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"Reconcile land system changes with planetary health"

## Phenotypic and molecular analysis of sea-buckthorn accessions reveal promising genotypes and candidate genes associated with micronutrient content

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## Abstract

Sea-buckthorn (genus *Hippophae* L., family Eleagnaceae), rich in vitamin C, anthocyanin, and 24 minerals including iron (Fe) and zinc (Zn), could be a potential candidate to combat micronutrient malnutrition. However, little is known about the genetic interplay between these nutrients, fruit morphology, and underlying genes. The aldo-keto reductase (AKR) gene family is known to contribute to Fe and Zn uptake, and vitamin C precursor synthesis. This study thus characterised seventy sea-buckthorn accessions from the Gilgit region of northern Pakistan (altitude: 2444–3172 m.a.s.l), representing seven distinct fruit colour groups, for iron, zinc, vitamin C, phytic acid, and AKR genes. Sea-buckthorn showed high diversity for Fe  $(39.7-341.5 \text{ mg kg}^{-1})$ , Zn  $(0.6-12.7 \text{ mg kg}^{-1})$ , and vitamin C (40 to) $595 \text{ mg } 100 \text{ g}^{-1}$ ). Orange/yellow fruit colours abundantly found at lower altitude regions showed the highest Fe concentration (250.03  $\pm$  61.5 mg kg<sup>-1</sup>). Phytic acid in berries was low  $(<2.0 \text{ mg } 100 \text{ g}^{-1})$  indicating high micronutrient bioavailability (PA: Fe/Zn < 0.8). Eighteen AKRs were identified in the sea-buckthorn genome, while ten were identified in the studied accessions. In-silico analysis indicated that AKRs were localised in the cytoplasm, chloroplast, mitochondria, nucleus, extracellular region, and plasma membrane suggesting their role in detoxification, metabolites synthesis, and membrane transport. Promoter analysis revealed MYC and MYB binding sites associated with stress tolerance, and core regulatory elements like CAAT-box, GA-biding sites and TATA-box, LTR, and GAREmotif for transcription initiation. Three HrAKRs were expressed in all the accessions while seven were differentially expressed in the leaf and fruit tissues. Notably, accessions from the Sost region having highest micronutrients and their bioavailability expressed maximum number of AKRs. These findings suggest AKR gene stacking could help improve nutritional status of sea-buckthorn. Further functional studies are needed to identify the function of each HrAKR gene to enable molecular marker development for sea-buckthorn breeding programs.

**Keywords:** Ascorbic acid, fruit colour, hidden hunger, iron, phytic acid, SDG goals 2 and 3, vitamin C, zinc

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