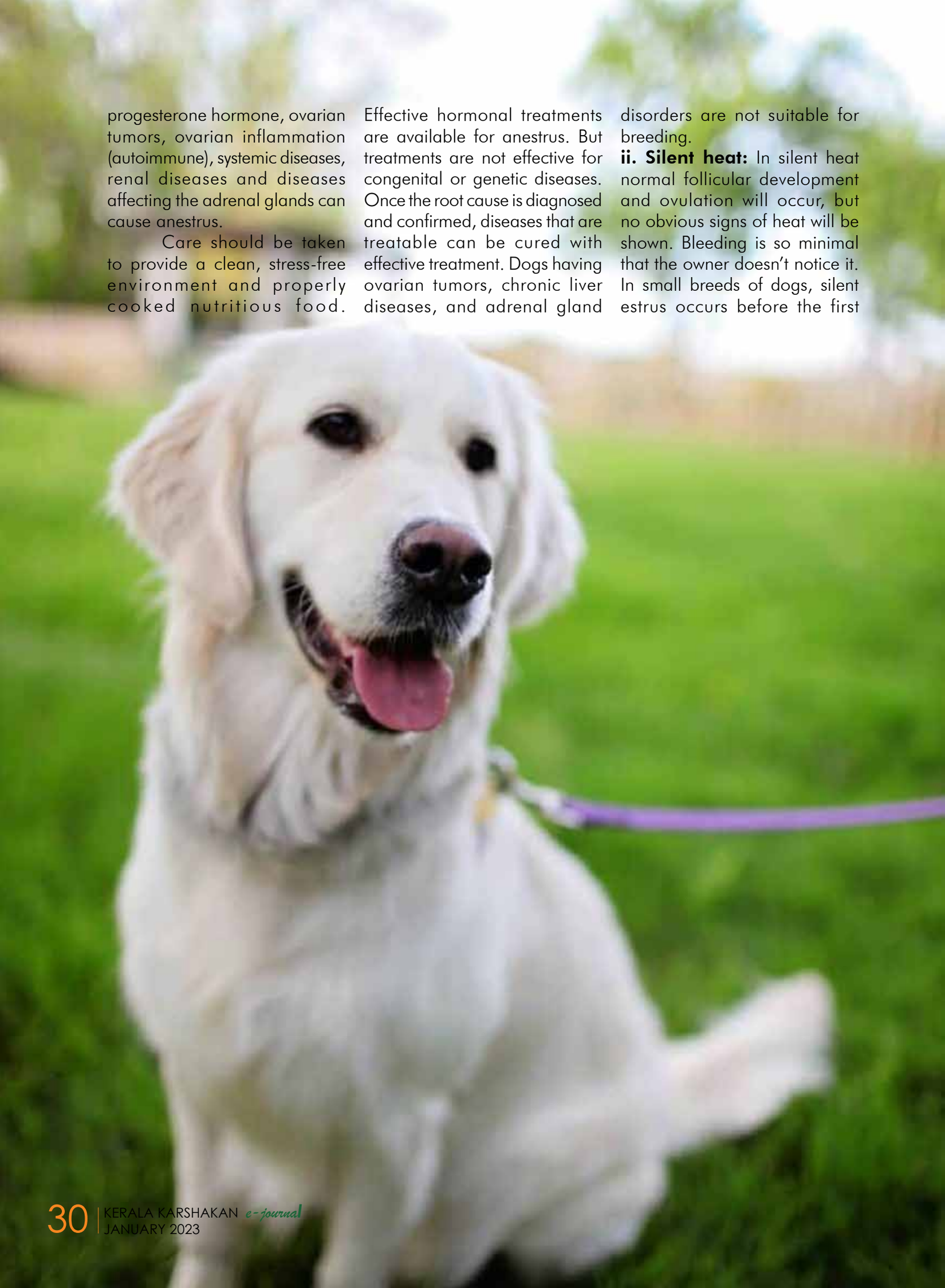
1. Disorders of the estrus cycle

i. Anestrus: In this condition, a complete absence of the signs of estrus is seen. Disorders of sexual differentiation, malnutrition, excessive physical activity, obesity, use of drugs that affect fertility (progesterone or glucocorticoids), nonexposure to females in estrus, congenital absence/defects of ovaries, congenital or non-congenital thyroid hormone deficiency, ovarian cysts that produce

progesterone hormone, ovarian tumors, ovarian inflammation (autoimmune), systemic diseases, renal diseases and diseases affecting the adrenal glands can cause anestrus.

Care should be taken to provide a clean, stress-free environment and properly cooked nutritious food. Effective hormonal treatments are available for anestrus. But treatments are not effective for congenital or genetic diseases. Once the root cause is diagnosed and confirmed, diseases that are treatable can be cured with effective treatment. Dogs having ovarian tumors, chronic liver diseases, and adrenal gland disorders are not suitable for breeding.

ii. Silent heat: In silent heat normal follicular development and ovulation will occur, but no obvious signs of heat will be shown. Bleeding is so minimal that the owner doesn't notice it. In small breeds of dogs, silent estrus occurs before the first



estrus. Autoimmune diseases can also cause silent estrus.

Blood progesterone levels can be checked every month to see whether the ovaries are functioning. (Serum progesterone/ SP4 level above 2 ng/mL). Biweekly testing of Exfoliative Vaginal Cytology (EVC) can detect estrus and the SP4 test can detect the time of ovulation.

iii. Prolonged estrus: In prolonged estrus, estrus signs last longer than usual (more than 21 days). Ovulation does not occur. The main reason is the increase in estrogen hormone levels. Ovarian tumors, cysts, estrogen hormone injections, the presence of phytoestrogens, insufficiency of other hormones, and various types of stress can cause prolonged estrus.

More than half of the disorders are reversible with proper diagnosis and hormone therapy. Those bitches with ovarian tumors and adrenal gland disorders are not suitable for breeding.

iv. Split heat: After showing proestrus signs, there are no signs of estrus in the following days. After a gap, the estrus signs will be shown again. This is usually seen in dogs showing estrus signs for the first time. Ovulation and pregnancy occur naturally in the estrus seen in the second round. There are dogs that show 'split heat' in every heat cycle. Rarely, split heat is caused by thyroid hormone deficiency and ovarian dysfunction. Effective

treatment can be done if the actual condition is diagnosed.

v. Irregular estrus cycle: This is due to fluctuations in hormone production and genetic disorders. Irregular hormone production indicates ovarian dysfunction. In such a condition, estrus signs will not be shown in time.

vi. Prolonged Interestrus Interval: The interval from one estrus cycle to the next is overly delayed. Delayed puberty, worm infestation, malnutrition, underdeveloped ovaries, inflammation of ovaries, congenital ovarian defects, and ovarian cysts (progesterone producing) are the main causes.

vii. Short Interestrus Interval: Ovarian disorders and cysts are the main causes.

2. Reluctance to mate

Dogs usually mate voluntarily only around the time of ovulation. Forced mating does not result in pregnancy. Due to some special characteristics of male and female dogs (Male preference / Female preference), they may be reluctant to mate.

Female dogs may also be reluctant to mate due to unfavorable environments, arthritis, congenital or genetic disorders of the vaginal canal, wounds, tumors, etc.

3. Failure to conceive: The reason for the failure to conceive even after mating with fertile male dogs around the time of ovulation or artificial insemination with their sperm may be due to congenital or genetic disorders of the reproductive organs, uterine infections, uterine polyps, and tumors. Treatment by people who are not skilled in hormone therapy and overuse of hormones can also cause uterine disorders. Estrogen hormone used for contraception or tubal locking, progesterone hormone used to hide estrus signs, and the presence of estrogen hormone in food and supplements can also cause infertility.

Since the cervix is open during estrus, germs can enter the uterus. In immunocompromised dogs, they can multiply in the uterus and cause pyometra. In this connection, the ability to conceive is lost due to structural defects occurring in the inner lining of the uterus (endometrium). Treatment for infertility is not effective for this cause.

4. Abortions

Even if the animal conceives, fluctuations in hormone production, hormonal insufficiency, imbalance, deficiency of thyroid hormone, diabetes, steroid medication, and diseases affecting the reproductive organs can cause abortion. In addition to this many other reasons can cause infertility.

How to diagnose?

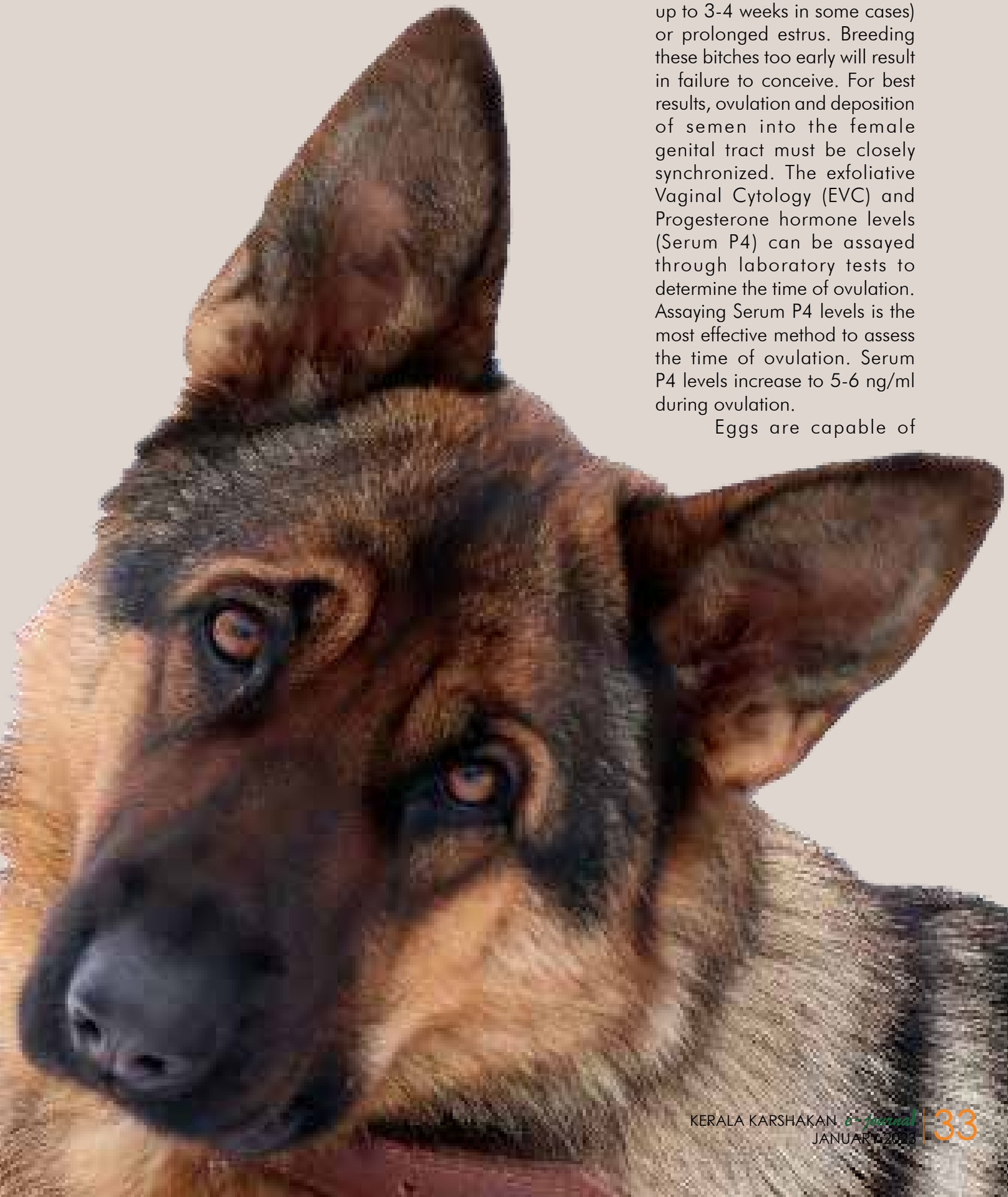
Tests commonly done to diagnose the cause of infertility include Blood count (CBC), screening for contagious diseases (Brucella, Mycoplasma Ureaplasma Screening, etc.), Hormonal tests (Progesterone, Oestrogen, Relaxin assay), X-ray, Ultrasound, EVC, Culture, and sensitivity test to determine the etiology and effective antibiotic. Hormonal disorders can be corrected with treatment. Ovarian cysts and tumors can be treated with surgery or hormone therapy. Ovulation timing can be determined through laboratory tests like EVC and Serum Progesterone.

What needs to be taken care of to prevent infertility in female dogs?

Defects in the estrus cycle can be diagnosed and treated appropriately or deficiencies in the management corrected. The most common cause of infertility in the bitch is improper or inadequate breeding management. Infertility treatment can only be effective if deficiencies/flaws in management are eliminated. Breeding based on receptive behavior or on the set day of a cycle may result in breeding outside the bitch's fertile period and this may result in decreased litter size or failure to conceive. Receptive behavior is a result of the change in estrogen: progesterone ratio and this doesn't always correlate with ovulation. Since dog semen has particularly good longevity, breeding a bitch prior to ovulation by a few days may still result in pregnancy because sperm will live in the bitch's tract for several days, but if semen quality is decreased or breeding occurs too early or after the bitch's fertile period, pregnancy rate and litter size will suffer. Most bitches will be in proestrus for 9 days, with the LH surge happening at the end of this stage, and then will be in estrus for 7-9 days. The fertile period is the last 4-5 days of estrus. At the very end of estrus or day 1 diestrus, the cervix will close to sperm, so that natural breeding or artificial insemination will not result in pregnancy. Not all bitches follow a textbook cycle, sometimes the first days of proestrus are missed because there is little bleeding or swelling, and sometimes proestrus may be

abbreviated, thus affecting when the fertile period may begin; alternatively, some bitches may have prolonged proestrus (lasting up to 3-4 weeks in some cases) or prolonged estrus. Breeding these bitches too early will result in failure to conceive. For best results, ovulation and deposition of semen into the female genital tract must be closely synchronized. The exfoliative Vaginal Cytology (EVC) and Progesterone hormone levels (Serum P4) can be assayed through laboratory tests to determine the time of ovulation. Assaying Serum P4 levels is the most effective method to assess the time of ovulation. Serum P4 levels increase to 5-6 ng/ml during ovulation.

Eggs are capable of



fertilization only 2-3 days after ovulation. As long as the female is ready to mate, mating can be done at 48-hour intervals. Spermatozoa remain motile in the female reproductive organs (uterus and fallopian tubes) for 5-7 days and are capable of fertilization. Infertility can be prevented to some extent by mating around the time of ovulation. If artificial insemination (AI) is performed, the timing of insemination should be done according to the mode of preservation of semen (Fresh, Refrigerated, Deep Frozen).

Hormonal induction of estrus is being done in dogs with high breeding values without giving adequate inter-estrus interval (IEI). IEI is the period where endometrium prepares for the next estrus cycle and pregnancy. If this interval is too short, it adversely affects the ability to conceive.

Treatment for Brucella infection is ineffective. It can be passed on to male dogs during mating. In addition, brucellosis is a zoonotic disease. Diseased dogs should not be used for breeding.

Infertility in male dogs

Another common cause of infertility is male factor infertility. Multiple matings with fertile female dogs close to ovulation and failure to conceive may be due to male infertility.

What are the reasons?

Male dogs are reluctant to mate for a number of reasons; lack of experience, nonattainment of sexual maturity, incomplete physical development, adverse environment, anxiety, slippery floor, physical incapacity, arthritis, diseases of the spine and hind legs, female preference, aggressive females, and female dominance. Even if the mating is successful, ejaculatory disorders and diseases affecting the penis can cause infertility. Failure to ejaculate and retrograde ejaculation(ejaculation to the urinary bladder) are the main problems caused.

Too few sperm, poor motility, or abnormal sperm may all result in poor fertility. Semen evaluation of the male prior to breeding is always recommended, but if it wasn't done before breeding and the bitch fails to conceive, it should be done after the bitch is determined to be not pregnant. Sometimes this can be overcome by intrauterine insemination or multiple inseminations, but in other cases, infertility may be too severe. Azoospermia, hypospermia, reduced motility, and structural defects may result in poor fertility. Similarly, bilateral obstruction of the sperm ducts can cause azoospermia and infertility. High environmental temperature and excessive conditioning resulting in increased body temperature can induce either temporary or permanent azoospermia. Scrotal dermatitis can have the same result.

Disorders of sexual differentiation result in infertility. Infertility can also be caused by diseases affecting the prostate glands and testicles, thyroid hormone deficiency, hormone imbalance, and indiscriminate use of hormones. Most of the causes of infertility (25 - 40%) are related to the prostate gland; prostatic hyperplasia (BPH), prostatitis, Lumps, and Cysts in the prostate, etc.

How to diagnose?

The cause of infertility in male dogs can be determined through blood tests (CBC), urinalysis, semen testing, brucella screening, ultrasound of the reproductive system, hormone analysis, and culture and sensitivity tests can be done to detect prostatic infection, and suitable antibiotics.

What needs to be taken care of to prevent infertility in male dogs?

It is effective to replace female dogs that are not interested in mating (male preference), use others, and provide environments that are conducive to mating. Kennel management should allow breeding males to remain cool during the summer. If necessary, artificial insemination can be performed for genetically superior female dogs using the semen of genetically superior males. Hormonal imbalances, malnutrition, and infections can be treated and cured. Azoospermia, oligospermia, reduced motility, and structural defects are difficult to treat.

Brucella infection can be transmitted to female dogs during mating. It is also a zoonotic disease for which treatment is ineffective. Do not use infected dogs for breeding. Up to 90% of infertility causes in domestic dogs are due to deficiencies and errors in breeding management. Confirmative diagnosis and correction of deficiencies in the management is the key to infertility control.

DECORATING BEHAVIOUR IN INSECTS

Sachin M. S [1]
Guruprasad G. S [2]
Bhuvaneshwari [3]
[1] Ph. D. Scholar, Dept. of Entomology, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga.
[2] Associate professor., Dept. of Entomology, University of Agricultural Sciences, Dharwad.
[3] M.Sc.Agri., Dept. of Entomology, University of Agricultural Sciences, Dharwad.

Introduction

Any animal that actively attaches foreign material to itself or to its biogenic structure. Thus, we exclude the passive accumulation of debris and structure building itself; for example, a polychaete tube of mucous-bound sand is not decorated, whereas a tube which is enhanced with shell and algal fragments is decorated. The behaviour that we call decorating has variously been called covering, ornamenting, masking, hatting, carrying, shield-carrying and trash-carrying.

Decoration in aquatic organisms

Crabs: Wicksten documented carrying behaviour in at least four families of brachyuran crabs. This involves shorter

Crabs

fifth and sometimes fourth legs that are no longer used for locomotion but to lift an object (e.g., a shell, piece of sponge or coral, or rock) over the dorsal aspect of the posterior part of the carapace. She speculated that this may act as a physical barrier against predators, as visual or chemical camouflage, or as food storage, but no direct evidence has been offered in support of any of these functions (fig 1)

Caddisfly larvae: The larvae of many caddisfly (insect order Trichoptera) construct cases out of various environmental materials bound together with silk. These cases are carried around, and even when feeding or moving most of the organism remains inside the case (fig 2)

Green lacewing larva: Larvae of some green lacewing species place extraneous materials (dead aphids and aphid's exuviae etc.) onto carrying structures that are located on the dorsal surface of the metathoracic and anterior abdominal tergites. This 'trash-package' of the green lacewing has been assumed to serve as an avoidance strategy against predation. (Fig3)

Assassin bug nymph: *Acanthaspis petax nymphs* create a large mound on their backs consisting predominantly of ant carcasses, but other small insect corpses, and bits of plants are often included. This is called masking (when an animal applies materials from the environment to its body), more specifically this is a type of masking called corpse camouflage. The nymphs use this camouflage to hide from predators and aid in the capture of their prey. (Fig 4)

Bag worm larva: The bagworm family Psychidae (*Lepidoptera: Tineoidea*) larvae construct portable cases. The materials used for constructing bags differ among bagworm species; e.g., tree/herb/grass leaves, lichens, twigs, petioles, bark fragments, wood debris, and sand particles. The portable bags are generally believed to play an important role as portable armour against natural enemies.(Fig 5)

Tortoise beetle grub: These larvae do not drop their exuviae after each of their four to five moults, but collect them, in many cases together with their faeces, on two spines at their 8th abdominal tip. The faecal shield varies in appearance and physical consistency in different larvae. The shields are highly manoeuvrable, capable of being oriented by the disturbed larvae in virtually any direction through rotation and flexion of the abdomen. (Fig 6)

Assassin bug nymph

Tortoise beetle grub

Decoration in extinct insect *Hallucinochrysa diogenesi* (Neuroptera: Chrysopidae)

In Chrysopidae, larvae exhibit the trash-carrying behaviour. There is evidence suggesting that these adaptations evolved several times within Chrysopidae. Such adaptations are present in the fossil, as a gibbous (humped) body, adapted for moving while carrying great loads. However, two of these adaptations are strikingly peculiar.

Hallucinochrysa diogenesi has: (i) pairs of dorsal setigerous tubercles, but these are extremely elongated, tubule-shaped; and (ii) setationon the tubular tubercles, but representing a special system for ensnaring the trash packet components. The trumpet-shaped setal endings and act as anchoring points among surfaces of tangled trichomes, whereas the extremely fine distal portions of the setae facilitate the setae to become flexible and to bend by gravity, enhancing their tangling and anchoring capacity.

In extant trash - carrying larvae, the lateral tubercles are often much more developed on the thorax than the abdomen, and the lengths of the thoracic tubercles never exceed the body width. By contrast, whereas those of the fossil are similarly developed on the thorax and

Caddisfly larvae

Green lacewing larva

abdomen, some of the preserved tubular tubercles not only exceed the body width but also the entire body length.

Materials used for decoration:

- Twigs, Petioles, Bark fragments, Wood debris, Sand particles used by caddisfly larva.
- Waxy secretions from woolly alder aphids/exuviae of aphids used by green lacewing larva.
- Rock, Stick, Leaf material used by bag worm larva.
- Exuviae / Feaces by tortoise beetle grub.
- Carcasses of its prey by assassin bug nymph.

Why do they decorate?

- Decoration broadly serves two functions that is protection and feeding. Protection can serve against predators or abiotic forces.
- Decoration may cover vulnerable body surfaces, thereby protecting against ultraviolet (UV) radiation, sedimentation, thermal stress, or other physical forces.

Conclusion:

Decoration is particularly diverse activity and it is difficult to produce an unambiguous definition that covers all cases effectively. Even though decoration has been studied under many taxa but there is a limited understanding of benefit and cost associated with this behaviour. Hence, there is a need to know the cost associated with this behaviour. Also, in many cases the benefits assumed rather than demonstrated. Till now the significance of this adaptation studied in relation to anti-predatory behaviour. Hence there is need to study how decoration helps to get protection from the abiotic factors such as temperature and harmful radiations.

Bag worm larva

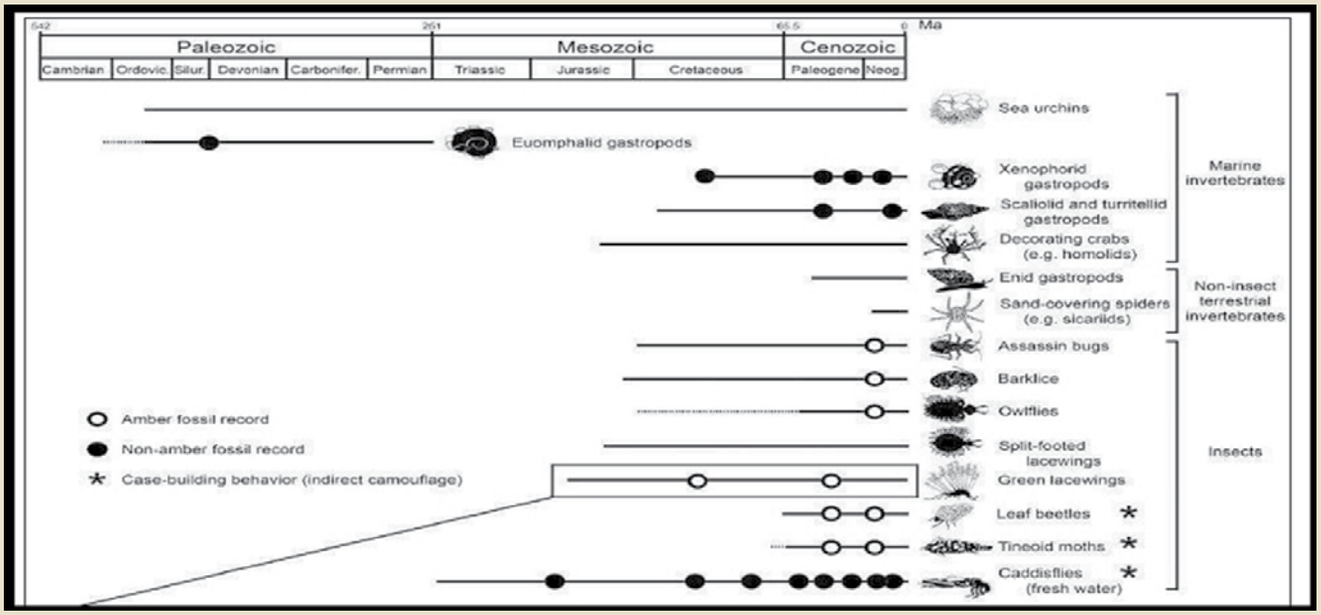
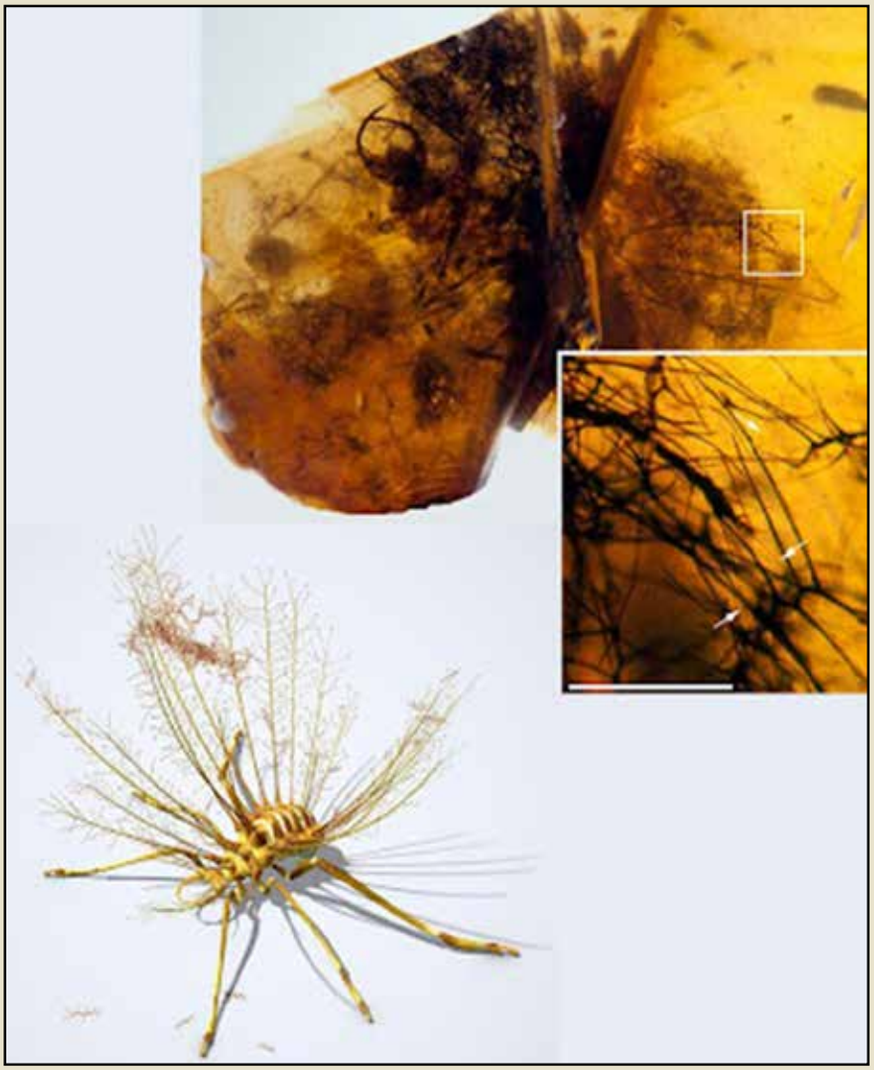


Fig. 7: Insect and other invertebrate groups in which th camouflaging behaviour by actively harvesting and carrying exogenous materials

- The decorating behaviour is exhibited by various marine invertebrates, non-insect terrestrial invertebrates and insects.
- Marine invertebrates are non-amber fossil records eg: Xenophorid gastropods from Mesozoic to Cenozoic period /era. Non insect terrestrial invertebrates like sand covering spiders are found in Cenozoic era.
- Insects from amber fossil record includes green lace wings, Assassin bug, Bark lice, Owl flies and case building behaviour exhibited byLeaf beetles, Tineoid moths, Caddis flies from Mesozoic to Cenozoic period (Fig.7).

Hallucinochrysa diogenesi



Chaithra T. N.[1]
Poornima G.[1]
Mahendiran G.[1]
Ramesha B.[2]
Nishalekshi V.[3]
[1]Division of Germplasm Collection and Characterization, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka
[2]Kerala Agricultural University, Extension Training Centre, Manjeshwar
[3]Kerala Agricultural University, Regional Agricultural Research Station.

RED PALM WEEVIL

Rhynchophorus ferrugineus (Olivier, 1791) (Curculionidae: Coleoptera): Keep them at bay

Introduction

Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier, 1791)native of South Asia was first reported as pest of Indian coconut palm in 1906 by Lefroy and the date palm in 1917 by Brand (Dosary et. al., 2016). They are commonly called as Asiatic palm weevil, coconut weevil, red palm weevil, Indian palm weevil, red stripe weevil, Indomalaiischer Palmen Ruessler (German), Picudo asiático de la palma (Spanish), Charançon asiatique du palmier (French). In the mid-1980s this pest was identified from Gulf region where it spread rapidly to other date producing countries through infested planting material (Faleiro et al., 2012). The

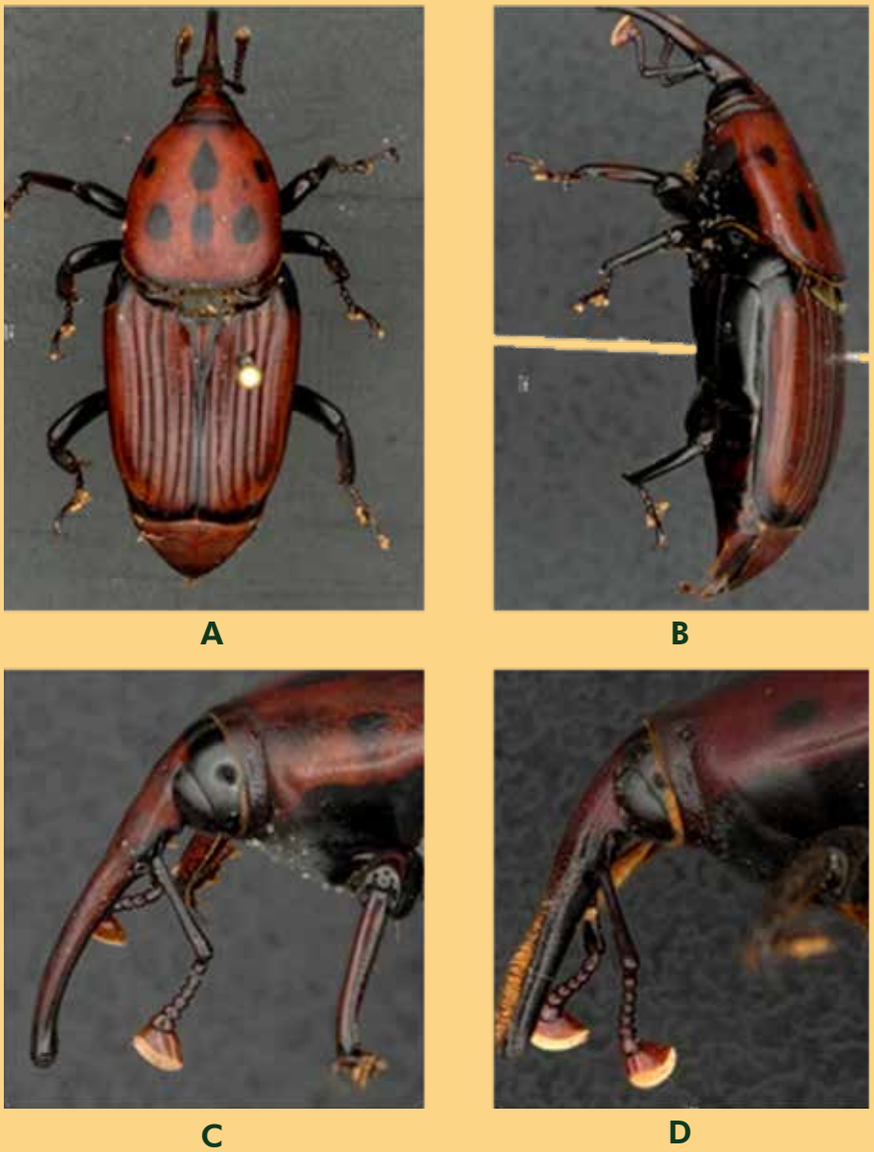


Fig 1. Habitus photographs of Red palm weevil, A. Dorsal view; B. Lateral view; C. Rostrum of female without tuft of hair; D. Rostrum of male with tuft of hair

geographical range of RPWis wide and is now reported from several countries of Asia, Africa, Europe, America and Oceania. RPW has been reported from 50 percentand 15percent of date palm and coconut growing countries all over the world respectively (Faleiro et al., 2012). This pest has wiped out many palm farms in various countries. FAO labelled RPW as a Category-1 pest in the Middle East and North Africa, where it is threatening the livelihoods. In March 2017, FAO through Rome Declaration called for the urgent need to take up arms at regional and global level against RPW and to stop its spread. It is one of the most destructive pests of coconut in India where it causes 12% of damage in 5 to 10 year old coconut palms (Sekhar, 2000). In the States of Karnataka, Kerala and Goa it is the most threatening pest to coconut growers (Prabhu and Patil, 2009). Ganapathy et. al. (1992) reported 34% of damage on coconut trees in Kerala. Nearly 8 million dollars are spent every year in Gulf and the Middle East countries only to remove infested palms. As per the reports of FAO(2017), by 2023 it is expected that RPW control and loss of benefits will amount to around 225 million dollars in Italy, Spain, and France.

Species described under the genus *Rhynchophorus*

Rhynchophorous comes under the subfamily Dryopthorinae, tribe *Rhynchophorini* and subtribe *Rhynchophorina*. Ten species have been reported under the genus *Rhynchophorous*. Among them *R. palmarum*(Linnaeus), *R. cruentatus* (Fabricius), R. ritcheri(Wattanapongsiri) and *R. quadrangulus* (Queden) are New world species, *R. phoenicis* (Fabricius) (African Palm Weevil) is African species, *R.ferrugineus*(Olivier) (Red Palm Weevil), *R. bilineatus* (Montrouzier) (Black Palm Weevil), *R. distinctus* (Wattanapongsiri), *R. lobatus* (Ritsema)and *R.vulneratus* are tropical Asian species (Mergawy et. al., 2011). Based on the morphological studies, Wattanapongsiri (1966) concluded that *R. ferrugineus* and *R. vulneratus* were valid species, whereas *R. schach* as a synonym of *R. vulneratus*. Genomic studies on DNA of mitochondrial cytochrome oxidase subunit I gene have shown the variation with a unique halotype in the Mediterranean type except Syria and several different types in the other Asian population (Mergawy et.al., 2011). Morphological studies by Ul Haq and co-workers have shown variation in the arrangement of

E

F

G

H

Fig 2. E. Larva; F.Pupa; G&H. Damage symptoms in coconut

prothoracic spots in male and female RPW and these spots are unique in some females which serves as useful guide in differentiating sexes. And also the size and number of black spots on prothorax has led to describe many different species (Wattanapongsiri, 1966).Till date only *R. ferrugineus* has been reported from India.

Synonyms

Curculio ferrugineus Olivier, 1790

Calandra ferruginea Fabricius, 1801

Rhynchophorus signaticollis Chevrolat, 1882

Identification

Adults are reddish brown, measurement ranging from 35-40 mm length and 10 -15 mm breadth. Rostrum is long and curved (Fig 1. A&B). Black spots are present on the pronotum and they vary from no markings to more than seven spots (Singh et. al., 2017). They are sexually dimorphic; males have tuft of hair (setae) on the dorsal surface of the rostrum (Fig 1. D), while it is bare in the female. Rostrum of female is slender, curved and slightly longer than the male (Fig 1.C). The foretibia of male bears comb like long hairs, whereas in females the hairs are short. In general, females are larger than males as the case in other weevils. The relative position of rostrum and foretibia differentiate male from female. When rostrum is placed perpendicular to the body axis, it extends beyond the foretibia in females whereas it ends at the same level or little before in males(Rochat et. al., 2017). Phenotypic variation is evident in adults where body colour varies from orange-red to black and the number and size of the black spots on prothoracic region varies. Larvae are white in colour

with brown head and apodus type (Menon and Pandalai, 1960).

Distribution

RPW being a native of tropical and subtropical climate has now extended its territory to temperate regions. This may be due to the intensive planting of palms and commercial trading across the boundaries.

World: Africa (Djibouti, Egypt, Libya, Mauritania, Morocco), Asia (Bahrain, Bangladesh, Cambodia, China, Georgia, India, Iran, Iraq, Israel, Indonesia, Japan, Malaysia,Myanmar, Oman, Pakistan, Philippines,Saudi Arabia, Singapore, Sri Lanka, Syria. Taiwan, Thailand, Turkey, United Arab Emirates, Vietnam, Yemen), Europe (Albania, Croatia, France, Greece, Italy, Portugal, Spain), North America (Aruba, Belize, Costa Rica, Cuba, Curacao, Dominica, El Salvador, Guadeloupe, Guatemala, Honduras, Martinique, Mexico, Netherlands Antilles, Nicaragua, Panama, Saint Vincent), South America (Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Tobago, Trinidad, Uruguay, Venezuela),Oceania (Papua New Guinea, Solomon Islands, Western Samoa) (Ajlan, 2004; Alonso-Zarazaga and Lyal, 1999; CABI).

India: Andaman and Nicobar Islands, Andhra Pradesh, Assam, Bihar, Daman and Diu, Goa, Gujarat, Karnataka, Kerala, Lakshadweep, Madya Pradesh, Maharashtra, Meghalaya, Odisha, Tamil Nadu, Tripura, Uttar Pradesh, and West Bengal (Singh et. al., 2017;CABI).

Biology

Male and female weevils mate many times in their life span. The pre oviposition period lasts for 1 to 7 days. Female scoops out a small hole in the soft tissues of the tree or in the wounds present in the trunk and in the petiole. It lays creamy white, oval and shiny eggs. A female can lay 200 to 500 eggs in her life span and it is noted that multiple inseminations are required to ensure the fertility. The eggs hatch in 2 to 5 days, upon hatching the whitish grub feed on the soft tissue and tunnel into trunk of tree. The fully grown larvae measures about 50 mm and have brown head and white body(Fig 2. E). The larval period lasts for about 2 to 4 months depending upon the availability of host nutrition, temperature and humidity. The last instar larvae spin oblong cocoon from the host fiber and pupate inside the cocoon(Fig 2. F). Pre-pupal period is about 2 to 11 days. Initially the pupae are cream in colour later turns to brown and are shiny, greatly furrowed and reticulated. Pupal period lasts for 12 to 20 days. After emergence, adults live inside the cocoon for about 4 to 7 days. Adults can live for 50 to 113 days where males surviving for longer period than females (Menon and Pandalai, 1960; Giblin-Davis et al., 2013).

Host plants

It is a polyphagous pest where palm species are primary hosts and *Agave americana* is secondary host. Hosts include *Cocos nucifera*(Coconut), *Areca catechu*(Areca nut), *Arenga pinnata*(Sugar palm),*Borassus flabellifer*(Toddy palm),*Sabal palmetto*(Cabbage palm), *Trachycarpus fortune* (Windmill palm), *Washington robusta*(Washingtonia palm) (Giblin-Davis et al.,2013), *Caryota cumingii*(Fishtail palm), *Caryota maxima* (Giant mountain fishtail palm), *Corypha gebanga*(Gebang palm),*Elaeis guineensis*(African oil palm), *Livistona decora* (Ribbon fan palm), *Livistona chinensis*(Chinese fan palm), *Metroxylon sago*(True sago palm), *Oncosperma horridum*(Thorny palm), *Oncosperma tigillarium*(Nibong palm), *Roystonea regia*,*Phoenix canariensis*(Canary island palm), *Phoenix dactylifera*(Date palm) (Murphy and Briscoe, 1999). In India it attacks *Cocos nucifera* (Coconut), *Areca catechu* (Areca nut), *Phoenix sylvestris* (Indian date) and *Metroxylon sago* (True sago palm).

Damage Symptoms

RPWs attack unhealthy, stressed and wounded palms, but they also reported attacking healthy palms.

The important symptoms are:

- Tunnels at base of fronds or the trunk of the palm.
- Gnawing sound caused by larvae is audible from the infested palms.
- Exudation of brown viscous fluids from tunnels and base of the frond is common. Later this solidifies and gives flaky appearance.
- Bored holes and chewed plant material is visible at the external sites of feeding and gives a characteristic fermented odour(Fig 2. G&H). This gives confirmation about the initial damage to palm trees.
- Dead adult RPW and empty

pupal cases are visible in the infested palms
- Breaking of the trunk or toppling of the palm crown is common.
- Yellowing or dried central leaves, damage of leaf base and damaged leaves that are under partial or total slope resembling an open umbrella.

Management

- Adopting 8x8 m and 7x7 m for planting tall and dwarf coconut trees,respectively helps to reduce odour cues.
- Entry of the weevil can be prevented by cutting the leaves at or beyond the region of leaf emergence.
- Placing of sachets containing ten *Heterorhabditis indica*-infected Galleria mellonella cadavers along with botanical cake on the uppermost three leaf axils of coconut (Joseph rajkumar et. al., 2017).
- In India, *Chelisoches morio* is a common predator on egg and larval stages of RPW found in the crown region of coconut tree (Abraham and Kurian, 1973). The earwig *Anisolabis maritima* and the anthocorid bug *Xylocorus galactinus* are recorded as predators on immature stages of RPW.
- Predatory bug, P*latymerus laevicollis* found to feed on *R. ferrugineus*.
- As per the reports of Hanounik (1998), genetically enhanced strains of EPNs Steinernema and Heterorhabdtis causes 95 to 100% and 50% larval mortality in laboratory and field condition, respectively.
- Important bird species found to feed on RPW are the Indian tree pie bird, *Dendrocitta vagabunda Parvula*, the crow pheasant bird, *Centropus sinensis* Stephens and the Eurasian magpie, *Pica pica L.*
- Deep cutting to completely remove the growing point of off-shoots then treating the cut surface with an insecticide and covering it with mud is known to minimize the level of infestation.
- Lufenuron at 0.01% leads to defective morphogenetic moults. This could be a long term biorational approach for the management of RPW (Joseph rajkumar et al., 2017).
- Trapping of adults with an aggregation pheromone, ferrugineol is an important tool in management. Pheromone traps installation @ 1trap/ha through in community mode.
- Pest attack can be recovered by spot application of imidacloprid 0.02% at 1 ml /L or indoxacarb 0.04% at 2.5 ml /L (Joseph rajkumar et. al., 2017).

Future prospects for management of RPW

Despite the global efforts in the management of RPW, many gaps and challenges need to be addressed. Important measures like early detection of pest infestation, proper removal and safe disposal of infested palms, development of effective and eco-friendly management technologies, making the biocontrol agents available to the farmers and active participation of farmers in RPW-IPM programmes will be helpful in managing the pest effectively. Similarly, at global level enforcement of phytosanitary and regulatory measures should be followed for further spread to the uninfested regions.

Further, in depth studies need to be done regarding early detection methods, identification OF effective natural enemies, nutrition management in palms, breeding resistant varieties and maintaining optimum palm population are helpful in keeping this noxious pest at bay.

References

Abraham, V.A. and Kurian, C., 1973. Chelisochesmoris F. (Forficulidae: Dermaptera), a predator on eggs and early instar grubs of the red palm weevil Rhynchophorusferrugineus F. (Curculionidae: Coleoptera). Journal of Plantation Crops, 1, pp.147-152.

Al-Ajlan, A. M., 2004. Red palm weevil, Rhynchophorus ferrugineus(Oliver)(Coleoptera: Curculionidae). In: Encyclopedia of Entomology. Springer, Dordrecht.

Al-Dosary, N.M., Al-Dobai, S. and Faleiro, J.R., 2016. Review on the management of red palm weevil Rhynchophorusferrugineus Olivier in date palm Phoenix dactylifera L. Emirates Journal of Food and Agriculture, pp.34-44.

Alonso-Zarazaga, M.A. and Lyal, C. H. C., 1999. A world catalogue of families and genera of Curculionoidea (Insecta: Coleoptera) (excepting Scolytidae and Platypodidae). Entomopraxis, Barcelona, pp. 64.

CABI, Centre for Agriculture and Bioscience International. Available at: https://www.cabi.org/isc/datasheet/47472. Accessed on: 4th November, 2022

[1]M.Ananthi
[2] M.Anitha
[1]Teaching Assistant,
Directorate of Planning
and Monitoring,
Tamil Nadu Agricultural
University, Coimbatore
[2] Assistant Professor,
Dept. of Biotechnology,
Selvamm Arts and Science
College, Namakkal

Tulsi

A Herb for all reasons

Tulsi, often referred to as "queen of the herbs", is native to India and cultivated throughout Southeast Asia. It is also called the "holy basil" as it is surrounded by legends and myths. The basil plant is revered by Hindus all over, and is considered to be a manifestation of the Goddess herself. Due to its antibacterial powers, the presence of tulsi in the vicinity of the house prevents the spread of germs and helps to keep the atmosphere clean. It is also grown in

Cultivation of Tulsi

Soil and Climate	• Sandy loam soil with good organic matter is considered ideal. • Preferred tropical and sub-tropical climates
Propagation	Seeds or Vegetative Propagation
a. Seeds	• For propagating through seeds, they are to be sown in the nursery beds. • For sowing of one hectare about 300g of seeds are required. • Well rotten farm yard manure is applied to the soil and prepared to a fine tilth and seed beds of 4.5x1.0x0.2 m size are prepared. • Seeds are minute, the required quantity of seeds are mixed with sand in the ratio of 1:4 and sown in nursery bed, 2 months in advance of the onset of monsoon. • Seeds germinate in 8-12 days and seedlings are ready for transplanting in about 6 weeks time at 4-5 leaf stage.
b. Vegetative Propagation	• Propagated by vegetative method using terminal cuttings • Cuttings with 8-10 nodes and 10-15 cm length are used. They are so prepared that except for the first 2-3 pair of leaves the rest are trimmed off. • Later, they are planted in the well prepared nursery beds or polythene bags. • In about 4-6 weeks time the rooting is complete and they are ready for transplanting into the main field.
Planting season	October-December months (Terminal cuttings)
Spacing	40 cm between the row
Manures and fertilizers	120:60:60 kg/ha of NPK
Irrigation	Twice a week till one month so that the plants establish themselves
Interculture operation	• First weeding is done one month after planting • Second after another 30 days.
Plant protection	• Organic practices include control measures using neem based formulations. • Fish oil resin soap can be used to manage such sucking pests. • Botanicals viz., extracts of garlic, *Vitex negundo*, *Lantana camera*, *Clerodendron inerme*, *Calotropis gigantean* are combined and sprayed periodically for controlling the pests.
Harvesting	• The first harvest is done after 90 days of planting and subsequently it may be harvested at every 75 days interval. • The crop is harvested at full bloom stage by cutting the plants at 15 cm from ground level to ensure good regeneration for further harvests. • The yield and oil content is more in plants harvested during bright sunny days.
Yield	• Tulsi gives about 10,000 kgs of fresh herbage per hectare per year. • The herb contains about 0.1 to 0.23 percent oil and it is about 10-20 kg of essential oil per hectare. • Irrigated tulsi gives higher herbage yield (upto 20 ton and oil yield (upto 40kg/ha).

temperate climates, the natural habitat of tulsi varies from sea level to an altitude of 2000 m. It grows naturally in moist soil. Tulsi is cultivated in semi urban areas and the fresh herbage is sold to the temples and worship centres. The major source of tulsi is from wild habitat including uncultivated field and roadside.

Every part of this plant contains some kind of spiritual significance - its roots symbolize a religious pilgrimage, its branches represent divinity, and its crown signifies an understanding of the scriptures. The leaves are definitely one of the most commonly used ingredients - a remedy for a cough, cold or congested chest.

About Plant

Tulsi is heavy branched having hair all over and it is a small annual or short-lived perennial shrub, up to 1 metre (3.3 feet) in height. The stems are hairy and bear simple toothed or entire leaves oppositely along the stem.

It has round oval shaped leaves which are up to 5 cm long. The leaves are 2- 4 cm in length. Its seeds are flat. Its flowers are purple – creamish in colour. The Tulsi with the green leaves is called the Shri Tulsi and one with the reddish leaves is called the Krishna Tulsi. Its seeds are yellow to reddish in colour. The small purple or white tubular flowers have green or purple sepals and are borne in terminal spikes. The fruits are nutlets and produce numerous seeds.

Chemical composition

Tulsi leaves contain a bright yellow volatile oil which is useful against insects and bacteria. The principle constituents of this oil are eugenol, eugenol methyl ether and carvacrol. The oil is reported to possess anti-bacterial properties and acts as an insecticide. It inhibits the in vitro growth of *Mycobacterium tuberculosis* and *Micrococcus pyogenes* var. *aureus*.

Properties of Tulsi

- It is an antipyretic (relieves fever) agent.
- It has anti-inflammatory activity.
- It is an antiemetic (prevents vomiting)
- It lowers the blood sugar (antidiabetic)
- It act as an hypotensive (lowers blood pressure)
- It has hypolipidemic (lowers cholesterol) activity.

Published by **Padmam S** Principal Information Officer Farm Information Bureau Owned by Department of Agriculture, Government of Kerala and Published at Farm Information Bureau, Kowdiar P.O, Thiruvananthapuram-3. Editor: **Sreekala S.** Mail: editorejournalkkfib@gmail.com Log on to http://www.fibkerala.gov.in Phone: 0471-2314358