



## Morphological and biochemical diversity among the *Malus* species including indigenous Himalayan wild apples



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### ABSTRACT

In the present investigation, 32 *Malus* species including 12 indigenous Himalayan wild apples were evaluated during 2014–2015, for their morphological (14 No.) and biochemical (14 No.) variabilities based on 28 parameters. The leaf related attributes like leaf shape, leaf apex shape, leaf base shape, leaf blade margin and tree attributes like tree habit, suckering tendency, burrknot tendency and presence of lenticels on shoot exhibited considerable variation. The maximum coefficient of variation value was recorded for foliar peroxidase activity (98.18%) followed by catechin (91.32%) content. Principal component analysis (PCA) revealed that the first five principal components (PC1, PC2, PC3, PC4 and PC5) together accounted for more than 50% variation of the total observed variations. The PC1 strongly integrated with the traits like tree habit, leaf blade length, leaf blade width, rutin, phloridzin, chlorophyll *a*, chlorophyll *b*, – chlorophyll *a:b*, total chlorophyll and total phenolics content, which were found to be effective parameters for explaining the natural variability among the studied *Malus* species. The dendrogram generated based on unweighted Neighbor-joining method clustered all the *Malus* species including the indigenous wild apples into three distinct clusters. Scatter plot based on PC1 and PC2 suggested high morphological and biochemical diversity among the *Malus* species including the indigenous wild apples. The cluster analysis and scatter plot revealed that the indigenous wild apples were quite diverse as observed for the exotic *Malus* species; though there were no separate groups detected. The higher diversity for endogenous biochemical compounds indicates differential physiological behaviour of *Malus* species under the prevailing environmental conditions. These findings revealed that there were several biochemical constituents, which could serve as effective indices for indirect selection of potential biotic and abiotic stresses resistance/tolerance *Malus* species for their potential use in genetic improvement programmes. Similarly, knowledge on graft compatibility with respect to wild *Malus* could help in exploiting them as new rootstock genotypes. These parameters are known to influence the expression of different traits in scion genotypes upon grafting and thus could help in sustaining the apple production in Indian Himalayan region under changing climate situations. The potential genotypes based on their morphological and biochemical parameters identified like *M. baccata* (Khrot), *M. baccata* (Kinnaur), *M. baccata* (Pangi), *M. baccata* (Rohru), *M. baccata* (Shillong), *M. hilleiri*, *M. micromalus*, *M. prunifolia* (Maruba), *M. prunifolia* (Ringo-Assami), *M. sieversii* and *M. spectabilis* suggested them to be quite diverse. Besides, some indigenous Himalayan crab apples were also identified, which have inherent potential to serve as valuable genetic resources for rootstock improvement programmes.

### 1. Introduction

The genus *Malus*, belonging to subfamily Maloideae of the Rosaceae or Rose family and the order Rosales. The genus *Malus* consists of varied number of species, which range from eight (Likhonos, 1974) to 78 (Ponomarenko, 1986). The existence of cross compatibility among different *Malus* species results in evolution of various sub-species. Thus,

taxonomy of genus *Malus* still remains complex, unclear and it is often felt that it needs revision in the future with deciphering of new information in the genomic era (Robinson et al., 2001). Among the *Malus* species, only *Malus × domestica* is a widely cultivated species, which is believed to be originated as a result of natural inter-specific hybridization amongst several progenitor species (Korban et al., 1992). The Central Asiatic wild apple *Malus sieversii* has been found as main

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**Table 1**

The name of the institutes, locations, coordinates, elevation, annual rainfall and temperature range where the field gene banks of *Malus* species are maintained.

Gene bank	Place	Coordinates	Elevation (m asl)	Annual rainfall (mm)	Temperature range (°C)
ICAR-Central Institute of Temperate Horticulture Regional Station (CITH RS)	Mukteshwar (Uttarakhand)	29°28'N, 79°39'E	2280	1300–1600	– 3.0 to 40.0
ICAR-Indian Agricultural Research Institute Regional Station (IARI RS)	Dhanda, Shimla (Himachal Pradesh)	31°06'21.6"N, 77°07'02.5"E	1975	1615	– 2.0 to 40.0
ICAR-National Bureau of Plant Genetic Resources Regional Station (NBPGR RS)	Phagali, Shimla (Himachal Pradesh)	31°05'53.89"N, 77°09'34.92"E	1924	1600	– 2.0 to 40.0

progenitor of *Malus* × *domestica* based on the fruits, morphological and molecular evidences (Coart et al., 2006; Velasco et al., 2010). Furthermore, molecular data revealed that the European crab apple *M. sylvestris* was to be the secondary contributor (Cornille et al., 2012). Later, some authors have emphasized that the *Malus baccata* and *M. orientalis* to have also contributed in the evolution of cultivated *Malus* × *domestica* (Luby et al., 2001; Forsline et al., 2003). Hence, different *Malus* species have played significant role in the evolution and diversity in *Malus* × *domestica* cultivars. Therefore, diversity and evolutionary studies on wild *Malus* species still remains pertinent and fascinating in fruit crop systematics.

A number of wild apples have been found growing in different agro-climatic regions of Himalayan range (Pramanick et al., 2012), which is recognized as one of the centre of origin of the genus *Malus* (Juniper et al., 1998). The indigenous wild Himalayan apples were botanically classified in two *Malus* species, viz. *M. baccata* and *M. sikkimensis* (Hooker, 1879; Anon 1962). As a part of apple germplasm collection, explorations were made to collect these diverse wild apples from the different Indian Himalayan states. These crab apples are suspected to be variants of *Malus baccata* and are thus named according to the place of their collection. Several of these variants have been collected and maintained at field gene banks. These *M. baccata* variants have been found to be quite diverse. This unique group of wild Himalayan apples are meagrely represented in the botanical classification attempted globally (Han et al., 2006; Velasco et al., 2010). At present, the existing genetic diversity in wild apples warrants a distinct species or varietal status (Kishore and Randhawa, 1993; Kishore et al., 2005). Since the commercial apple production in India thrives in the Indian Himalayan states, a number of exotic *Malus* species have also been introduced in this region as collection in field gene banks for genetic improvement.

The wild *Malus* species are important genetic resources for further improvement of cultivated apples since they carry genes, which are responsible for tolerance to various biotic and abiotic stresses (Forte et al., 2002) as well as various novel horticultural traits (Kishore et al., 2005). Therefore, conservation and maintenance of wild apple germplasm is essential for future apple breeding programmes. Evaluation and characterization of genetic diversity existing in the wild apple germplasm is equally important to make the decision on efficient germplasm management systems (Oraguzie et al., 2001). The genetic diversity among wild apple species can be analysed by evaluating morphological, phenological and agronomic traits, as well as using biochemical and molecular markers (Höfer et al., 2014). Morphological parameters are one of the most decisive factors for taxonomic classification and agronomic value of the plants (Jannatabadi et al., 2014). Plant breeders mostly use the morphological parameters for assessment of desirable genetic material due to their simplicity in adoption and are also economical (Geleta et al., 2006). However, these parameters were highly sensitive to phenotypic plasticity; on other hand, they also allow the evaluation of diversity in the occurrence of environmental variations (Mondini et al., 2009).

In the present investigation, effort was made to analyse the morphological and biochemical diversity among the *Malus* species including some indigenous Himalayan wild apples, so as to establish the phylogenetic relationship among them. The information generated

might be useful for the taxonomical classification of wild *Malus* species. Furthermore, the information on variation in biochemical parameters would assist in establishing pre-selection criteria for distinguishing potential *Malus* species to be used as parent in the rootstock improvement programmes.

## 2. Materials and methods

### 2.1. Plant materials

The present study was undertaken on 32 *Malus* species including 12 indigenous Himalayan wild apples (*M. baccata* variants and *M. sikkimensis*), one *M. × domestica* cultivar Golden Delicious as reference of cultivated *Malus* species, while remaining 19 were exotic wild *Malus* species. Single accession of each species were taken in the study except *M. sikkimensis* and *M. baccata* var. *mandshurica*, which were maintained separately as duplicates in the two different gene banks. The 32 species were chosen for both morphological and biochemical diversity analysis are the ones which have been *ex situ* conserved in three public funded field gene banks located in two Indian Himalayan states (Table 1). The details of *Malus* species along with name of their field gene banks are presented in Table 2. The study was undertaken for two consecutive years, i.e. 2014 and 2015.

### 2.2. Analysis of morphological parameters

Total of 14 morphological traits were analyzed; broadly divided into qualitative and quantitative traits, which included leaf and tree attributes. The morphological characterization of *Malus* species was performed based on the Apple Descriptor developed by the International Union for the Protection of New Varieties of Plants (UPOV), International Board for Plant Genetic Resources (IBPGR) (now Bioversity International) and additional references were also included for identification of the traits. The details of qualitative and quantitative traits with their gradation are presented in Table 3.

### 2.3. Biochemical parameters

The biochemical parameters were grouped as leaf photosynthetic pigments, viz., chlorophyll *a*, chlorophyll *b*, ratio of chlorophyll *a*: *b*, total chlorophyll, total carotenoids; antioxidant enzyme activities, viz., peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), total soluble protein, total phenolics and phenolic components, viz., phloridzin, rutin, catechin and epicatechin. The biochemical compounds were estimated from the recently matured leaves (45 day after bud break) of selected *Malus* species. Healthy leaf samples of each *Malus* species were collected in ice box from the field gene banks and stored at deep freeze (– 20 °C). Different biochemical compounds except the phenolic fractions were estimated for two consecutive years, i.e. 2014 and 2015. The details of methods opted for their estimations are given hereunder.

#### 2.3.1. Leaf photosynthetic pigments

The leaf chlorophyll contents (chlorophyll *a*, *b* and total) were

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