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Strategies for rootstock and varietal improvement in apple: A review

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Abstract
Low productivity in apple is mainly due to old senile orchards, low yielding varieties, poor orchard and canopy management as well as improper technical facilities. Rootstocks play an important role by influencing flowering, yield, nutrient uptake, canopy architecture and thereby fruit quality. Plant grown on seedling rootstock generally develop into large and vigorous canopy architecture and hence, is difficult to manage than the clonally propagated ones. For instance, the apple rootstock M 9, M 27, etc. induces larger fruit size and good quality fruits by better light interception and good aeration within the canopy than seedling rootstocks. Rootstocks provide a mean of controlling scion cultivar by modifying the size and shape of the trees through shorter intermodal length, altering the angle of the branches etc. and tend to make a cultivar adapted to wider climatic conditions, reduces the maturation time and also infers resistant to abiotic as wells as biotic stress and hence serve as an essential component in modern fruit production. Furthermore, it makes the orchards more uniform and productive and there is a need to complex underlying mechanisms which would ease future rootstock as well as cultivar breeding programme and therefore more concerted efforts are required to research of rootstocks and varieties of apple which will help to mitigate as well as exploit the beneficial effects and to overcome the problem facing by the fruit crop.

Keywords: Apple, rootstocks, variety, improvement, quality

Introduction
Plant genotypes used for exploiting their inherent traits such as dwarfness, biotic and abiotic stress tolerance, earliness, fruit quality improvement etc. imparted on the scion variety are referred as rootstock and are divided in two groups based on propagation method as seedling rootstock i.e. develop from germinated seeds and clonal rootstocks which are vegetativaly propagated in nature. Both seedling and clonal rootstocks have their own advantages as well as disadvantages. The major problem associated with seedling rootstock is genetic variability which cause lack of uniformity, unsuitability for intensive orcharding due to vigorous nature, graft incompatibility etc. However clonal rootstocks overcome these barriers because of similar genetic makeup as and can be expected to have identical growth characteristics in a given environment (Hartmann *et al.*, 2009) [5]. Furthermore, these rootstocks impart resistance to pest and diseases, adverse soil conditions, drought conditions and also to replant soil. But clonal rootstock also possess its own set of disadvantages as it is very difficult to propagate through cuttings and stooling techniques mostly in temperate fruit crops (ex. cherry, apple) and thus lacks in availability. Most of them are successfully propagated in areas with deep soil, flat and fertile land with assured irrigation facilities. They have brittle or shallow roots which results in poorly anchored plants susceptible to drought conditions and some of them are highly susceptible to certain pests and diseases thus restricting their use ex: rootstock 'Colt' rootstock of cherry is highly susceptible to crown gall.

Characteristics of an ideal rootstock
1. An ideal rootstock should be easy to propagate (either vegetatively or by seeds) and must produce well, clean, easy-to bud or graft with upright stem.
2. Good root system is prerequisite to provide adequate anchorage and support the scion tree.
3. It should have wider adaptability (soil as well as climatic), winter hardiness, salt tolerance, diseases and insect-pests resistance, dwarfing, precocity and heavy cropping effects to the scion cultivar.

Characteristics of an ideal variety

1. It should be regular and precocious bearer
2. It should produce quality fruits of uniform size, good colour and acceptable texture with good keeping quality.
3. It should be resistant to biotic as well as abiotic stresses and should not have pre- harvest fruit drop.
4. It should have wider climatic adaptability.

Strategies for rootstock improvement

1. Evaluation of Indigenous seedling rootstocks

Several indigenous biotypes of *M. baccata* have the potential to serve as a suitable rootstocks and there is a need of systematic evaluation of these rootstocks.

Table 1: Promising characters of some Indian rootstocks

Indian Rootstocks	Promising characters
Malus bacatta var himalica (Maxim.) Schneid	Partially resistant to collar rot, root rot and wooly aphid. Could be more dwarfing than M 9.
Malus bacatta (Linn.) Borkh Shilong	From Shilong (Meghalya). Similar in vigour to MM 106. Show high degree of resistance to powdery mildew.
Malus bacatta (Linn.) Borkh (Giabung)	Almost similar in vigor to M9.Show more dwarfing with Golden delicious scion than M9.
Malus bacatta (Linn.) Borkh (Rohru)	From Rohru H.P. May be more dwarfing than M9. Moderately resistant to Powdery mildew.

Srivastava *et al.* (2016) [15] carried out an experiment to find the suitable exotic as well as indigenous apple rootstocks for temperate zone of India for screening of collar rot, burr knots and woolly apple aphid. They reported highest collar rot incidence in M27 and M-26 and lowest in indigenous root stocks (CITH-Apple rootstocks). They found highest incidence of wooly apple aphid infestation in EMLA-106, M-9, M-27 and ALNARP whereas least in indigenous apple rootstock (CITH-ARS-01, CITH-ARS-02, CITH-ARS-03, CITH-ARS-10). Similarly burr knot was present in most of apple rootstocks except CITH-Apple-Rootstock01 and ALNARP. On the basis of the results obtained by them they concluded that the CITH-AR selections have better superiority to exotic Malling rootstocks for better suitability to climatic conditions of the North Western Himalayan regions of Kashmir.

2. Apomictic seedlings as a rootstock: Seed formation in flowering plant is carried through sexual or less commonly by asexual or apomictic processes. In Apomictic plants embryo is derived from somatic cells of the ovule that have not undergone the events of meiosis and fertilisation and therefore produce seeds with maternal genotype. This associative formation of endosperm may or may not involve fertilization. Apomixs serves as a means of clonally propagating plants through seeds due to absence of the meiotic process and lack of paternal contribution in embryo and if available in crop plants it can enable the propagation of hybrids by fixing hybrid vigour through many seed generations. Apomixis is genetically controlled phenomenon and therefore there is possibility that many genes controlling sexual development are also responsible for the induction of apomixis. As far as utility of apomixis in plant breeding is concerned it can help in rapid multiplication of genetically uniform individuals without risk of segregation, fixation of hybrid vigour, exploitation of maternal effect, development of homozygous inbreed lines etc. Examples of apple apomictic rootstocks includes *M. sikkimensis, M. hupehensis, M. rockii, M. toringoides, M. serqentili.* Schmidt (1988) [13] demonstrated that by crossing *M. Sergentii* with M 9 produces 60-90 per cent of maternal clones but gave low yields and small fruits and there needs to further research carried out in this aspect.

3. Development of drought tolerant rootstock

Introduction, evaluation, development and multiplication of drought tolerant rootstock may serve as an efficient tool for rootstock improvement particularly in water scarce areas and some of the examples includes M7, MM111, KC-1, KC-1-48-41, KC-14, Mark and M 26 which is intermediate drought tolerant. Atkinson *et al.* (1999) [1] conducted a study to assess drought tolerance of the commercial apple (*Malus domestica* Borkh.) rootstocks i.e M9, M26, M27 and MM111 and some new selections (AR69-7, AR295-6, AR36019, AR486-1 and AR628-2 from the rootstock breeding programme at HRI-East Malling by reducing the amount of irrigation after an initial period of growth to simulate natural drying in the soil and observed that the dwarfing rootstocks tend to have smaller amounts of both coarse (>2 mm diameter) and fine roots (<2 mm diameter), than the more vigorous rootstocks. They observed a close linear relationship between coarse and fine root, irrespective of rootstock or irrigation treatment and there was also no change observed in the length/weight relationship for fine roots.

4. Screening of newly developed rootstocks from abroad under Indian conditions

There is a need of properly screening and evaluating of potential rootstock from abroad under Indian conditions to facilitate efficient rootstock improvement programme. Robinson and Aldwinckle (2003) [12] evaluated Geneva series of apple rootstocks for tolerance to fire blight and *Phytophthora* root rot, high yield efficiency and good tree survival and reported that 'G.11' had the highest cumulative yield efficiency, good tree survival and also had good average fruit size. They also observed tree size to be similar as 'M.9', but exceeded the yielding parameters however 'G.65' was more dwarfing than 'M.9' with smaller fruit size and significantly lower cumulative yield 'M.9'.

5. Utilization of biotechnological approaches (marker assisted selection techniques (MAS)

Several potential sources of genes resistant to various biotic and abiotic factors have been present in apple germplasm which can be efficiently utilized for rootstock improvement programme. Some of these are mentioned below:

Table 2: Potential gene sources in apple germplasm

Gene	Effect	Source	Reference
Er	Wooly apple aphid	'Northern Spy'	
Pl_1	Powdery mildew	*M.* × *robusta*	Knight & Alston (1968) [8]
Pl_2	Powdery mildew	*M.* × *zumi*	
Vb Vf	Apple scab	*M. baccata M. floribunda* 821	
S1S2	Self incompatibility	'Northern Spy'	Knight *et al.* (1962) [9]

A Case study - Introduction of rolC into Marubakaidou apple rootstock via *Agrobacterium tumefaciens*

Igarashi *et al.* (2002) [6] introduced the rolC gene derived from *Agrobacterium rhizogenes* into apple cuttings in attempt to produce new dwarf rootstock cultivars with high rooting ability. They integrated 1-3 copies of the transgene into the plant genome of rolC transformants which resulted in short plants with smaller leaves, short internodes and reduced apical dominance whereas, adventitious root formation was induced earlier in rolC transformants than in pIG121Hm-transformants. They reported when the shoot apex was cut from rolC transformants and transferred to medium containing 1 mg/l BA, there was a alteration in cytokinin-related response in rolC transformants due to callus formation with abnormal multi-shoot primordia which cease growth. However, they reported that when cultured on plant growth regulator free medium these could develop into whole plants.

Strategies for Varietal Improvement

As the basic requirement for development of new cultivars changes fast and becomes tougher constantly i.e. better performance than existing cultivars, early maturity, large fruit size, firm skin without resetting, high sugar content, better preservation capacity etc, there need to implement different strategies for their improvement and some of these strategies are mentioned below:

Introduction and evaluation of low chill varieties

Climate change had led to shift in apple belts in Himachal Pradesh and effects of Heat wave Apple had led to following effects:

- Flowering was early by 15 days.
- Flower drop resulting in poor fruit set due to excessive rainfall during 2nd fortnight of April followed by fall in temperature.
- Optimum temperature for fruit blossom and fruit set is 24°C while the region experienced above 26 °C for 17 days.

Singh *et al.* (2016) [14] reported that introduction of low chill varieties may serve as an alternative for mitigating the effect of climate change on apple cultivation.

Development / Introduction of precocious and regular bearing variety

Through selection: Das *et al.* (2012) [4] reported a variety CITH Lodh Apple clonally selected from Red Delicious, matures early as compared to Red Delicious and high yielding with better fruit colouration under marginal chilling zones.

Main features of the variety are as under

- The variety has been developed through clonal selection from the old Red Delicious plantation and has been identified by the Central Institute of Temperate Horticulture (CITH), Srinagar in 2012.
- It is very precocious, regular bearing and mid-season blooming variety. Trees have spreading growth habit with large to medium, oblong or conical shaped fruits.
- Calyx end is prominent and bluntly lobed. Ground colour yellow and over colour dark shiny red with presence of medium lenticels on the skin. Fruit stalk is short-medium in length with 8.5 kg/cm^2 firmness and 13.6 ^{0}B Total soluble solids.
- Tolerant to major pests and diseases with good shelf life. The variety is very much suitable for cultivation in mid to high hills of North Western Himalayan agro-climatic zones where low fruit set has been recorded on traditional high chilling apple cultivars due to climate change.

Through Hybridisation: Brown and Maloney (2015) [3] developed a variety through hybridisation and named it as SnapDragon, gets its juicy crispness from its Honeycrisp parent and it has a spicy-sweet flavour that was a big hit with taste testers. It was released as New York 1 in 2010 and is similar to Honeycrisp, but without leaf disorders and bitter pit. It is an early-ripening, firm, good quality variety with high sugar level.

Gene transformation (for early flowering induction): Kotoda *et al.* (2010) [10] identified, isolated and characterized two flowering locus (*FT*)-like genes of apple i.e. *MdFT1* and mapped, them on distinct linkage groups (LGs) (LG 12 and LG 4) with partial homology. The found difference in expression pattern of *MdFT1* and *MdFT2* as former was mainly expressed in apical buds of fruit-bearing shoots during adult phase, whereas later one was expressed mainly in reproductive organs. Since transgenic Arabidopsis which expressed *MdFT1* or *MdFT2* flowered earlier than wild-type plants therefore both the genes also had the potential to early flowering induction. Furthermore, overexpression of *MdFT1* altered the expression of other endogenous genes and conferred precocious flowering in apple which suggests that *MdFT1* could function to promote flowering by alteration of gene expression and which suggests that other genes may play a crucial role in flowering regulation of apple. The long juvenile period of fruit trees prevents early cropping and efficient breeding and these findings will be useful information to unveil the molecular mechanism of flowering as well as developing new methods to juvenile period in various fruit crops.

Development of varieties having high yield and excellent quality

Brown and Maloney (2015) [3] developed a variety RubyFrost (NY2) through hybridisation and Parents are braeburn and Autumn Crisp. It was released in 2010 as New York 2. The fruits are good sources of vitamin C and have excellent storage and shelf life. The apples are ideal for fresh eating.

Development of firm Royal Gala apple

Atkinson *et al.* (2012) [2] studied the effect of down regulating the expression of PG1 in apple cultivar 'Royal Gala' (has high levels of PG1 and typically softens during fruit ripening). They observed 'Royal Gala' apples harvested at various seasons, in which *MdPG1* is supressed, were more firm than the controls. They found higher intercellular adhesion and analysis of cell wall indicated a higher molecular weight distribution of CDTA-soluble pectin, change in yield and composition of pectin. Whereas, ruptured cells with free juice in pulled apart sections as revealed by structural analyses suggested improved integrity of intercellular connections and subsequent cell rupture. They further observed reduced expansion in hypodermis of apple in PG1-suppressed lines resulting in densely packed cells in this layer and this change appeared to be linked with reduced transpiration loss in fruit.

Development of arctic apple- non browning apple: A company in Canada (Okanagan,) has genetically modified 'Granny Smith' and 'Golden Delicious' by inhibiting four *MdPPO* genes using the *RNAi* approach. The resulting

transgenic lines (Arctic Granny Smith and Arctic Golden Delicious) showed a strong reduction of the browning process compared with the control (Xu, 2015) [16].

Development of varieties having wider adaptability and resistance to biotic and abiotic stress

A new variety named 'Jazz' has been developed in New Zealand which is a cross between Braeburn x Royal Gala and is adapted to a range of climates. Fruit are extremely firm at harvest with rich flavour and moderate acidity. Laurens *et al.* (2005) [11] reported 'Ariana' to have good resistance to powdery mildew and to fire blight which is a hybrid of Florina x Prima developed in France. It contains Vf gene which imparts resistant to *Venturia inequalis,* has bright blush coloured skin with excellent flavour, eating quality and storage life.

Conclusion

With advances in molecular breeding and other biotechnological approaches, development and improvement of rootstock as well as varietal status of apple in accordance with market as well as consumer preferences have become much simpler and easier than previously employed conventional breeding methods. Recent approaches such as genome editing consists of inserting, replacing and removing from a genome using artificially engineered nucleases can be utilized efficiently for varietal improvement in apple. Other technologies such as ZFN, TALENS CRISPR/Cas9 which allows direct manipulation of target genetic sequences and thus leading to the desired phenotype can also be employed further in future breeding programmes (Jia and Wang, 2014) [7].

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