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Comparison of foliar photosynthetic pigments in indigenous Himalayan *Malus baccata* (Shillong) with different apple cultivars

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Abstract
In the present investigation, the photosynthetic potential of the wild and cultivated apples was examined. The indigenous Himalayan wild apple *Malus baccata* (Shillong) showed the highest photosynthetic pigments, *viz*., chlorophyll *a* (2.53 mg/g FW), chlorophyll *b* (0.78 mg/g FW) and total chlorophyll content (3.33 mg/g FW), which was at par with disease resistant apple genotype Liberty. The photosynthetic potential and disease resistance ability of the indigenous Himalayan wild apple *M. baccata* (Shillong) accessions make them fit for further exploration as commercial rootstock with dwarfing ability besides biotic and abiotic stress tolerance types.

Keywords: *Malus* sp., chlorophyll, pigments, variability

Introduction
The Indian Himalayan region represents the great diversity for fruit crop species including the wild apple species (Dhillon and Rana 2004; Rana *et al*. 2007) [4, 15]. The wild apples distributed across the Indian Himalayas are popularly termed as indigenous Himalayan crab apples (Kishore *et al*. 2005) [8]. The indigenous Himalayan crab apples are distinctly classified in to two species, *viz*., *Malus baccata* and *M. sikkimensis* (Hooker 1879; Anon. 1962) [6, 1]. The several ecotypes of the *M. baccata* were collected from the Indian Himalayan states (Himachal Pradesh, Jammu and Kashmir, Uttarakhand and Sikkim). These ecotypes have been collected and conserved in the Field Gene Banks at different ICAR research institutes. These indigenous Himalayan crab apples are the important source for the biotic and abiotic stress tolerance (Sharma *et al*. 2006) [13, 19] as well possesses important horticultural traits (Kishore and Randhawa 1993; Kishore *et al*. 2005; Kishore *et al*. 2015) [7-9]. Apart from these, crab apples are well adapted under the Indian Himalayan conditions being naturally distributed over the high steep slopes. Thus, these indigenous Himalayan crab apples offer the genetic resources for the development of the new rootstock and scion genotypes, which would assist in sustaining apple production in this region.

The indigenous Himalayan crab apples *Malus baccata* (Shillong) is one of the ecotypes of *Malus baccata* collected from the North-eastern India- Shillong, Meghalaya (Pramanick *et al*., 2012) [14]. The ecotype of *M. baccata* (Shillong) was evaluated for various biotic and abiotic stresses resistance and found resistance for wooly aphid, powdery mildew and scab (Randhawa and Kishore 1981; Kishore and Randhawa 1993; Pramanick *et al*. 2006) [16, 7]. Thus, *M. baccata* (Shillong) can be widely explored as suitable rootstock or can be used as important genetic resource for the development of new rootstock or scion genotypes. However, photosynthetic efficiency of *Malus baccata* (Shillong) has never been estimated in the past. The photosynthetic pigments are important indices for measuring photosynthetic ability of a plant genotype. Various organic solvent, *viz*., dimethyl-formamide (DMF), dimethyl sulphoxide (DMSO), acetone, methanol, ethanol and chloroform *etc*. are used to measure the photosynthetic pigments in the crop plants and DMSO was found advantageous and efficient for chlorophyll extraction (Parry *et al*. 2014) [12]. Keeping this view, DMSO based photosynthetic pigments extraction was tried among the wild and cultivated apples.

Material and Methods

Plant material

The four *Malus* genotypes, *viz*., an indigenous Himalayan crab apple *M. baccata* (Shillong), commercial apple cultivars Golden Delicious, Red Spur and Liberty were collected from the Field Gene Bank located at Indian Agricultural Research Institute, Regional Station, Amartara Cottage Shimla (H.P.) Dhanda farm. The location is situated at the coordinates of 31°06'21.6″N, 77°07'02.5″E with an elevation of 1975 m AMSL and receiving mean annual rainfall 1615 mm and annual temperature ranged from -2.0 to 40.0˚C.

Photosynthetic Pigments

The photosynthetic pigments *viz*., chlorophyll *a*, chlorophyll *b* and total chlorophyll were estimated from the collected leaf samples of the *Malus* genotypes. The leaf chlorophyll contents (chlorophyll *a*, *b* and total) were estimated as per the method described by Barnes *et al*. (1992) [3] with some modifications. Exactly 200 mg of leaf samples were weighted, chopped in small pieces and kept in the test tubes. The dimethyl sulphoxide (DMSO, SRL Mumbai) was used as organic solvent for the extraction of photosynthetic pigments. The leaf samples incubated in DMSO for 4 hr at 70˚C and absorbance was read on UV-VIS double beam PC 8 scanning auto cell spectrophotometer (UVD 3200; Labomed, INC, USA). Chlorophyll *a*, chlorophyll *b* and total chlorophyll were calculated on fresh weight basis as per the following formulae.

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{f.w.)} = \frac{(12.7 \times OD_{663}) - (2.69 \times OD_{645}) \times \text{volume} \times \text{dilution}}{1000 \times \text{Weight of the sample}} \times 100$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{f.w.)} = \frac{(22.9 \times OD_{645}) - (4.68 \times OD_{663}) \times \text{volume} \times \text{dilution}}{1000 \times \text{Weight of the sample}} \times 100$$

$$\text{Total chlorophyll (mg g}^{-1}\text{f.w.)} = \frac{(20.7 \times OD_{645}) - (8.02 \times OD_{663}) \times \text{volume} \times \text{dilution}}{1000 \times \text{Weight of the sample}} \times 100$$

Ratio of chlorophyll *a* and *b* was estimated by dividing the values of chlorophyll '*a*' by that of chlorophyll '*b*'.

Statistical analysis

The experiment was conducted in Randomised block design with five replications. Data were subjected to analysis by using statistical analysis system software (SAS version 9.3 SAS Institute, Inc., USA).

Results and Discussion

The photosynthetic pigments content of the studied wild and cultivated apple genotypes were differed significantly. The estimated photosynthetic pigments *viz*., chlorophyll *a*, chlorophyll *b*, Chlorophyll *a*; *b* and total chlorophyll content of wild and cultivars of apples are given in the Table 1. The chlorophyll *a* content of the wild and cultivated apples are differed significantly and the highest content (2.53 mg/g FW) was recorded in the indigenous Himalayan wild apple genotype *Malus baccata* (Shillong), which was at par with the genotype Liberty (2.25 mg/g FW), while minimum (0.31 mg/g FW) in the commercial apple cultivar Golden Delicious (Fig. 1 and Table 1). The chlorophyll *b* content of the wild and cultivated apple cultivars were also showed significant variations and indigenous Himalayan wild apple *M. baccata* (Shillong) had highest (0.78 mg/g FW) content of photosynthetic pigment chlorophyll *b* which was at par with the apple cultivar Liberty (0.66 mg/g FW), while lowest (0.10 mg/g FW) in the commercial apple cultivar Golden Delicious which was at par with Red Spur (0.21 mg/g FW) (Fig. 2 and Table 1). The total chlorophyll content of the wild and cultivated apples which differed significantly and the maximum total chlorophyll content (3.33 mg/g FW) was estimated in the indigenous Himalayan wild apple *Malus baccata* (Shillong), which was statically at par with the another genotype Liberty (2.93 mg/g FW), while minimum was in the commercial apple cultivar Golden Delicious (0.41 mg/g FW) (Fig. 3 and Table 1). Further, the ratio of chlorophyll *a* and chlorophyll *b* was calculated among the studied wild and commercial apple cultivars though it was not significantly different (Fig. 4 and Table 1). Among the photosynthetic pigments, chlorophylls are important, which are stored in the chloroplast and retained in the greener parts of the leaf, stem, root and flower (Srichaikul *et al*. 2011; Mirza *et al*. 2013) [20, 10]. The chlorophyll *a* and chlorophyll *b* are the major chlorophyll fractions and actively involved in the conversion of light energy to chemical energy (Richardson *et al*. 2002) [17]. Thus, chlorophyll content directly decides the photosynthesis and production ability of the plant genotypes (Whittaker and Marks 1975; Curran *et al*. 1990; Filella *et al*. 1995) [5]. The content of photosynthetic pigments particularly chlorophyll content also indicates the nutrient status of the plant because most of the nitrogen is incorporated into the chlorophyll (Moran *et al*. 2000) [11]. Besides, the chlorophyll content also indicates the senescence and stress status of the plant (Hendry *et al*. 1987; Merzlyak *et al*. 1999; Tripathi *et al*. 2007) [21]. In the present investigation, the indigenous Himalayan wild apple genotype *M. baccata* (Shillong) showed high photosynthetic pigments i.e. chlorophyll *a*, chlorophyll *b* and total chlorophyll, which indicate the high photosynthetic potentiality of the indigenous wild apple genotype. Furthermore, *M. baccata* (Shillong) confirmed the tolerance / resistance to various biotic stresses, which make them fit for commercial rootstock or the important genetic material for improvement of both rootstock and scion genotypes. The present study elucidated the usability of the *M. baccata* (Shillong) as potent rootstock, which could be explored for the improving the photosynthetic efficiency of the scion genotype, because it could be clearly deciphered since the rootstock genotype affects the photosynthetic pigment fractions in the scion genotypes (Šabajevienė *et al*. 2006) [18].

Table 1: Variation in chlorophyll content (*a*, *b*, total and *chl a*:*b*) of wild and cultivated apples

Genotype	Chlorophyll *a* (mg/g FW)	Chlorophyll *b* (mg/g FW)	Total chlorophyll (mg/g FW)	Chlorophyll *a*:*b* ratio
Golden Delicious	0.31^{c}	0.10^{b}	0.41^{c}	3.68^{a}
Liberty	2.25^{a}	0.66^{a}	2.93^{a}	5.15^{a}
Red Spur	1.36^{b}	0.21^{b}	1.58^{b}	6.35^{a}
M. baccata (Shillong)	2.53^{a}	0.78^{a}	3.33^{a}	3.48^{a}
LSD ≤ (0.05)	0.46	0.29	0.52	3.15

Note: Superscript in same letters are not statistically significant.

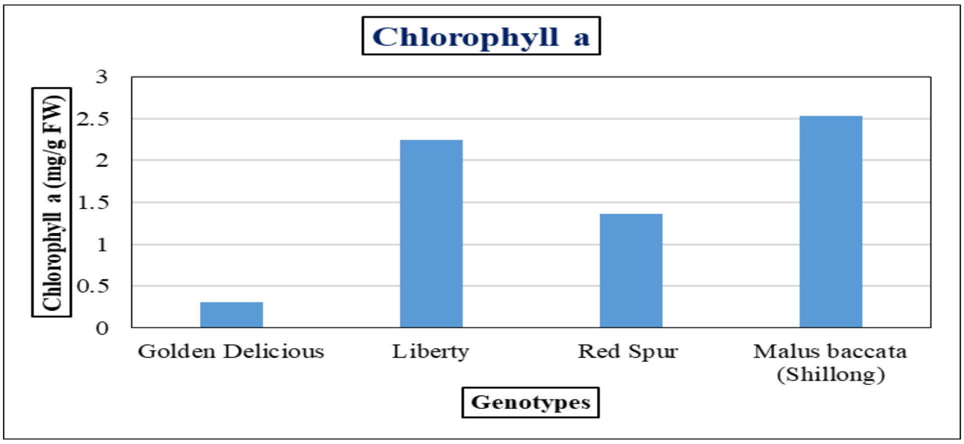

Fig 1: Variation in chlorophyll *a* content of wild and cultivated apple genotypes

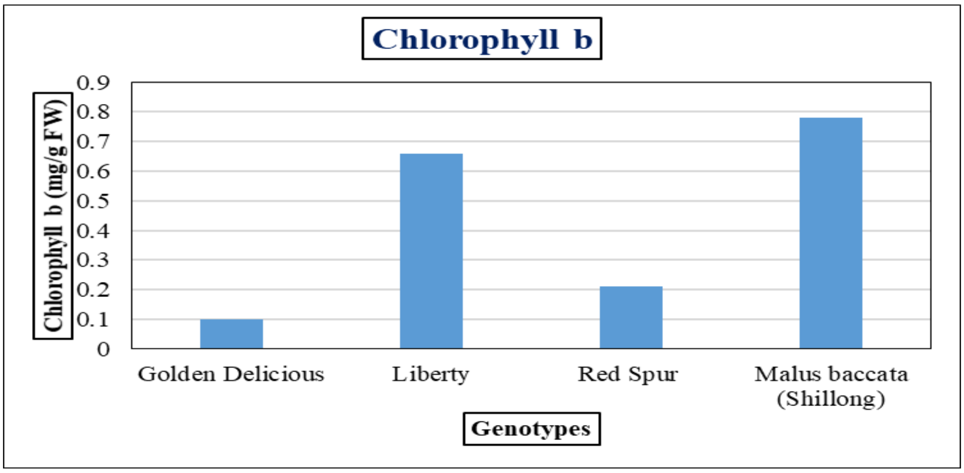

Fig 2: Variation in leaf chlorophyll *b* contents of wild and cultivated apple genotypes

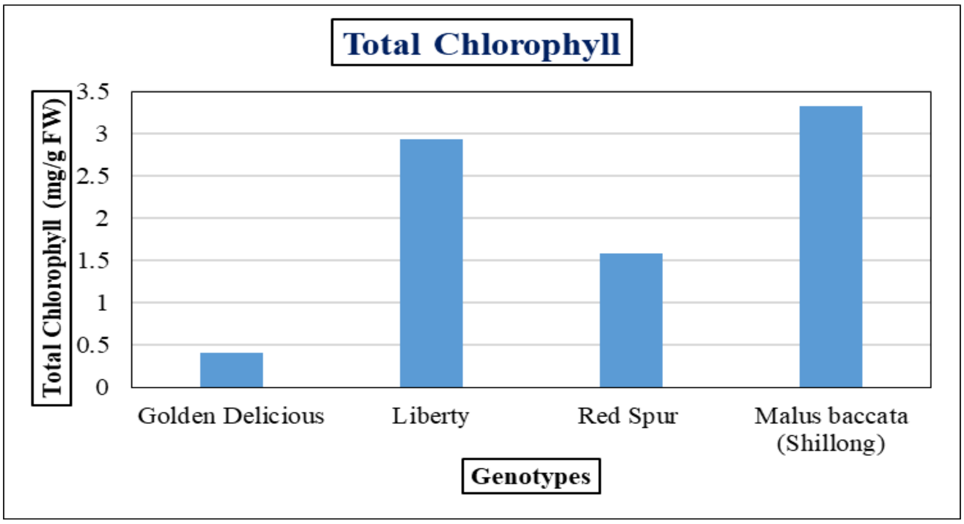

Fig 3: Variation in leaf total chlorophyll content in wild and cultivated apple genotypes.

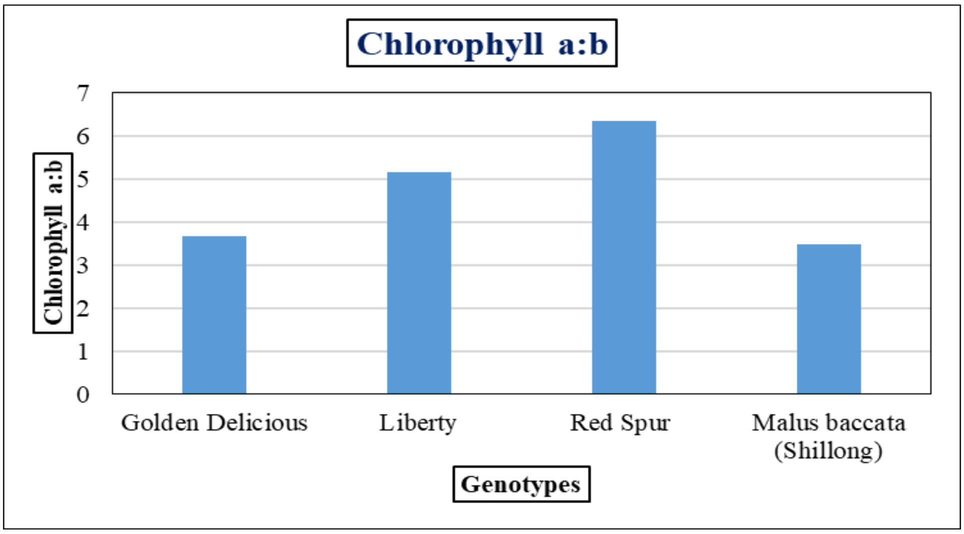

Fig 4: Variation in chlorophyll *a*:*b* of wild and cultivated apples

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