ASSESSMENT OF GENETIC VARIATION FOR MINERALS, METABOLITES, AND ANTIOXIDANT PROPERTIES OF MULTI-COLOURED CARROTS AND STABILITY ANALYSIS OF ELITE EUROPEAN CARROTS

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Introduction:

Carrot (*Daucus carota* L.) is one of the most economically important root vegetables globally, valued for its rich nutritional composition and wide genetic diversity in root colour- ranging from black, purple, red, yellow and white to orange. The distinctive pigmentation in each colour type is attributed to specific classes of bioactive compounds: anthocyanins in purple/black, lycopene in red, xanthophylls in yellow and beta-carotene in orange- making these genotypes excellent sources of natural antioxidants and nutraceuticals.

Multi-coloured carrot genotypes represent a vast natural repository of minerals, phenolic compounds, vitamins, carotenoids and secondary metabolites. This biochemical diversity is strongly influenced by genetic factors and genotype x environment (G x E) interactions, making multi-coloured carrots an ideal model for studying nutritional quality and phytochemical complexity. Despite this diversity, comprehensive research integrating mineral profiling, targeted metabolomics, biochemical assays and genetic marker analysis across multi-coloured carrot genotypes remains limited. Existing studies highlight substantial variation in mineral accumulation, pigment biosynthesis pathways and antioxidant potential. However, systematic characterization using advanced tools such as ICP-MS, LC-MS, GC-MS and functional InDel markers is still needed.

Additionally, while elite european carrot lines are recognized for superior root quality, they often exhibit inconsistent adaptability under tropical and subtropical Indian conditions. Multi-location evaluation is therefore essential to identify stable, high-yielding genotypes suitable for cultivation across Karnataka's diverse agro-climatic zones. The present study addresses these gaps by integrating phenotyping, biochemical analysis, metabolomic profiling, molecular marker-based diversity assessment and multi-location trials of elite European carrots.

Objectives:

1. To carry out mineral profiling and targeted metabolite analysis (phenolic compounds and vitamins) across multi-coloured carrot genotypes using ICP-MS, LC-MS, and GC-MS platforms.
2. To characterize genetic variability among multi-coloured carrot genotypes using functional InDel markers associated with specific metabolites and mineral accumulation pathways.
3. To evaluate antioxidant and anti-inflammatory properties of multi-coloured carrots through in vitro biochemical assays: DPPH radical scavenging and protein denaturation inhibition assays.
4. To conduct multi-location evaluation of elite European carrot genotypes for stability, adaptability, and performance under diverse agro-climatic conditions of Karnataka.

Methodology:

The experiment utilizes six multi-coloured carrot genotypes (G1: Black- Pusa Asita; G2: Dark Orange- EC-3/Kuroda; G3: Light Orange- Krishna Prabha Vriddhi; G4: Red- EC-21/Pusa Rudhira; G5: Yellow- EC-16; G6: White- EC-1) grown under controlled conditions at the College of Horticulture, Bagalkot. Five elite European carrot genotypes (EC-3, EC-26, EC-27, EC-30, EC-34) along with two checks (Kuroda-1, Kuroda-2) were sown in October 2025 for multi-location trials across three centres: (1) KRCCH, Arabhavi; (2) College of Horticulture, Bagalkot; and (3) College of Horticulture, Bengaluru.

1. **Mineral Profiling (ICP-MS/AAS):**

   Roots harvested at 90 DAS will be washed, peeled, freeze-dried, and powdered. Samples will undergo acid digestion (HNO3/H2O2) via microwave digestion. Minerals (K, Ca, Mg, P, Fe, Zn, Mn) will be quantified using ICP-MS or AAS in triplicate with certified reference standards for accuracy.

2. **Targeted Metabolic Profiling (LC-MS/GC-MS):**

   Freeze-dried root powder will be extracted using 80% methanol and analyzed in ESI+ and ESI- modes to cover phenolics, vitamins, carotenoids, and secondary metabolites. Data will be processed using MetaboAnalyst, PCA, and OPLS-DA software pipelines for chemometric analysis.

3. **Biochemical Assays:**

   Antioxidant activity will be assessed using the DPPH radical scavenging assay (0.1 mM DPPH, absorbance at 517 nm, 30-minute dark incubation). Anti-inflammatory activity will be evaluated through protein denaturation inhibition assay (egg albumin/BSA, 70 degrees C for 5 minutes, absorbance at 660 nm). Percentage inhibition: [(A_control - A_sample)/A_control] x 100.

4. **Functional InDel Marker Analysis:**

   Genomic DNA will be extracted using the CTAB protocol optimized for carrot. A panel of 100 functional InDel markers linked to metabolite and mineral pathways will be screened by PCR, resolved on agarose gel, and scored. Molecular diversity indices

(Na, Ne, PIC, Ho, He) will be computed and phylogenetic relationships visualized using Neighbour-Joining (NJ) dendrograms.

5. **Multi-location Evaluation of Elite European Carrots:**

   Five elite European genotypes (EC-3, EC-26, EC-27, EC-30, EC-34) and two checks will be evaluated across three environments in Randomized Complete Block Design (RCBD) with three replications. Traits recorded: root length, width, diameter, weight, colour, cracking, shape; petiole number, petiole length, shoot/vegetative weight, plant height; and yield traits (root weight/plot, days to maturity). Stability will be analyzed using AMMI, Eberhart and Russell model, and GGE biplot analyses.

Result and Conclusion:

In conclusion, this study represents a comprehensive, multi-disciplinary approach integrating advanced mineral profiling, metabolomic characterization, biochemical functional assays, molecular marker-based diversity analysis, and multi-location performance evaluation of carrot genotypes. The integration of ICP-MS, LC-MS, and GC-MS with functional InDel markers will provide a robust understanding of the biochemical and genetic diversity across multi-coloured carrot genotypes. Multi-location trial data from Karnataka's diverse agro-climatic zones will enable identification of elite European carrot lines with stable yield and superior nutritional quality. The outcomes will serve as a strong scientific foundation for nutritional breeding, biofortification strategies, and development of climate-resilient, high-value carrot cultivars suitable for commercial cultivation and functional food applications.

References:

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Future Scope:

The future scope of this project includes:

1. Development of molecular breeding tools using validated InDel markers for marker-assisted selection (MAS) of nutritionally superior carrot genotypes in formal breeding programmes.
2. Expansion of metabolomic studies to untargeted metabolomics for discovery of novel bioactive compounds in coloured carrot types.
3. Utilization of stable European carrot genotypes as parental lines for hybrid development targeting high yield, nutritional quality, and agro-climatic adaptability.

4. Exploration of nutraceutical and pharmaceutical applications of identified bioactive compounds from multi-coloured carrots for functional food and health product development.
5. Scaling up multi-location trials across more environments in India for national-level variety release and broadened adaptability assessment of elite carrot genotypes.