**Morphological Diversity Assessment and Characterization of Cultivated and Indigenous germplasm of Apple (*Malus* × *domestica* Borkh.) in India**

**Abstract**

Apple cultivation is one of the major means of subsistence in the Himalayan region of India. The existence of delicious group of cultivars has a tendency to dominate, resulting in a mono-cropping system. In order to diversify cultivation and extend the harvest season, there is a need to evaluate new cultivars based on physico-chemical attributes of their fruit, which determine the market prices for the same. This needs to be done before the recommendation of new cultivars to farmers for adoption. Exploration of indigenous genetic resources may also help to identify promising selections suitable for cultivation. The present study aimed at assessment of variability and germplasm characterization of forty-eight apple cultivars and indigenous selections in the Bhaderwah region of Jammu & Kashmir (J&K). The study evaluated different morphological characteristics, which showed that growth habit of the genotypes was predominantly spreading (62.5%), followed by upright (33.33%), and drooping (4.17%) forms. Tree vigor was strong (50%), medium (39.58%), and weak (6.25%). Tree height ranged from 3.25 meters (BA-1) to 5.68 meters (BA-19), and leaf area ranged from 22.78 cm² (BA-39) to 41.44 cm² (BA-3). The flowering period ranged from 21 to 25 days. Out of 48 genotypes, 32% were early bloomers, 52% were mid-bloomers, and 16% were late bloomers. Fruit set was as high as 87.57% (BA-4) and as low as 37.89% (BA-19). The PCA and Cluster analysis showed positive correlations among different morphological traits, indicating their potential for direct or indirect selection in breeding programs. The PCA highlighted the most diverse germplasm, which was genotype BA-46 and BA-47, while Cluster analysis grouped the genotypes into distinct groups with Cluster II showing notable variation. Based on the results of this study, it is concluded that there is wide diversity among the germplasm under investigation for morphological traits. Notably, the seedling-origin plants differed markedly from the commercial apple cultivars. It is recommended that these promising seedling strains be considered for further breeding programs to improve apple production and quality in J&K, particularly in the Bhaderwah region.

**Keywords**: Clustering, Variability, Morphological Characterization, genetics.

**Introduction**

Apple (*Malus* x *domestica* Borkh.) is one of the leading fruit crops of international trade. The species is believed to have originated in the Tien Shan mountains of Central Asia and spread to Asia and Europe via trade routes. Being a deciduous fruit grown in temperate regions, apples are of great economic importance and are highly profitable. They constitute the most important tree fruit crop of the temperate zones and display a great range of commercial cultivars. Apples are grown all over the world, including Central and Western Asia, India, Western China, Europe, and parts of America and Africa (Juniper *et al*., 1999). In India, apples are cultivated over 315,000 hectares, producing around 2,506,000 metric tonnes annually (Anonymous, 2023a). The main apple-producing states in India are Jammu and Kashmir (the leading region), Himachal Pradesh, Uttarakhand, Arunachal Pradesh, and Nagaland. In Jammu and Kashmir, the total area under apple cultivation is about 164,744 hectares, producing around 1,882,320 MT of apples, with a productivity rate of 11.42 metric tonnes per hectare.

In the Jammu region, the area under apple cultivation is approximately 18,416 hectares, producing around 30,596 MT (Anonymous, 2023b). The future of apple breeding relies on maintaining genetic diversity, which enables species to adapt to changing environmental conditions (Martinelli *et al*., 2008). The collection and evaluation of local germplasm is a fundamental step in cultivar improvement (Damyar *et al*., 2007; Forte *et al*., 2002; Mratinic and Fotric-Aksic, 2012). Breeders worldwide annually produce new cultivars, but every country and region once had its own native varieties of apples. Similar efforts to conserve and evaluate the diversity existing in the gene pool of Malus have been carried out in countries such as Estonia (Kask, 2002), Portugal (Bettencourt, 2002), Spain, and Belgium (Lateur, 2002). The germplasm collections need to be well characterized with international descriptors (UPOV, 2005; IBPGRI, 2002) for effective management and utilization. The traditional methods of cultivar characterization based on agronomic and morphological parameters are in use for differentiation of cultivars within the same species (Cantini *et al*., 1999; Barranco and Rallo, 2000; Farrokhi *et al*., 2011). Likewise, studies on evaluation of native apple germplasm in the Jammu region showed the presence of variation for agronomic traits among accessions studied (Mratinic and Fotric-Aksic, 2012). Multivariate analysis such as Principal Component Analysis (PCA) is an ideal tool for studying correlations among variables, cultivar evaluation, and interpretation of the relationship among genotypes for germplasm characterization (Pereira-Lorenzo *et al*., 2003; Aljane and Ferchichi, 2009).

**Materials and Method**

**Study site:** Present study was carried during 2023-2024 at Bhaderwah region of doda district of (J&K), India. It is located 320 53’ and 340 21’ latitude and 750 01’ and 760 47’ E longitudes with an altitudinal 1600 m above the sea level. The climate is characterized as sub humid temperate region, rainfall restricted mostly to winter months. Snowfalls during January and February months are also experienced.

The aim of this study was to evaluate morphological traits of apple germplasm. The material comprised 48 apple germplasm (30 known varieties and 18 indigenous apple acsessions) examined using apple descriptor IBPGR, (2002). All the accessions were examined for tree characters viz., tree vigour, tree growth habit, type of bearing, number of lenticels present; leaf characters viz., leaf shape, extent of anthocyanin colouration from base and fruit characters viz., fruit shape, bloom of fruit skin, ground colour, firmness, eating quality. Other characters viz., leaf Area, petiole length, fruit length, fruit diameter was measured by using Vernier Calliper; flowering characters viz., silver tip stage, initial bloom and final bloom were studied. The initial bloom was observed when 10% of flowers were open and the date was recorded. The full bloom was observed when 80% of flowers were open and the date was recorded. Time for harvest (days) was calculated out of time lag between the date of full bloom and the date of actual harvest and the genotypes were classified as early (100-120 days), mid (120-150 days) and late (>150days).

**Table:1 Name and code of forty-eight apple (*Malus* × *domestica* Borkh.) germplasm evaluated.**

|  |  |  |
| --- | --- | --- |
| Sr. no | Genotypes | Variety/Location |
| 1 1. 1. | BA-1 | Starking delicious |
| 2. | BA-2 | Red delicious |
| 3. | BA-3 | Oregon spur |
| 4. | BA-4 | Red spur |
| 5. | BA-5 | Red gold |
| 6. | BA-6 | Jona gold |
| 7. | BA-7 | Coop-IV |
| 8. | BA-8 | Vista Bella |
| 9. | BA-9 | Super chief |
| 10. | BA-10 | Lodh |
| 11. | BA-11 | Vance delicious |
| 12. | BA-12 | Silver spur |
| 13. | BA-13 | Mollies delicious |
| 14. | BA-14 | Red chief |
| 15. | BA-15 | Gold spur |
| 16. | BA-16 | Royal delicious |
| 17. | BA-17 | Well spur |
| 18. | BA-18 | Top red |
| 19. | BA-19 | Lal Ambri |
| 20. | BA-20 | Firdous |
| 21. | BA-21 | Golden delicious |
| 22. | BA-22 | Hybrid-29 |
| 23. | BA-23 | Starkrimson |
| 24. | BA-24 | Hybrid-60 |
| 25. | BA-25 | Akbar |
| 26. | BA-26 | Shireen |
| 27. | BA-27 | Amarican Trail |
| 28. | BA-28 | Hard France |
| 29. | BA-29 | Ambari |
| 30. | BA-30 | Maharaji |
| 31. | BA-31 | local germplasm of Bhaderwah region |
| 32. | BA-32 | local germplasm of Bhaderwah region |
| 33. | BA-33 | local germplasm of Bhaderwah region |
| 34. | BA-34 | local germplasm of Bhaderwah region |
| 35. | BA-35 | local germplasm of Bhaderwah region |
| 36. | BA-36 | local germplasm of Bhaderwah region |
| 37. | BA-37 | local germplasm of Bhaderwah region |
| 38. | BA-38 | local germplasm of Bhaderwah region |
| 39. | BA-39 | local germplasm of Bhaderwah region |
| 40. | BA-40 | local germplasm of Bhaderwah region |
| 41. | BA-41 | local germplasm of Bhaderwah region |
| 42. | BA-42 | local germplasm of Bhaderwah region |
| 43. | BA-43 | local germplasm of Bhaderwah region |
| 44. | BA-44 | local germplasm of Bhaderwah region |
| 45. | BA-45 | local germplasm of Bhaderwah region |
| 46. | BA-46 | local germplasm of Bhaderwah region |
| 47. | BA-47 | local germplasm of Bhaderwah region |
| 48. | BA-48 | local germplasm of Bhaderwah region |

**Results and Discussion**

**Morphological characteristics**

For the phenotypic characterization of 48 apple germplasm on the basis of morphological traits is done by using IBPGR 2002 apple descriptor (table 2). The germplasm were assessed for twelve different traits. The results of tree characters were accessed in H’ index are given in table 2.

**Table 2: Descriptor utilized for the morphological evaluation of apple germplasm and estimated Shannon-Weaver diversity index (H’) value.**

|  |  |  |  |
| --- | --- | --- | --- |
| S. No. | Descriptor | Descriptor state | H’ index |
| 1 | Tree growth habit | Spreading (5), Upright (3), Drooping (7) | 0.79 |
| 2 | Tree vigor | Strong (7), Medium (5), Weak (3) | 0.99 |
| 3 | Branch angle | Narrow (1), Medium (3), Wide (5) | 1.08 |
| 4 | Bearing habit | Spur (1), Mixed (3), Shoot (4) | 1.19 |
| 5 | Shoot tip colour | Dark brown (), Medium brown (), Light brown () | 1.05 |
| 6 | Leaf shape | Ovate (1), Oval (2), Lanceolate (3) | 0.99 |
| 7 | Fruit shape | Ellipsoid conical (10), Globose (1), Globose conical (2), Intermediate conical (8), Ovate (12), Oblong (11), Oblate (13) | 1.85 |
| 8 | Fruit color | Orange red (1), Pink red (2), Red (3), Purple (4), Yellow (6), Brown red (5) | 1.42 |
| 9 | fruit skin lenticels | High (7), Medium (5), Low (3) | 1.09 |
| 10 | Fruit apex | Smooth (1), Wrinkled (2), Grooved (3) | 0.85 |
| 11 | Leaf blade |  | 0.89 |
| 12 | Juiciness | Less (3), Medium (5), High (7) | 0.93 |

**Statistical analysis**

The Shannon-Weaver diversity index (H’) was used to measure phenotypic variability by analyzing the qualitative traits frequencies by using formula (Jain et al. 1975).

H’ = −Σpi (log2 pi)∕log2 n

where the proportion of genotypes accessions in the ith category of an n-class characteristic is denoted by “pi”, “n” represents the number of phenotypic categories for a trait. This proportion ranges from 0 to 1. The diversity index, H′, is categorized as high (H′≥0.60), intermediate (0.40≤H′≤0.60), or low (0.10≤H′≤0.40), based on the classification system described by Eticha *et al*. (2005). Correlation and Principal Component Analysis (PCA) is done by using R-Studio software, Cluster analysis was carried out by NTSYS and cluster mean is done by indostat. Correlation analysis among quantitative traits is done by R software (Olivoto and lucio 2020).

**Frequency distribution of apple germplasm for morphological traits**

For morphological traits the frequency distribution of germplasm is represented in fig. 1. total of 48 germplasms were characterized as having good early plant vigor (Fig. 1A). Most of the germplasm exhibited a semi-erect plant growth habit (Fig. 1B). Dark purple fowers were observed in the majority of accessions (Fig.  1C), while green leaves were predominant (Fig. 1D). Most accessions displayed light green immature pods (144 genotypes) and pale tan

A B

C D

E F

G H

I J

Fig. 1: Frequency distribution of Apple germplasm for morphological traits.

**Variability parameters**

The diversity among apple genotypes for qualitative traits (Table 3) was accessed by estimating the Shannon-Weaver diversity index (SDI). SDI ranged from 0.79-1.85. Among all the traits the highest diversity was observed in fruit shape (1.85) followed by fruit colour (1.42) and fruit skin lenticels (1.09). the lowest diversity was observed in tree growth habit (0.79) followed by fruit apex (0.85) and leaf blade (0.89).

The results of this study were in close confirmation with (Mratinic and Aksic, 2012), who reported that the selections of wild apple showed variability in their tree behavior and tree size. Wohner *et al*. (2014) also described the morphology of *Malus × robusta* as a small to medium-sized tree with a weak to strong vigor and upright to spreading crown and medium to absent pubescence on the upper side of the shoot surface.

**Table 3: Morphological Correlation**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Fruit length | Fruit width | Fruit weight | Fruit firmness | Petiole length | Leaf area | Number of flowers | Fruit set | Fruit drop | Number of seeds |
| Fruit width | 0.808\*\* | -0.952\*\* | -0.817\*\* | 0.923\*\* | -0.946\*\* | 0.893\*\* | 0.145 | -0.939\*\* | -0.938\*\* | 0.938\*\* |
| Fruit weight |  | -0.787\*\* | -0.704\*\* | 0.754\*\* | -0.788\*\* | 0.701\*\* | 0.095 | -0.779\*\* | -0.773\*\* | 0.781\*\* |
| Fruit firmness |  |  | 0.859\*\* | -0.923\*\* | 0.984\*\* | -0.933\*\* | -0.173\* | 0.952\*\* | 0.958\*\* | -0.947\*\* |
| Petiole length |  |  |  | -0.814\*\* | 0.846\*\* | -0.834\*\* | -0.241\*\* | 0.812\*\* | 0.835\*\* | -0.794\*\* |
| Leaf area |  |  |  |  | -0.916\*\* | 0.901\*\* | 0.111 | -0.910\*\* | -0.910\*\* | 0.890\*\* |
| Number of flowers |  |  |  |  |  | -0.926\*\* | -0.150 | 0.947\*\* | 0.954\*\* | -0.939\*\* |
| Fruit set |  |  |  |  |  |  | 0.135 | -0.921\*\* | -0.919\*\* | 0.917\*\* |
| Fruit drop |  |  |  |  |  |  |  | -0.109 | -0.126 | 0.129 |
| Number of seeds |  |  |  |  |  |  |  |  | 0.947\*\* | -0.933\*\* |
| Cropping efficiency |  |  |  |  |  |  |  |  |  | -0.933\*\* |

Conclusion:

The present study confirmed that there is huge diversity of apple at Bhaderwah region of J&K, thus it becomes necessary for preserving these unique genetic resources and continuing its study to ensure its conservation, exchange and utilization in future breeding programmes for future development of innovative, market-driven cultivars.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

Option 2:

Author(s) hereby declares that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1. I hereby declared that no generative I technology such as Large Language Models (Chat GPT, manuscript.

2.

3.

**References:**

Aljane, F. and Ferchichi, A. (2009). Assessment of genetic diversity among some southern Tunisian fig *(Ficuscarica L*.) cultivars based on morphological descriptors. *Jordan Journal of Agricultural Sciences*, **5**(1):1-16.

Anonymous, (2023a). Indian Horticulture Database. National Horticulture Board, Gurgoan.

Barranco, D. and Rallo, L. (2000). Olive cultivars in Spain. *Hort Technology*, **10**(1): 107-110.

Bettencourt, E. (2002). Status of Malus/Pyrus germplasm collections in Portugal. *compilers. 2004. Report of a Working Group on Malus/Pyrus*, 46.

Bignami, C., Vagnoni, G. and Magro, P. (2003). Field Evaluation of Old Italian Apple Cultivarsfor Scab Susceptibility. *Acta Horticulture*, **598**:91-96.

Bozbuğa, F. and Pırlak, L. (2012). Determination of phenological and pomological characteristics of some apple cultivars in Niğde-Turkey ecological conditions. *Journal of Animal and Plant Sciences*, **22**(1): 183-187.

Cantini, C., Cimato, A. and Sani, G. (1999). Morphological evaluation of olive germplasm present in Tuscany region. *Euphytica*, **109**:173- 181.

Damyar, S., Darab, H., Raana, D., Hassan, H., Ali, A.Z. and Esmaeil, F.(2007). Evaluation of Iranian native apple cultivars and genotypes. *Journal of Food Agriculture and Environment*, **5**: (3):211-215.

Eticha, F., Bekele, E., Belay, G., Börner, A. (2005). Phenotypic diversity in tetraploid wheats collected from Bale and Wello regions of Ethiopia. *Plant Genetic Resources*-C, **3**(1):35–43.

Farrokhi, J., Darvishzadeh, R., Naseri, L., Azar, M.M. and Maleki, H.H. (2011). Evaluation ofGenetic Diversity Among Iranian Apple (*Malus× domestica* Borkh.) Cultivars andLandraces Using Simple Sequence Repeat Markers. *Australian Journal of Crop Science*, **5**(7): 815-821.

Forte, A.V., Ignatov, A.N., Ponomarenko, V.V., DoroKhov, D.B. and Savelyev, N.I. (2002). Phylogeny of the Malus (Apple Tree) species, inferred from the morphological traits andmolecular DNA analysis. *Russian Journal of Genetics*, **38**(10):1150-1161.

IBPGRI. 2002. Descriptor List for Apple (*Malus*). IBPGRI Secretariat, Rome, Pp.32-34.

Jain, S.K., Qualset, C.O., Bhatt, G.M., Wu, K.K. (1975). Geographical patterns of phenotypic diversity in a world collection of durum wheats 1. *Crop Science*, **15**(5):700–704.

Juniper, B. E., Watkins, R., and Harris, S. A. (1999). The origin of the apple. *Acta Horticulture*, **48**:27–33.

Kask, K. (2002). An inventory of apple and pear cultivars of Estonian origin. In L. Maggioni, M. Fischer, M. Lateur, E. J. Lamont and E.Lipman, eds., Report of a working group on Malus/Pyrus. IPGRI, pp.22-26.

Lateur, M. (2002). Short note on Malus/Pyrus genetic resources in Belgium. InL. Maggioni, M. Fischer, M. Lateur, E. J. Lamont and E.Lipman, eds., Report of a working group on Malus/Pyrus. IPGRI, P.19.

Lateur, M., Ordidge, M., Engels, J. and Lipman, E. (2012). Report of a working group on Malus/Pyrus. In *Fourth meeting*, 7-9.

Maggioni, M. Fischer, M. Lateur, E. J. Lamont and E.Lipman, eds., Report of a working group on Malus/Pyrus. IPGRI, Pp.56-57.

Martinelli, F., Busconi, M., Camangi, F., Fogher, C., Stefani, A. and Sebastiani, L.(2008). Ancient Pomoideae (*Malus domestica BorKh.* And *Pyruscom munis* L.) cultivars in “AppeninoToscano” (Tuscany, Italy): molecular (SSR) and morphological characterization. *Caryological*,**61**(3):320- 331.

Mratinic E. and Fotric Aksic, M. (2011). Evaluation of phenotypic diversity of apple (*Malus* sp.) germplasm through the principal component analysis. *Genetika*, **43**(2): 331-340.

Mratinic, E. and Fotric Aksic, M. (2012). Phenotypic diversity of apple (*Malus* sp.) germplasm in South Serbia.  *Brazilian archives of biology and technology*, **55**(3): 349-358.

Olivoto, T. and Lúcio, A. D. C. (2020). metan: An R package for multi‐environment trial analysis. *Methods in Ecology and Evolution*, **11**(6): 783-789.

Pereira-Lorenzo, S., Ramos-Cabrer A. M. and Ascasibar- Errasti, J. 2003. Analysis of apple germplasm in Northwestern Spain. [*Journal of the American Society for Horticultural Science*](https://journals.ashs.org/jashs/view/journals/jashs/jashs-overview.xml), **128**(1): 67-84.

Reim, S., Proft, A., Heinz, S. and Hofer, M. (2012). Diversity of the European indigenous wild apple *malus sylvestris* (L.) Mill. in the east Ore Mountains (Osterzgebirge), Germany: morphological characterization. *Genetic Resources and Crop Evaluation*, **59**(6):1101- 1114.

UPOV International union for the protection of new varieties of plants., 1974. Draft guidelines for the conduct of tests for distinctness, homogeneity and stability (Apple). Intl. Union Protection New Varieties Plants, pp 23.

UPOV Apple Descriptor. 2005. Guidelines for the conduct of tests for Distinctness, uniformity and Stability by International Union for The Protection of New Varieties of Plants, Geneva. pp: 45.