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A REVIEW

Clonal propagation in apple

YAMINI SHARMA, SANDEEP KUMAR SINGH* AND NIDHIKA THAKUR
Department of Fruit Science, Dr.Y.S.Parmar University of Horticulture and Forestry, Nauni,
SOLAN (H.P.) INDIA (Email : nidhika991@gmail.com, yaminisharma811@gmail.com)

Abstract : Clonal propagation is to select a source of superior plant characteristics and to reproduce population of progeny with identical genotypes. Most of the fruit plants are heterozygous in nature if they are propagated through seed their unique characteristics are changed. Propagation through cuttings is the most common means of clonal regeneration of number of horticultural crops. Adventitious root formation is pre-requisite to successful cutting propagation. Vegetatively raised clonal rootstock, on other hand are not only uniform but they are also precocious, productive and resistant to biotic and abiotic factors. Thus in apple the use of clonal rootstock has become an acceptable practice of eliminating variability arising from the use of variable seedling rootstocks and of reducing tree size and increasing precocity and productivity. In this review, the scattered information on clonal propagation of apple through growth regulators, bio-inoculants, pre-conditioning (blanching and girdling) on rooting of cuttings and pre-conditioning treatments with IBA is enlightened. This could eventually be helpful in drawing the attention of the researchers and scientists to work on it, besides would be benefitted by utilizing the knowledge reviewed in this paper.

Key Words : Apple, Growth regulators, Bio-inoculants, Blanching, Girdling, Clonal propagation

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INTRODUCTION

Apple is the most important fruit crops of the temperate region. It is a member of family Rosaceae is native to South West Asia, which occupies nearly 47.11 lakh hectare area with 702.97 lakh metric tonnes production in the world (NHB, 2011). India ranks 7th in the world with a production of 28.91 lakh metric tonnes from an area of about 2.89 lakh hectare (NHB, 2011). In India apple is mainly grown in North Western Himalayan region comprising states of Jammu and Kashmir, Himachal Pradesh and Uttarkhand. Himachal Pradesh on account of its production has been designated as “Apple Bowl of India”. Clonal propagation of rootstocks through cuttings is of special significance as it is additional tool to increase the production of rootstocks. Whereas, during grafting top portion of rootstock (> 80 %) goes waste. If this part is converted into plantlets through cuttings the multiplication rate of rootstock can be increased manifold. As the old plantations are declining, there is an increase in the demand of quality planting material which has necessiated the need for development of an easier, quicker and economical method of propagation. Hardwood cuttings in apple rootstocks have been very successful (Howard, 1971), as they result in true to type, uniform in growth, quick, less expensive, require less space and skill. According to Frey *et al.* (2006) multiplication through stem cuttings is based upon number of factors such as age, condition, health of mother plant, time of planting, rainfall, humidity, temperature, rooting media and after care. Various pre-conditioning treatments such as blanching and girdling have been reported to improve rooting capacity of cuttings (Hartmann *et al*., 2009). Blanching is practiced where stock plants are initially grown in light and then shaded either entirely or at a localized area usually the base of the stem. Banding of MM106 shoots for 7 days promoted rooting, negated the inhibitory effect of high IBA (Sun and Bassuk, 1991). In case of girdling, outer tissue of vascular cambium (bark, cortex and phloem) is disturbed (Hartmann *et al.,* 2009). Using these techniques of girdling

*** Author for correspondence and Present Address** Department of Vegetable Science, Dr.Y.S.Parmar University of Horticulture and Forestry, Nauni, SOLAN, (H.P.) INDIA (Email: sandeephort0233@gmail.com)

and ringing, downward translocation of carbohydrates, hormones and other possible root promoting substances are restricted (Evert and Smittle, 1990). The relevant literature on clonal propagation in apple has been reviewed as under :

Growth regulators :

Various naturally occurring compounds possessing hormonal properties are effective for root initiation. All classes of plant growth regulators like auxins, gibberellins, cytokinin, ethylene and abscisic acid as well as ancillary compound such as growth retardants, inhibitors, polyamines and phenolic substances influence root initiation, growth and development either directly or indirectly. Among these, auxins have the greatest effect on rooting of cuttings. Rooting potentiality of apple rootstocks have been reported to vary according to endogenous content of inhibitors and promoters, however, the inhibitors were stronger in difficult-to-root species (Nanda, 1975). Promotion of adventitious root formation by applying synthetic auxins to shoot cuttings is well tested technique in the rootstock production of fruit trees rootstock (Howard, 1967). In this respect cultivar Bramley's seedling hardwood cuttings is a typical example which roots only in very low concentration of auxin under a standard condition of propagation (Withnall, 1968). Tustin (1976) found a positive relationship between rooting in M12 apple rootstock cuttings and an endogenous IAA like substance while studying anatomical basis of rooting potentiality in apple hardwood cuttings. Pandy and Pathak (1979) observed that forced cuttings of apple clonal rootstock treated with IBA 2500 ppm and with adequate bottom heat, rooted better than unforced ones, because in forced cuttings there was only a single layer of sclerenchymatous ring, whereas the unforced cuttings contained invariably a double layer. According to Pontikis *et al*. (1979) high level of rooting was observed in unrooted stool shoots of M27 apple rootstock when treated with IBA. IBA was applied between 0.5 and 0.8 cm on the untrimmed base of apple shoots for 5 second in early January or in early March gave the best results. Randhawa and Nobumasa (1980) reported that *Malus prunifolia* cuttings when treated with IBA 2500 ppm gave highest rooting percentage, number and length of roots per cutting and maximum percentage of bud break. However, the cuttings of M9, M26 and Starking Delicious failed to root.According to Pandey and Upadhyay (1981) highest rooting, survival percentage, number and length of roots per cutting were obtained by treating peach hardwood cuttings with IBA 2000 ppm. In the same year, Pandey and Pathak (1981) while studying the biochemical basis of rooting potentiality in apple hardwood cuttings found that forced cuttings rooted better than the unforced ones when treated with IBA. In another experiment, they also found that application of IBA and cinnamic acid, each at 2500 ppm induced rooting in MM115, M104, M25, M2 and MM109 apple rootstock cuttings.With a view of rapid production of apple rootstocks through softwood cuttings, Hansen (1990) reported that 1.0 per cent IBA talc was more effective in softwood cuttings of MM106 and M26 apple clonal rootstock as compared to 0.5 and 2.0 per cent IBA talc. Cuttings which were treated with 1.0 per cent IBA produced more number of roots per cutting and shoot length was found to be inversely related with IBA concentrations *i.e.* concentration of 0.5, 1.0 or 2.0 per cent produced longer shoots (20, 14 and 10 mm, respectively). Similarly, in apple clonal rootstock MM106, highest rooting percentage and root number was recorded with the application of 500 to 1000 ppm IBA (Sun and Bassuk, 1991). Noor *et al.* (1995) concluded that IBA 3000 ppm was the best concentration for rooting in M26 and M27 apple rootstock cuttings as it resulted in highest sprouting, survival percentage, shoot length, number of roots and root length per plant. Howard (1985) applied IBA in granular form to leafless winter cuttings of MM111 and Myroblan rootstock of apple and plum, respectively and observed that IBA 2500 $mgkg^{-1}$ gave the highest rooting percentage and number of primary roots per cuttings. Nyomora and Manzara (1982) observed that juvenile cuttings of apple and peach rooted better than adult cuttings, when each cutting was treated with IBA. Abd *et al*. (1992) recorded the highest rooting in hardwood cuttings of apple clonal rootstock MM106 when treated with 2000 ppm IBA. Shawky *et al.* (1993) reported that best rooting was obtained in semi - hardwood cuttings of apple clonal rootstock MM106 after treating with IBA 2000 ppm. Sharma *et al.* (2005) studied the effect of IBA at 0, 1000, 2000 and 3000 ppm on the production of apple clonal rootstock by semi-hardwood cuttings and observed that IBA at 3000 ppm produced the highest rooting with good quality roots in terms of rooting per cent, days to rooting, primary root number, primary root length and increased the emergence of new leaves in apple rootstock. Imtiyaz and Sofi (2007) studied the effect of IBA concentrations (1500, 2000, 3000 ppm) on rooting efficiency and influence of time of collection of two clonal Malling apple rootstock *viz.,* M9 and M26. Cuttings were planted in three stages of growth *viz.,* second week of June, second week of July and second week of August. Best results were found with the application of 2000 ppm IBA from the cuttings that were collected at second week of June. Karakurt *et al*. (2009) conducted an experiment to evaluate the effect of single, double and triple treatments of IBA, bacteria and carbohydrates, which may affect the rooting performance of hardwood cuttings of MM106. Three different concentrations of IBA (1000, 2000, 4000 ppm) along with PGPR was applied. The result indicated that double and triple combination of IBA, bacteria and carbohydrates were more effective in increasing rooting capacity and better quality roots. Suriyapananont (1990) reported that apple rootstock *viz.,* MM106, Marubakaida N-1 and Indonesian were treated with 4 root promoting chemicals (IAA, IBA, NAA and 2,4, 5-T). MM106 gave significantly better rooting with IBA than any other chemical and Marubakaido N-1 cuttings with IBA significantly increased rooting percentage and quality of roots that were formed. Studies on rooting response of hardwood cuttings was carried out by Divin *et al.* (2011) with a view to determine the most suitable IBA concentration and rooting medium for MM111 hardwood cuttings. They recorded maximum rooting percentage (37.03%) and root number (11.33) with 2500 ppm IBA in cocopeat + perlite as rooting medium. Rahimi *et al.* (2011) conducted a study with the purpose of determining the most suitable IBA concentration and rooting medium for MM111 hardwood cuttings. They recorded maximum rooting percentage (37.03%) and root number (11.33) with 2500 ppm IBA in cocopeat + perlite rooting medium.

Bio-inoculants :

Plant growth promoting rhizobacteria (PGPR) are the free living bacteria which are beneficial to number of agriculture crops. The PGPR are known to impart beneficial effects on plant growth, suppress disease causing microbes and accelerate nutrient availability and their assimilation (Babalola, 2010). There are several ways in which different PGPR may directly facilitate the prolification of their host plant. They may (i) fix atmosphere nitrogen and supply it to plants (ii) synthesize siderophores which provide iron to the plant (iii) synthesize various phytohormones. Host plants are benefited by improved root development and subsequent increase rates of water and mineral nutrient uptake (Okon and Kapulnik, 1986). Caesar and Burr (1987) reported 65 per cent growth increase in apple seedling and up to 179 per cent in rootstock when treated with PGPR. Growth responses were correlated with detection of fewer roots associated with fungi and more active lateral nodes. Karakurt and Aslantas (2010) studied the effects of plant growth promoting rhizobacteria (*Agrobacterium rubi* A18, *Bacillus subtilis* OSU-142, *Burkholderia gladioli* OSU-7 and *Pseudomonas putida BA-8*) strains on plant growth and leaf nutrient content of apple. The application of bacteria increased the leaf number and area as well as annual shoot length and diameter was increased, although OSU-7 application suppressed annual shoot length. BA-8 application resulted in the highest annual shoot number (52.4) and OSU-142 in the largest leaf area (16.12cm^2). The application of A-18 bacteria decreased the concentration of N, K and Cu and increased the concentration of P and Zn in the leaves.

Blanching :

It has long been known that stem tissues developing in dark are more likely to initiate adventitious roots than tissue exposed to light. The mechanism of blanching in promotion of rooting is not clear, but the harmful effects of light on rooting may be due to photo inactivation of one or more natural rooting co-factor in the stem tissues. Doud and Carlson (1977) studied etiolation stem anatomy and starch reserves on root initiation on layered *Malus* clone and reported that mounding of *Malus* shoots throughout the growing season assured early etiolation of the developing tissues in sandy loam soil. Etiolation during layering increased stem starch and decreased the degree of sclerification of the cortex. Etiolated stem cuttings rooted in 7 days under mist chamber, while non- etiolated cuttings failed to root, indicating that etiolation provided a stimulus to root initiation. Howard *et al.* (1985) studied the response of apple summer cuttings to severity of stock plant pruning and to stem blanching. They investigated reasons for relatively free rooting of shoot on stool beds compared to less ready rooting of cuttings obtained from hedges. Leafy cuttings of apple rootstock M9 and the easier to propagate MM106 rooted better under mist when obtained from ultra-severely pruned stools stocks plants in the form of non-earthed stools than from conventional hedges with a permanent frame work. Improved rooting was expressed as either an increased rooting percentage or more roots per rooted cutting. A positive response was obtained to blanching the stem base by excluding light as it occurs when soil is placed around the stems of stool shoots. Black adhesive tape was most effective experimentally for blanching. Blanching effect was expressed in four weeks of tape application. Hartmann *et al.* (2009) observed anatomical and physiological changes that occur in etiolated stem tissue which enhances rooting. Etiolation may reduce the production of lignin, thus instead of forming lignin, phenolic metabolites may be channeled to enhance root initiation (Englert *et al.,* 1991). In the same year, Sun and Bassuk (1991) reported that the banding in apple clonal rootstock M9 and MM106 with velcro bands for 10-20 days significantly improved per cent rooting, number of roots per cutting and also stimulated bud break. As the duration of banding treatment increased from 0-20 days, per cent rooting and number of roots per cutting also increased. In M9, banding increased cutting survival rate and increased new growth in transplanted cuttings during first four months after planting. The effect of auxin treatment and etiolation in relation with difficult to root cultivar of apple 'Bramley's seedling' was investigated by Delargy and Wright (1979). Un-etiolated cuttings of the scion variety rooted at comparatively lower level as compared to six synthetic hormones applied. Localized etiolation of the shoot, stimulated more root formation in proportion to the length of the stem segment. Howard (1983, 1984) found that rooting per cent and root count were proportional to shade level. The beneficial effect of stock plant shading on adventitious root initiation does not appear to be related to changes in spectral composition. Maynard and Bassuk (1987) observed that stem banding along with stock plant etiolation with the self-adhesive fabric velcro increased per cent rooting, number of roots per cutting and rate of rooting in several difficult to root woody species. Evidence also indicates that growing stock plants of woody species under reduced irradiance enhance the rooting of stem cuttings taken from these plants. Grzyb *et al.* (1989) demonstrated that covering the hard pruned mother plants of apple rootstock P2, B9 and P22 in the spring with black polythene increased the rooting of cuttings compared with uncovered controls. Exclusion of light from shoots intended for propagation has been shown to stimulate adventitious rooting in apple. Localized etiolation of the shoot base, if carried out early in the developing stem then it was effective in inducing rooting in several scion cultivars (Gardner, 1937), and ring barking treatment further enhanced the effect (Delargy and Wright, 1979). Christensen *et al.* (1980) found that rooting percentage of apple clonal rootstock Malling 26 cuttings (*Malus pumila* Mill.) decreased at higher irradiance, whereas, Harrison (1981) reported that etiolation in M9 apple clonal rootstock increased rooting in softwood cuttings. Banding of MM106 shoots for seven days not only promoted rooting, but also negated the inhibitory effect of IBA, while simultaneously it stimulated lateral bud break and increased the root number of the cuttings was studied by Sun and Bassuk (1991). Harrison and Howard (1982) reported that technique of blanching on the cutting base before detachment from the stock plant aid the rooting of apple rootstock MM106, M9 and MM111. Morphological characters of four clones of M9 rootstock and propagation of M9 Nagano in stool bed was assessed by Tamai *et al.* (2002) and found that 'M9 Nagano' rooted well in the stool bed by etiolating young shoots and on an average about ten rooted shoots were harvested in 3 to 5 year old stool beds. Sivaci *et al.* (2007) showed that improvement in root formation was due to reduction of total phenols in the etiolated plants.

Girdling :

According to Kossuth *et al.* (1981) girdling of cuttings, 2 weeks before collection enhanced rooting of apple cuttings. Girdling and chemical treatment forces accumulation of food reserves and pre callusing in pine cuttings. Girdled cuttings with preformed root initials generally developed large root system within a month. Fachinello *et al.* (1988) have shown that girdling shoots of MM106 on stock plants, 15 weeks before their collection as cutting may enhance dry weight and also subsequent rooting.

Pre-conditioning treatments followed by IBA on rooting of cuttings :

Studies on root formation in cuttings using etiolation was carried by Thomas (1988). It was found that blanching of shoots of *Malus pumila* cv. Northern Spy improved the efficiency of IBA applied at 2500 ppm and recorded higher rooting percentage (80%), number of roots per cutting (19.7) as compared to blanching (60%) alone. The investigation by Sabrout and Shazly (1994) while studying the propagation of MM106 apple rootstock cuttings showed that maximum rooting (52.25%) was obtained from dipping the girdled shoot in 2500 ppm IBA solution. Sun and Bassuk (1991) demonstrated that blanching of apple rootstock M9 and MM106 could be achieved by using bands of velcero tape. Application of the IBA to the blanched cuttings significantly improved both the per cent rooting and number of roots formed per cutting. They further suggested that slight wounding caused by velcro tape may have aided the IBA uptake by the stem cutting. The treatment also stimulated bud break on cuttings and amount of shoot growth made in first month after planting. Sinha *et al*. (1986) studied the effect of IBA concentration and the biochemical aspects of rooting in three commercial cultivars of apple propagated through air layering. Application of 5000 ppm IBA for Fanny and 7500 ppm for Early Shanburry and Rymer was found to be the best as they resulted in 100 per cent survival of rooted shoots. Joshi *et al.* (1987) observed that concentration of IBA at 2500ppm in lanolin paste applied to ringed shoot was ideal for clonal multiplication of different apple rootstock through stool layering.While studying root formation in *Malus pumila* 'Northern Spy' cuttings, Deering (1990) found that combination of IBA treatments, etiolation and localized banding proved best and resulted in 84 per cent rooting, production of 19.7 roots per cutting and length of 36.5 mm. Exclusion of light from shoots intended for propagation has been shown to stimulate adventitious rooting in apple. Localized etiolation of the shoot base if carried out early in the development of the stem was effective in inducing rooting in several scion varieties (Gardner, 1937). Ringing done 10 days earlier to air layering and IBA and NAA applied immediately after instant ringing was compared to advanced ringing resulted in higher survival percentage as compared to advance ringing. Instant ringing had more fresh (0.59 g) and dry (0.15 g) weight of roots than advanced ringing (0.50 and 0.12g) (Chuvatia and Singh, 2000). Maximum rooting percentage (70.0%) was registered in treatment blanching + girdling + IBA 2000 ppm. Multiplication of MM106 apple rootstock by using different combinations of ringing alone or ringing combined with IBA treatments *viz.*, 500, 1000, 2000, 2500 and 5000 ppm was undertaken in Kashmir valley by Srivastava *et al.* (2006).

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