


# Characterization and Evaluation of Citrus Genotypes for Nutrition-rich and Zero-waste Utilization

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# 1. Introduction

| Crop | Production ('000 MT) | Area ('000 ha) |
|---|---|---|
| Banana | 37614 | 948 |
| Mango | 22398 | 2396 |
| Citrus | 14553 | 1076 |
| Mandarin | 6076 | 474 |
| Sweet oranges | 3852 | 240 |
| Lime and lemon | 3836 | 319 |
| Others | 789 | 93 |

Source: Area and Production of Horticulture crops for 2023-24, MoA&FW, GoI

**Improved citrus cultivar**

- ❑ **Processing qualities**
  - ✓ Medium fruit size (250-400g)
  - ✓ Thin peel (<10 mm)
  - ✓ High juice recovery (>42%)
  - ✓ Less number of seeds (<6-8 seeds)
- ❑ **Reduced bitterness (Naringin and Limonin)**
- ❑ **Enhanced flavour and aroma**
- ❑ **Health promoting properties (Antioxidants, Micronutrients Fe & Zn)**

2012

2017

**Pummelo (*Citrus maxima*)**

- ➢ **Health promoting properties**
- ➢ **Large size fruit**
- ❑ **Thick peel**
- ❑ **Large hollow core**
- ❑ **High number of seeds**
- ❑ **Less juice content (<30%)**

×

**Sweet Orange cv Mosambi (*Citrus sinensis*)**

- ➢ **Sweetness**
- ➢ **High juice content (>40%)**
- ➢ **Thin peel**
- ➢ **Small solid core**
- ❑ **Bitterness**
- ❑ **Low bioactive compounds**

**Pusa Swaras**
**SCSH11-15**

Pusa Swaras has 19.20% higher yield and 14.63% higher juice than Mosmabi

No infestation of citrus psylla was observed. Other pests/Diseases as noted in other sweet citrus cultivars

# 2. Project Objectives:

1. To carry out physico-biochemical characterization of citrus hybrids

2. Identification of key bioactive compounds and nutraceuticals in juice and peel of citrus hybrids

3. Development of molecular fingerprint of citrus hybrids

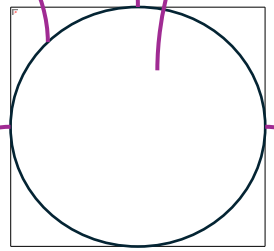
# 3. Methodology

**(A)TREE(2)**
Tree growth habit
Tree shape

**(B) BRANCH AND SPINE CHARACTERS(4)**
Density of branches
Branch angle
Spine density on tree
Spine shape

**(C) LEAF(9)**
Lamina length (mm)
Lamina width (mm)
Leaf lamina attachment
Ratio of leaf lamina length/width
Leaf lamina shape
Leaf lamina margin
Leaf apex
Petiole wing shape
Petiole wing width

**Morphological and Pomological Characterization**
Total parameters:36
Qualitative: 23
Quantitative: 13



**Molecular Characterization**

DNA Extraction (Doyle and Doyle, 1987)

PCR as per standard protocol using HvSSRs

Development of fingerprint for the potential hydrids

**(E)FRUIT(13)**
Weight (g)
Length (mm)
Width (mm)
Shape
Shape of fruit base (stalk end)
Shape of fruit apex (styler end)
Fruit surface colour
Fruit surface texture
Rind thickness (mm)
Fruit axis
Diameter of fruit axis (mm)
Number of segments per fruit
Pulp colour

**(F)SEED(8)**
Average no. of seeds per fruit
No. of false seeds per fruit
Shape
Colour
Surface
Embryony status
Seed weight (10 seeds)
Seed length (mm)

**Experimental Site**
**Main Citrus Orchard, IARI, New Delhi**

Orangelo Hybrids (*Citrus maxima*[Burm. f.] Osbeck× *Citrus sinensis*(L.)Osbeck)

Parents: *Citrus sinensis* cv Mosambi, *Citrus maxima* cv. Pummelo (Red flesh) and Pummelo white flesh, *Citrus* reticulata cv. Tangerine

The characterization and evaluation of available citrus hybrids (150 hybrids) will be carried out at morphological (based on DUS traits), physico-chemical, and molecular levels following standard protocols and by utilizing hypervariable microsatellite markers

Promising citrus hybrids will be further evaluated through biochemical and secondary metabolite profiling of juice and peel

The potential hybrids will be multiplied for various downstream applications

The standardization of peel and juice organelolaptic properties will be tasted on hedonic scale

# 3. Methodology

## Biochemical Parameters

| Parameters | Methods |
|---|---|
| Juice pH | AOAC, 2000 |
| TSS (°B) | Hand refractometer |
| Titratable acidity (%) | AOAC,1990 |
| TSS : acid ratio | AOAC, 2000 |
| Ascorbic acid (mg/100ml) | AOAC, 2000 |
| Total flavonoid content (qe mg/100ml) | Spectrophotometer (Zhishen *et al.*, 1999) |
| Total phenolic content (GAE mg/100ml) | Spectrophotometer (Singleton *et al.*, 1999) |
| Total antioxidant activity (µmol trolox/g) | Spectrophotometer (Sanchez-Moreno *et al.*, 1998) |
| Total sugars (%) | Lane-Eynon methods (Lane *et al.*, 1923) |
| Reducing sugars (%) | Lane-Eynon methods (Lane *et al.*, 1923) |
| Non-reducing sugars (%) | (Total sugars – reducing sugars) × 0.95 |

## Mineral Nutrient in Leaf, Juice and Peel

| | |
|---|---|
| Phosphorus (%) | Vanadomolybdophosphoric yellow colour method (Jackson, 1980) |
| Potassium (%) | Flame Photometer (Jackson,1980) |
| Calcium (%) | Flame Photometer (Jackson,1980) |
| Sulphur (%) | Turbidimetric method (Tabatabai and Bremner, 1970) |
| Magnesium (%) , Iron, Zinc, Mangenese, Copper (ppm) | AAS<br>Ince and Coskun (2008) |

**Naringin content, Hesperidin-HPLC**

**Essential Oil estimation-peel-Limonene-GC-FID**

**Limonin content UPLC-QTOF-ESI-MS**

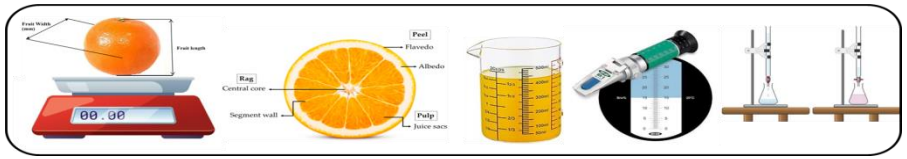
**Sensory analysis of juice**

(1) Sweetness (1-5 scale)

(2) Sourness (1-5 scale)

(3) Bitterness (1-5 scale)

(4) Acceptability (1-9 hedonic scale)




- **Number of genotypes-150**
- **Number of replication:** 3
- **Fruits per replication:** 15
- **Seasons/Year:** Two harvesting seasons 2026 and 2027

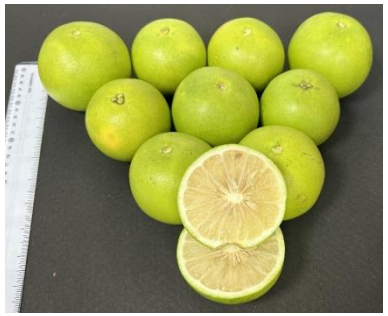
# 6.Social and nutrition impact pathway – Project outcomes/Impact:

The proposed project will boost the demand, consumption, propagation, creation of database, conservation and characterization of highly nutritious and nutraceutical loaded citrus hybrids

The promising citrus hybrids and improved traits will be introduced and multiplied in some pockets of citrus growing areas in a planned manner in collaboration with KVK, line departments and local stakeholders to enrich the genetic diversity and increase the yield without eroding the diversity of existing citrus germplasm

The proposed interventions will certainly promote sustainability; genetic enrichment for enhanced production and recovery of bioactive compounds such as naringin, limonene and hesperidine which is having medicinal properties and enhanced quality, appeal and nutritional values of interspecific citrus hybrids, besides promoting total utilization through processing of peel

*The project is in line with CFI's mission of sustainability, utilization of underutilized fruits, ensuring nutritional and livelihood security and involvement of local communities*



Non of the hybrids having the infestation of citrus psylla, the vector of HLB

Nutrient Fortified, Antioxidant rich, ant diabetic, ecofriendly, Central India suitable planting

# Slide 6. Environmental and climate advantages

Water management

Soil Health Conservation

Biodiversity Conservation

Waste Management

Agricultural Diverification

**Fruit trees store significant amounts of carbon in above-ground biomass**

- Simple, easy-to-use allometric equations are provided to help farmers estimate the amount of carbon present in their trees
- This will empower the farmers to negotiate better results-based payments and hopefully evade exploitation by carbon marketing schemes
- Using fruit trees in carbon projects can help prevent unintended loss of livelihoods and food security as a result of sequestration efforts
- The DBH (measured at 1.3 m above the ground), collar diameter (CD, measured at 30 cm above the ground), diameter below the graft union (DBGU), and diameter of the primary branches (DPB) were measured on trees with DBH 2.5 cm or height ≥2 m using a diameter tape.
- Perennial crops such as citrus have the potential to absorb and sequester carbon dioxide from the atmosphere, save for the carbon released back through the application of agro-chemical inputs and use of fossil fuels in running farm machinery in the management of citrus production systems
- citrus trees carbon sequestration in biomass ranged from 23.99 Mg CO2e/ha for young trees to 109 Mg CO2e/ha for mature trees

# 7. Scaling, sustainability and cost efficiency:

Citrus agriculture has significant potential to promote social, economic, and rural development because of its strong market demand, year-round yield, and variety of product applications

**Social Sustainability**
Community Development
Health and Nutrition
Research and Training
Infrastructure Development
Skill Development

**Economic Sustainability**
Employment oppurtunity
IncomeGeneration
Market Access and Trade
Value Addition and processing
Agribusiness Growth

Like CFI's mission present project proposal based on sustainability, complete utilization of citrus fruits, ensuring nutritional and livelihood security
A large set of meticulously characterized citrus hybrids for various downstream including citrus improvements
This will also help tremendously to release citrus hybrids as variety. Identification of key bioactive compounds and nutraceuticals in juice and peel of citrus hybrids to diversify its usage and promote zero-waste utilization

**Multiple Sustainable Development Goals (SDGs)**

SDG 1 (poverty eradication), SDG 2 (zero hunger), and SDG 3 (supporting excellent health and well-being), climate adaptation (SDG 13), sustainable urban development (SDG 11), responsible consumption (SDG 12), and international partnerships (SDG 17), inclusive education (SDG 4), gender equality (SDG 5), and sustainable economic growth (SDG 8)

# Key Deliverable

- A large set of meticulously characterized citrus hybrids for various downstream including citrus improvements. This will also help tremendously to release citrus hybrids as variety.
- Identification of nutritionally and bioactive compounds rich citrus hybrids for their potential utilization as processable genotypes.
- Identification of key bioactive compounds and nutraceuticals in juice and peel of citrus hybrids to diversify its usage and promote zero-waste utilization.
- Refined protocol for bioactive compounds estimation
- Others

Planting Material Development of identified variety and their distribution
Environmental Sustainability-Pathogen free genotypes will be identified
Waste Utilization
Soil Health Restoration
Market Access-Demonstration, Field Day, KVKs, Farmers

# 8.Budget summary and risk and mitigation strategy:

✓At IARI, New Delhi a 'Field Gene Bank of citrus hybrids' housing more than 200 has already been established. Characterization of 70 citrus hybrids for morphological (in coherence with DUS guidelines), biochemical and molecular traits has already been carried out

✓ Standarization of Naringin, Hesperidin and Limonin extraction protocol were carried out and published

✓ Both peel and pulp could be utilized to extraction of these compounds

*The proposed research team has ample experience of technology to execute the proposed work plan*

| | 1st yr (USD) | 2nd yr(USD) | 3rd yr (USD) |
|---|---|---|---|
| **Field and Lab Assistant** | 3,484.47 | 3,484.47 | 3,484.47 |
| **Consumables** | 4,572.83 | 3,429.85 | 2,286.56 |
| **Travel** | 285.86 | 285.86 | 285.86 |
| **Field Work** | 1,143.36 | 1,143.36 | 1,143.36 |
| **Contingency** | 571.68 | 571.68 | 571.68 |
| **OverHead (15%)** | 1,509.14 | 1,680.52 | 1,509.14 |
| **Total Cost** | **36,025.86 USD,/31,97,132.96 Rs** | | |

# Publications, Variety, Sequence Deposited, Protocol Standarized in the Proposed Area of Work

Chavda, D., **Sharma, N.*,** Sharma, R.M., Tripathy, V., Joshi, N., Vittal, H., Kumar, N. and Dubey, A.K. 2025. High-performance liquid chromatography (HPLC) method for standardization and quantitative analysis of naringin in interspecific citrus hybrids. *Journal of Food Composition and Analysis*, 142, p.107467.

Chand, L., **Sharma, N*.**, Sharma, R.M., Pandey, R., Sathee, L. and Dubey, A.K. 2024. Physio-biochemical and growth response of contrasting reciprocal grafting in citrus under water deficit and rehydration. *Journal of Plant Growth Regulation*, **43(4):**1230-1246.

Kumar, R., **Sharma, N.*,** Dubey, A.K., Sharma, R.M., Sharma, N., Mishra, G.P., Vittal, H., Shivran, M. and Sethi, S. 2025. Generation of denovo EST-SSRs and Validation in Newly Developed Interspecific Citrus Hybrids (*Citrus maxima*Merr.×*Citrus sinensis* (L.) Osbeck). *National Academy Science Letters*, pp.1-5.

Kumar, R., **Sharma, N*.**, Dubey, A.K., Sharma, R.M., Sethi, S., Mishra, G.P., Mathur, S., Vittal, H., Shivran, M. and Sharma, N. 2024. Fruit quality assessment of novel hybrid Pummelo× Sweet Orange and its molecular characterization using acidity specific markers. *Food Technology and Biotechnology*, **62(1)**: 35-45.

Kumar, R., **Sharma, N*.**, Dubey, A.K., Sharma, R.M., Vittal, H., Mishra, G.P., Shruti, S. and Shivran, M. 2024. Morphological characterization of newly developed orangelo (*Citrus* spp.) hybrids. *Indian J Agricultural Sci*, **94(2):**174-180.

**Sharma, N**., Sharma, R.M. and Dubey, A.K. 2023. Production and verification of lemon× acid lime hybrid populations via embryo rescue. *Indian Journal of Horticulture*.**80(1):**24-29.

Soni, A., Dubey, A.K., Gupta, A., Sharma, R.M., Prakash Awasthi, O., Bharadwaj, C. and **Sharma, N**. 2019. Optimizing embryo age and media for enhancing hybrid seedling recovery in sour orange (*Citrus aurantium*)×*Sacaton citrumelo* (*C. paradisi*× *Poncirus trifoliata*) crosses through embryo rescue. *Plant Breeding*, **138(3)**:368-376.

Dubey, A.K., Sharma, R.M., Awasthi, O.P., Srivastav, M. and **Sharma, N**., 2016. Genetic diversity in lime (*Citrus aurantifolia*Swing.) and lemon (*Ctruslimon* (L.) Burm.) based on quantitative traits in India. *Agroforestry Systems*, **90**:447-456.

Chavda, D**., Sharma, N**., Sharma, R. M., Khandelwal, A., Joshi, N., Singh, N., ... & Lal, C. (2025). Identification of Novel Interspecific Citrus Hybrids with High Hesperidin Content and Favorable Sensory Quality. *Analytical Letters*, 1-15.

Kumar, R**., Sharma, N**., Dubey, A. K., Sharma, R. M., Sharma, N., Mishra, G. P., ... & Sethi, S. (2025). Generation of denovo EST-SSRs and validation in newly developed interspecific citrus hybrids (Citrus maxima Merr.× Citrus sinensis (L.) Osbeck). *National Academy Science Letters*, *48*(3), 399-403.

**Varieties developed:** Institutevariety release committee (as associate breeder)recommendedPusaSwaras (SCSH 11-15) crossed between Pummelo (*Citrus maxima* ( Burm. ) Merr. ) × sweet orange cv Mosambi (*C. sinensis* (L.) Osbeck for variety release dated 9-4-2025.

**Process optimized: Naringin** extraction protocol from interspecific citrus hybrids were optimized
**(Published :** Food Composition and Analysis, NAAS-10).
**Hesperidine** Extraction protocol ( Analytical Letters NAAS-7.4)
**Limonin** (IJGPB NAAS-7.2)
**Limonene** (Analytical Letters NAAS-7.4)

Mahawer, A.K., Sharma, R.M., Dubey, A.K., Awasthi, O.P., Singh, D., Kesharwani, A.K., Kashyap, A.S., **Sharma, N.,** Kulshreshtha, A., Kumar, A. and Yadav, J. 2025. Deciphering the Genetic Response: Differential Gene Expression Profiling in *Citrus aurantifolia* (Chrism.) and *Citrus limon* (L.) Burm Infected with *Xanthomonas citripv. citri. Tropical Plant Biology*, 18(1), p.22.

Singh, N., Sharma, R.M., Dubey, A.K., Saha, S., Awasthi, O.P., Bharadwaj, C., Sevanthi, A.M., Kumar, A., **Sharma, N**., Kumar, R. and Kumar, V. 2025. Bioactive Compounds and Bitterness Properties of Newly Developed Interspecific Citrus Hybrids (*Citrus maxima* [Burm. f.] Osbeck× *Citrus sinensis* [L.] Osbeck). *Horticulturae*, **11(2)**.

Singh, N., Sharma, R.M., Dubey, A.K., Awasthi, O.P., Porat, R., Saha, S., Bharadwaj, C., Sevanthi, A.M., Kumar, A., **Sharma, N**. and Carmi, N. 2023. Harvesting maturity assessment of newly developed citrus hybrids (*Citrus maxima*Merr.×*Citrus sinensis* (L.) Osbeck) for optimum juice quality. *Plants*, **12(23):** p.3978.

Mahawer, A.K., Dubey, A.K., Awasthi, O.P., Singh, D., Dahuja, A., Sevanthi, A.M., Kumar, A., Goswami, A.K**., Sharma, N**., Yadav, J. and Kesharwani, A.K. 2023. Elucidation of physio-biochemical changes in *Citrus* spp. incited by *Xanthomonas citri*pv. citri. *Horticulturae*, **9(3):** 324.

Kadam, D.M., Dubey, A.K., Sharma, R.M., Morade, A., **Sharma, N.** and Bhardwaj, C. 2022. Response of citrus (*Citrus*sps.) rootstock hybrids to PEG induced drought under hydroponic system. *Indian Journal of Agricultural Sciences*, **92(10):**1230-1236.

Dubey, A.K., Kholia, A., **Sharma, N**. and Sharma, R.M. 2021. Assessing genetic diversity in Indian pummelo collections utilizing quantitative traits and simple sequence repeat markers (SSRs). *Indian Journal of Horticulture*, **78(1):**3-8.

Dubey, A.K., Gupta, A., Sharma, R.M. and **Sharma, N**., 2020. Maximizing hybrid seedlings recovery and early identification of highly polyembryonic acid lime (*Citrus aurantifolia* swing.)× lemon (*Citrus limon*burm.) hybrids using SSR markers. *Journal of Horticultural Research*, *28*(2). ): DOI: 10.2478/johr-2020-24

Mallick, M., Bharadwaj, C., Srivastav, M., **Sharma, N**. and Awasthi, O.P. 2017. Molecular characterization of Kinnow mandarin clones and mutants using cross genera SSR markers.*Indian Journal of Biotechnology*,**16(2)**:244-249

Singh N, Sharma R M, Dubey AK, **Sharma N,** Awasthi O P, Saha S, Bharadwaj C, Sevanthi AM, Shivran M, Akshay, Kadam DM, Kumar A. 2025. Developing new interspecific citrus hybrids for improved fruit quality: physico-chemical and sensory characterization. *Journal of Agriculture and Food Research*. 24:102453.https://doi.org/10.1016/j.jafr.2025.102453 (NAAS-12.2).

Singh, N., Sharma, R. M., Dubey, A. K., Awasthi, O. P., **Sharma, N.,** Saha, S., ... & Kumar, A. (2025). Molecular insights to discover the nucleotide variations related to limonin-associated delayed bitterness in new interspecific citrus hybrids. *Indian J. Genet. Plant Breed*, *85*(3), 512-520.

**Students Guided 5 (M.Sc. And Ph.D. Completed, 4 continue Ph.D. 4 Co-chairman )**

**Sequence deposited in NCBI** for Limonin Content in Citrus Hybrids(As Co-author):
BankIt2736627 Seq1_NCLPS3_SCSH-11-9/13_P9 OR474192
BankIt2736674 Seq1_NCLPS3_WP P9 OR474193
**BankIt2735646 Seq1_NCLPS1_WPP7 OR465050**

Alliance Bioversity & CIAT

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# Thanks