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Propagation

Advances in fruit crop propagation in Brazil and worldwide – apple trees

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Abstract - Apple production has been an important economic activity worldwide. The wide dissemination and cultivation of apple trees in several regions of the world is due to the development of propagation techniques from its domestication to the present time. Apple trees can be propagated by seeds, restricted to the use in breeding programs, and multiplied by several methods of vegetative propagation, both for rootstocks and apple scion cultivars. Considering the high technological level currently used in apple crop management, plant propagation plays a very important role in order to enable seedling production with high morphological quality and free of diseases, requiring new advances and propagation improvements in new releases of rootstocks and apple scions.
Index terms: *Malus domestica*, production of seedlings, grafting.

Avanços na propagação de fruteiras no Brasil e no mundo – macieira

Resumo – A produção de maçãs é uma atividade de grande importância econômica em nível mundial. A ampla disseminação e cultivo da macieira em várias regiões do mundo deve-se ao desenvolvimento de técnicas propagativas desde sua domesticação até a atualidade. A macieira pode ser propagada por sementes, com restrita a utilização em programas de melhoramento genético, e multiplicada por vários métodos de propagação vegetativa, tanto para portaenxertos como para cultivares copa. Levando-se em consideração o elevado nível tecnológico atualmente empregado na cultura da macieira, a propagação de plantas tem papel de grande relevância, no sentido de viabilizar a produção de mudas de elevado padrão morfofisiológico e fitossanitário, demando novos avanços e aprimoramentos a medida que novas cultivares copa e portaenxertos são desenvolvidos.
Termos para indexação: *Malus domestica*, produção de mudas, enxertia.

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Introduction

Apple tree (*Malus domestica* Borkh.) is one of the main temperate climate fruits produced worldwide, with great economic importance in the fruit growing activity of many countries. The development of present-day species is presumed to have begun about 20,000 years ago in the Caucasus region, Asian mountains and eastern China is the center of origin of the apple tree. Apple tree is a perennial, deciduous fruit species that developed the bud dormancy mechanism as an adaptive strategy to survive the low temperature conditions during fall and winter periods, characteristic of its region of origin, and to resume growth / development in spring. Until the 20th century, the cultivation of apple trees was restricted to typical temperate regions, with climatic conditions more similar to those observed in their region of origin. However, there are reports that the Greeks in classical antiquity cultivated apple trees, and in the Roman Empire, the apple culture was already widespread.

The first records of apple tree cultivation in Brazil date back to 1926 in the region of Valinhos, SP. However, the commercial expansion of apple tree occurred from the 1970s, when crops were observed in countries with subtropical climate characterized by a lower chilling requirements due to the increase in the demand for this fruit in developing countries.

Seminiferous propagation was an important propagation method at the beginning of the domestication and expansion of apple tree, but the vegetative propagation is the main propagation method of this species, emphasizing grafting, in which the union of a rootstock with canopy cultivar is made.

The maintenance and increase in apple tree productivity are directly related to the planting of high quality seedlings, as it will have repercussions during the economic exploitation of the orchard. A quality seedling should have genetic identity, varietal purity, proven origin, high sanity and be within established standards. The use of seedlings with high morphological and phytosanitary quality standard in orchard implantation is decisive for the viability of apple tree production systems. Quality seedlings increase the level of response to all the technology used in the orchard, being a decisive component in the reduction of costs, mainly with agrochemicals, and increase fruit production with quality and productivity (OLIVEIRA et al., 2004).

Apple tree propagation methods

Apple tree can be propagated by means of sexual and asexual propagation. The following are among the main propagation methods of apple trees: seminiferous (seed propagation), cutting, air layering, grafting and micropropagation. Aspects related to each propagation method are given below:

Sexual propagation -Sexual propagation is performed by means of seeds. At the beginning of commercial plantings, the most common method of propagation of rootstocks was by means of seeds. Semeniferous multiplication is no longer used in commercial crops, since it presents intense genetic segregation, forming very vigorous plants, providing heterogeneous plants of different sizes, slowing fruiting, and plants are not resistance to diseases and pests. Seed multiplication can be used to produce virus-free plants that can be used as rootstock for the formation of mother plants, where fruit production is not targeted.

Seed multiplication is still used in genetic improvement to obtain new cultivars. For seed use for propagation purposes, the extraction process is quite simple. Seeds should be manually removed from medium to large sized mature fruits with completely dark seeds and washed in water, immersed in a broad spectrum fungicide solution and dried in the shade. The optimum drying temperature of seeds should be around 32^{o}C, and should not exceed 43^{o}C. Rapid drying can cause cracks and hardening of the integument (HARTMANN et al., 1997). In order to ensure good germination and obtain uniform plants of good standard, it is recommended to select larger and good quality seeds (WERTHEIM; WEBSTER, 2003). After removed from fruits, apple seeds are dormant and cannot be planted directly (DENNIS, 1994). To remove dormancy, seeds must undergo a process called stratification. Seeds should be stored at 2-6^{o}C for a period ranging from 30 to 90 days, and should be kept in an open container with adequate humidity and aeration. After this period, they can be germinated at temperature of 20^{o}C in substrate composed of sand or other compounds, maintaining adequate humidity and phytosanitary controls for both soil and shoot diseases. Treatment with growth regulators such as gibberellic acid, benzyladenine or ethephon can be used to help promote germination. After 90 to 120 days of germination, seeds will be suitable for transplanting and with another 90 to 120 days to grafting, if they have reached the diameter of 1 centimeter. If growth is interrupted after germination, when plants have 4 to 6 pairs of leaves, 100 mg.L^{-1} gibberellic acid (GA_3) should be used in two to three applications.

Vegetative propagation - Vegetative (asexual) propagation, in its various methods, presents as main advantage obtaining individuals with the same genetic identity as the plant from which the propagation material was used. Stems and roots are plant parts that have the capacity to regenerate, form roots and give rise to new plants, enabling vegetative multiplication that transmits the genetic characteristics of a single individual and form uniform plants. Asexual propagation is especially useful to maintain the genetic constitution of a clone throughout generations. The clone is defined as the genetically uniform material derived from a single individual that propagates exclusively by vegetative means such as cuttings, roots and explants.

In apple tree, vegetative propagation is used both in the multiplication of rootstocks and in canopy cultivars.

The following are the main propagation methods for obtaining apple rootstocks:

a) Propagation by cutting

Among the various techniques of vegetative propagation, cutting stands out, which is a propagation method in which detached segments of a plant, under appropriate conditions, form roots and originate a new plant, with characteristics similar to the one that gave rise to it (PASQUAL Et al., 2001). Propagation by cutting in apple crops can be done using woody, cuttings, vegetative cuttings and root cuttings.

Woody cutting is made with dormant cuttings after the fall of leaves. Vegetative multiplication by means of woody cuttings provides low rooting rate in most rootstocks of commercial interest, except for 'Marubakaido' and 'JM 7', two rootstocks of Japanese origin. In this system, dormant cuttings should be collected from plants aged at least two years. The survival percentage of cuttings removed from one-year-old plants or from the top of rootstocks may not exceed 10-20%.

Cuttings should be cut to a length of 20 cm and a minimum diameter of 5 mm during the winter period (July-August). At the base of the cutting, the peel is removed, exposing the tissue and the cutting base is immersed into indolebutyric acid solution (IBA) at 2000 $mg.L^{-1}$ for 30 seconds (Figure 1). The rooting potential is higher in the basal section of the branch and decreases along the upper portion (HOWARD, 1987; SABIN, 1983).

Figure 1. Apple tree rootstock cuttings prepared to be emerged into indolebutyric acid solution, aiming at the rooting of cuttings.

After immersing cuttings into indolebutyric acid solution, they were planted by leaving two buds uncovered or vertically stored in bins with moistened sawdust for 30 days at 18-20°C to aid in the process of callus formation in the basal portion of the cutting and rooting and then in a cold chamber until planting. The rooting index is variable according to the rootstock. ‘Marubakaido’ shows rooting rates close to 100%, but in clonal rootstocks of EM and M series, rates range from 10 to 30%. This technique allows higher rooting percentage when using the base of the cutting that did not take root in the layering system.

The multiplication of woody cuttings through root grafting at the cutting base is not the most indicated, because it may be transmitting viruses. It should only be used in special cases that require rapid multiplication. Cuttings are cut with 20 cm of length grafting in its base a root with 5 cm in length. Preference should be given to grafting the root of the same rootstock or root from a seed plant. In this case, care should be taken to eliminate the grafted root in the following year, when the rootstock is removed. In the grafting, one must use degradable material, avoiding the bottleneck that can favor the entrance of diseases. The cutting should be performed just below the bud.

Multiplication by semi-woody cuttings refers to the use of cuttings collected in late summer and early fall. In general, the term “semi-woody” refers to intermediate cuttings, between herbaceous and woody. The necessary care refers mainly to dehydration, since it is a material that has a more pronounced metabolism and is often a more herbaceous but without leaves.

Vegetative cuttings are collected in the period of vegetative growth, when tissues present high meristematic activity and low lignification degree. Vegetative cuttings should contain two to three pairs of 10 cm long leaves, and should be planted in greenhouses with high humidity, porous substratum and good drainage. Larger leaves should be reduced by half to avoid water loss and also to facilitate handling. This type of cutting takes root 20 to 30 days after planting. After rooting, they should be pricked to a less humid environment and on their own substrate. It is not a conventional system of multiplication of apple-tree rootstocks, but can be used when rapid multiplication is desired.

Root cutting is an uncommon method in apple trees, and requires the use of roots that are free of viruses. The method consists of the use of roots with length varying from 5 to 10 cm, which must be vertically placed in substrate beds for rooting and sprouting, originating a new plant.

Rooting by apple cuttings is affected by several factors, especially the physiological conditions of the plant and factors related to the environment. The nutritional condition of the mother plant affects rooting, since the nutritional balance tends to favor rooting. One of the important elements for the rooting of apple cuttings is zinc (Zn), which is an activator of tryptophan, an auxin precursor necessary for root formation. The age of the mother plant also exerts an influence on the propagation capacity of rootstocks, since younger plants root more easily rooted. The genetic potential is one of the factors that most influence the rooting of apple cuttings and presents a great variability among the different rootstocks, and in general, less vigorous rootstocks (dwarfing) present lower rooting rate. Regarding the hormonal aspect, it is necessary to have a balance between auxins, cytokinins and gibberellins, which can be exogenously applied, such as indolebutyric acid or naphthalene acetic acid, which increase auxin content and consequently increase the rooting rate.

Regarding environmental factors, substrate temperature is one of the most important factors to define the rooting potential, in which temperatures ranging from 21 to 26°C have higher root formation. In herbaceous and semi-woody cuttings, very high temperatures must be avoided, as they significantly increase the transpiration of tissues, which can cause the wilting of cuttings and also stimulate sprouting before rooting actually takes place. Water loss is one of the main causes of the non-rooting of cuttings, especially in vegetative cuttings. The use of nebulization allows reduction and consequently favors rooting. The substrate influences both the rooting rate and the development of roots, which for this purpose should provide water retention and adequate porosity.

b) Layering system

The most widely used system for the multiplication of apple tree rootstocks is layering. In this system, the new plant is not detached from the mother plant until the formation of adventitious roots. The system is characterized by adaptability to mechanization, high quality of vegetative material from this system, low cost, easy maintenance and high rooting rate and number of tillers (Table 1).

Table 1 - Production of rootstocks rooted in the layering system, per mother plant.

Rootstock	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year
M – 106	2	4	4	9	11	10	13
M – 111	3	5	7	11	10	8	12
M – 25	2	5	8	12	12	9	11

Source: INRA, Angers

Two layering methods are used: stool layering and Chinese layering. Stool layering requires much more plants for the establishment of mother plants when compared to the Chinese system. However, in the Chinese system, greater care is required with the phytosanitary quality of plants to avoid empty spaces between plants due to their loss. For both systems, the first year is generally used only for plant growth. No action is necessary until the first shoots are cut, which is done in the winter, leaving the branch tips at 5 cm from the soil surface. However, some nurseries perform the substrate approach in the first year of planting of mother plants to induce rooting. Although the rooting percentage is quite low in the year of planting, the collected tillers can be used to start a new nursery.

The substrate used may be the local soil or sawdust. In the case of sawdust, it must be neutral, coming from trees that do not produce any kind of chemical substance that could harm rooting.

Stool layering consists of planting a rootstock rooted at approximately 30 cm, with minimum diameter of 1 cm. The rootstock is planted by leaving two buds above ground level. The planting density is variable and depends on each nursery and the machinery available for cultural operations. Mother plants are usually planted in two parallel rows 10 cm apart between plants and the row. When buds reach 10 cm in length, a substrate protection is made, leaving from 2 to 3 cm from the end of buds out of the soil. During the vegetative cycle, two or three more protections should be done in order to obtain a rooted area of 10 to 15 cm (Figure 2). In the dormancy period, tillers should be detached from the mother plant, being ready for grafting.

Substrate protection as well as the collection of rootstocks (tillers) can be done manually or with the aid of specialized equipment which is often manufactured by nursery workers themselves.

The Chinese layering system differs from the stool layering in the planting system, where the rootstock is planted with a slope of 30-40º in relation to the ground level and a larger spacing 30-40cm (Figure 5). Before the beginning of sprouting, it should be tilted to the ground level and covered with soil, and the other procedures are the same as the stool layering system. In the Chinese system, rootstocks from 50 to 60 cm in length can be used, with the advantage of producing greater number of shoots in the first year.

For one hectare, it is necessary from 15 to 25 thousand rootstocks (mother plants) depending on the spacing. Each mother plant can produce from the fourth year 5-8 rootstocks of good quality and well rooted. This number increases until the eighth year, when stabilization begins (Table 2). The same mother plants can be used for a period of 15 or more years, depending on their phytosanitary conditions.

Some care should be taken in production by air layering, emphasizing the phytosanitary control and in relation to aphid (*Eriosoma lanigerum*), because some rootstocks are very susceptible and infestation in the nursery may turn mother plants unviable. Substrate protection requires soil moisture in order to avoid the formation of air pockets and wounds in new shoots. Weed competition is one of the biggest problems at the beginning of sprouting and should be controlled as soon as it emerges.

In the winter, when rootstocks are removed, mother plants should be covered again with soil or sawdust, avoiding dryness at the cutting points. In early spring, this substrate should then be removed ("swept") to allow the emergence of new shoots.

The rootstocks removed should be stored in cold chamber until the classification process is completed. Rootstocks are classified by cutting diameter and rooting.

c) Micropropagation

In the 1970s, micropropagation became an important technique of vegetative multiplication for the production of rootstocks. The process involves the culture of apical meristems or axillary buds in culture medium containing cytokinins to initiate and promote the growth of branches and the proliferation of meristems (cytokinins) and auxins to promote rooting (AIA) (MILLER, 2009). Micropropagation is a technique that allows the rapid and large multiplication of rootstocks or canopy material (DOBRÁNSZKI; SILVA, 2010), also for the production of virus-free material. This technique generated great expectation, but commercially it has been used for the multiplication of rootstocks for the formation of mother plants, later followed by the traditional multiplication system. In addition, micropropagation facilitates the import / export of plant material since the phytosanitary conditions of propagules kept under aseptic conditions significantly reduces or even eliminates the quarantine period.

As the micropropagation process takes place in an environment with controlled climatic conditions, the propagation of plants does not depend on the season of the year and therefore can be performed multiple times during the year. It is a very specialized process and procedures can vary from cultivar to cultivar. However, regardless of cultivar, there are four stages of development involved in tissue culture: 1) *in vitro* establishment; 2) multiplication; 3) rooting; and 4) acclimatization.

The secrecy of micropropagation is totipotency, in which each cell has the potential to divide and produce all the differentiated cells of an organism (HARTMANN, 1997). Basically, the establishment phase is characterized by the isolation of explants in a suitable and aseptic environment for the production of propagules. The explant type should be chosen according to its ability to conform to *in vitro* conditions, and those with the highest proportion of meristematic tissue are preferred. Several explants such as seeds, cuttings, buds, meristems, stem apices and nodal segments can be used in the *in vitro* establishment and their choice should consider the level of tissue differentiation used and the purpose of micropropagation.

Disinfestation of explants with calcium or sodium hypochlorite before being introduced into the culture medium is essential for the success of the establishment. The culture medium consists of four main components: inorganic salts (nitrogen, phosphorus, potassium, calcium, magnesium, boron, cobalt, copper, manganese, iodine, iron and zinc), organic compounds, natural complex ingredients and inert substrates, which include carbohydrates, vitamins, and hormones. Moderate doses of cytokinin are used in the establishment (0.5-1 mg L-1) and have a primordial function. Generally, the establishment phase comprises around 4-6 weeks.

The second phase involves the multiplication process, where propagules are multiplied in scale during successive subcultures in proper multiplication means, so that the parts formed are either subdivided into smaller parts or are individualized to form new explants. At this phase of formation of new buds / explants, cytokinin levels should be higher.

Once sufficiently multiplied, the material is transferred to cytokinin-free medium for adventitious root formation. Indole-3-butyric acid (AIB) is the type of auxin typically used in this phase.

Light and low temperatures usually have a negative effect on root formation. Seedlings kept in the dark (etiolated) root more easily than those grown directly in the light (HARTMANN et al, 1997). Etiolation causes a series of anatomical and physiological modifications, such as reduction of phloem fibers, promotion of cell wall elongation, and increase of undifferentiated tissues of the parenchyma.

In the case of apple trees, there is an increasing tendency to promote *in vivo* rooting. Soon after the multiplication phase, explants are treated with rooting hormones (auxins) and then directly transferred to trays with commercial substrates of preference (pine bark, sand, peat, etc.) and treated in the same way as when propagated through vegetative cuttings. The main auxins used for rooting of cuttings are indoleacetic acid (IAA), indolebutyric acid (IBA) and naphthalene acetic acid (NAA). In the present study, it is possible to observe the difference responses of these phytoregulators in the rooting of cuttings of the same rootstock (PASQUAL; ISHIDA, 1994; CASTELI, 1986; SONI et al., 2011). According to Soni et al. (2011), this response can be justified by the differentiated age of the propagating material, which may present differentiated sensitivity to growth regulators.

In vitro rooting is followed by acclimatization of plants, which consists of transferring plants from *in vitro* conditions to the substrate in order to provide a certain tolerance to water stress, resistance to certain pathogens and to convert the plant from heterotrophic to autototrophic stage (SONI Et al., 2011). Acclimatization of seedlings is considered the last micropropagation process, and depending on the crop, it is the most difficult process due to the survival rate. At this stage, seedlings are transferred to greenhouse equipped with fogging system to avoid dryness. Due to the variability in the size of rootstock seedlings, it is recommended to classify them to obtain uniformity of mother plants. Apple tree seedlings propagated in culture medium may be of great advantage in the establishment of mother plants due to the greater vigor of seedlings (Figure 2).

Figure 2. Micropropagated apple tree plants

d) Grafting

Grafting is the process by which the apple tree seedling is produced and consists of the union of the rootstock with the canopy material. There are several uses for grafting in agriculture, including plant propagation. Through the use of grafting, it is possible to propagate the same genotype, obtaining clones of the mother plant. Among the advantages of the use of grafting in plant propagation, the significant reduction in the size of trees due to the possibility of using a rootstock of less vigor with a particular canopy cultivar to obtain plants of lower vigor and height stands out (MELNYK, MEYEROWITZ , 2015). In addition, grafting may prevent or minimize the juvenile period in which propagules of adult plants grafted on juvenile rootstocks will maintain their adult state and ability to produce fruits.

Although there are many ways to carry out grafting in apple trees commercially, cleft grafting and budding grafting are used, the first being the most used.

Cleft grafting - Cleft grafting can be performed at the final location of the nursery, if rootstocks have been transplanted or tabletop, which consists of removing the rootstock, which can be done in sheds, which, and has the advantage of not depending on climatic conditions. Forks should have 3 buds or length of 5 to 8 cm, with the cut at the top just above the last bud. The cleft grafting method used is the double cleft (Figure). Cleft grafting is performed during dormancy. Rootstock and forks of the same diameter should be preferably used, but if there is a difference in diameter, the important thing is that one side should be in contact with the shell (figure). After grafting, it should be tied, preferably with biodegradable tape, if plastic tape is used, it should be removed after the seedling reaches 30 to 40 cm in height.

Budding grafting is usually carried out in the vegetative period directly in the nursery. It is used to make use of the rootstocks that in winter would not reach diameter for cleft grafting, being re-nursed for later budding grafting. Budding grafting can be classified as vegetative and dormant. Vegetative is when it takes place in late spring and early summer and after 15 to 20 days, the rootstock is cut above the grafted bud, making it to start its development. In dormant, it is carried out at the beginning of fall, keeping the dormant bud during the winter, and the rootstock will only be cut in late winter at the beginning of spring. The method of budding grafting used in the apple tree is normal T or inverted T, and in rainy regions the inverted T has the advantage of avoiding the penetration of water at the grafting point, which would compromise the healing process (Leite et al., 2002).

e) Interstocking

The reduction of the final plant size can be obtained through the use of a dwarfing rootstock interlayer between the vigorous rootstock and the canopy cultivar. This is a technique that, as a rule, aims to reduce plant vigor, increase productive efficiency and improve fruit quality (MARCON FILHO et al., 2009).

Interstocking is a technique used to reduce the vigor of vigorous rootstocks and consists of placing a dwarfing rootstock cutting between the rootstock and the canopy cultivar. In addition to reducing vigor, this technique is used to take advantage of some interesting characteristics of the rootstock such as being resistant to pests and diseases, anticipate fruiting, improve fruit quality, facilitate plant management, use the more developed root system and allow planting in high density without the use of a support system.

It is commonly used with vigorous rootstocks such as Marubacaido, but can also be used with semi-vigorous rootstocks such as M-7, MM-111 and others. The interstocking used is the M-9. It is mentioned the

M-26 with possibility of use, but has the disadvantage of developing many burrknots, vigor superior to M-9, and showing greater variability in plant size.

The reduction in vigor depends on the interstocking length, where the greater, the more influence it has on the decrease of the plant size. On Marubacaido rootstock, cutting of M-9 20 cm, it has shown good results. Interstocking over 20 cm has the disadvantage of favoring the emission of sprouts, making plants vulnerable to breakage.

Care must be taken to avoid that the roots are facing upward, which favors the emission of sprouts from the rootstock. The interstocking should be close to ground level, but it should not be buried avoiding its rooting.

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3. Rootstocks for apple tree crop

Rootstocks for apple tree crop began to be used 2000 years ago in central and eastern Asia, and were exclusively of seminal and unknown origin (WEBSTER, 1997; WEBSTER; WERTHEIM, 2003). The first clonal rootstocks originated from selections of these plants of unknown origin.

The use of rootstock is the most efficient and cost-effective method to control vigor and keep the apple tree cultivar under control and to maintain productive efficiency. Of temperate fruit trees, apple tree is the one with the greatest variability of rootstocks, being widely used in commercial crops. The rootstock produces several effects on grafted cultivars, such as vigor, flowering, fruiting, fruit quality, longevity, adaptation to climatic and soil conditions, and tolerance to certain diseases.

Due to the work of genetic improvement, since the early nineteenth century, apple tree crop has a large number of clonal rootstocks, widely spread in all producing regions. Given the existing variability, it is important to know the most relevant agronomic characteristics that should be taken into account when choosing the rootstock. The following are among the main characteristics:

Vigor control - considering a seed plant with maximum vigor (100%), this characteristic may vary up to 20% of maximum vigor, which is very important to determine planting density.

Entry into fruiting - a quick entry into fruiting stage should be sought, which is essential for high density plantings.

Production stability - constant production, without production alternation.

Fruit quality - related to size, color and maturation period.

Resistance to diseases and pests - Resistance to *Phytophthora cactorum* and wooly apple aphid (*Eriosoma lanigerum*) is critical for Brazilian conditions. This feature gains importance when we think of integrated production, which restricts the use of certain fungicides and insecticides.

Sprouting – it is a harmful factor, especially in high-density plantations.

Root system - rootstock with abundant root system, preferably deep and well distributed should be sought.

Nutrient absorption capacity - for the Brazilian conditions, it is necessary to search for rootstocks with good calcium absorption capacity.

Physiological disturbance 'Burrknots' - Burrknots is a physiological disorder that causes the formation of aerial roots, which can occur both in the canopy cultivar and in the rootstock. Symptoms of this disorder begin as small cream-colored warts near the grafting point or along the trunk branches, especially in shaded and humid places and later on with aging, these warts rupture forming a large number of roots in shoots; in contact with the ground, these aerial roots are able to become functional (normal) roots. The preferred sites for the development of burrknots are those rich in starch, which is why they are more frequent near the grafting point.

The occurrence of burrknots is peculiar to the genetic constitution of the cultivar, not being due to infection by biological agents (KUDELA et al., 2009). Rootstocks differ in the sensitivity degree to this disorder, being very marked in MM-111, MM-106, M-7 and M-26. With the appearance of the aerial roots near the grafting point, there is discontinuity in the sap circulation, causing weakening and unevenness of plants. The site may serve as a shelter for wooly apple aphid (*Erisoma lanigerium*) and susceptible to infection for colon rot (*Phytophthora cactorum*).

The presence of burrknots is related to genetic constitution, thus, there are no forms of control, but measures that reduce their effects. The intensity of occurrence of burrknots in plants is influenced by the environment conditions, being intensified in response to high humidity, low temperatures and reduced luminosity levels (CUMMINS and ALDWINCKLE, 1983). Thus, the use of rootstocks less susceptible to this disorder (such as Marubakaido), avoiding shading or humidity near the grafting region, leaving the grafting point close to the ground level in the orchard are preventive measures for the appearance of this disorder.

Presence of sprouts - Sprouting of rootstocks can also be considered unfavorable, as it may also harbor certain pests, such as wooly apple aphid. There are several sensitivity degrees among the different rootstocks in the emission of sprouts, with M-7 and Marubakaido presenting a large number of sprouts. The use of Marubakaido rootstock with M-9 interstocking favors the emission of sprouts. As a means of reducing sprout formation, roots should not be bent during planting and more deeply planting should be performed. The elimination of sprouts should be carried out preferentially during the vegetative phase, when it is easier to remove. Sprouts should not be cut at ground level, and cutting or pulling should be carried out at the base of the insertion to prevent further sprouting.

Replanting - Apple trees may be sensitive to planting in places where apple orchards previously existed. Under replanting conditions, plants develop less and plant growth is not uniform. The following are measures to reduce the effects of the replanting problem: to maintain the soil with grass cultivation for two years after eradication the apple orchard, to make good soil preparation, to use good quality seedlings and to avoid the emergence of seedlings. Marubakaido rootstock or Marubakaido grafted with M-9 interstocking is less sensitive to the replanting problem, allowing planting in the same year that the orchard is eradicated. MM-106 rootstock stands out due to the great sensitivity to development problems in replanting conditions. Recently, the CG series shows tolerance to replanting diseases (KVIKLYS et al., 2016).

Grafting compatibility with canopy cultivars - Most of the rootstocks are compatible with the different apple tree cultivars. This is due to the fact that rootstocks belong to the same species (*Malus domestica*). When other Malus species are used as rootstocks, incompatibility problems can be observed (WERTHEIM, 1998).

The difference in growth in grafting union, common in dwarfing rootstocks such as M-9 is not a symptom of incompatibility, but rather a characteristic of the combination with some cultivars. This growth difference does not cause problems if support is used, avoiding the break in the grafting point.

Description of the main rootstocks

In the case of the apple trees, the availability of rootstocks is very large, being usually grouped in series of origin, with great differences in their characteristics within the same series. The vigor control is considered the most important characteristic, and also one of the most used forms for classification. For simplification purposes, it will be presented in five categories: vigorous, semi-vigorous, semi-dwarfing, dwarfing and very dwarfing apple rootstocks.

Vigorous rootstocks present above 90% of vigor established for seed plants. With the development of fruit trees with new planting systems due to the great development and lack of precocity that provides apple trees, its use is restricted to situations of low fertility soils, some situations of replanting with rootstocks that are resistant to *Phytophthora* Spp. Semi-vigorous rootstocks present 80 to 85% of vigor established for seed plants. They do not adapt to high density plantings, but some have some interesting characteristics regarding resistance to pests and diseases.

Rootstocks characterized as semi-dwarfing present 50 to 70% of vigor established for seed plants, being the most planted in Brazil at the beginning of the apple tree cultivation, since in general, they induce precocity and great productive efficiency, which contributes to control vegetative growth. However, dwarfing rootstocks present from 25 to 40% of the vigor established for seed plants. They adapt to the new planting systems of high density with more than 2,500 plants per ha. They need support systems, because in general, their root system is superficial. They induce production precocity, high productivity and fruit quality. Dwarfing rootstocks suffer more from the effects of viruses for which one must be careful to only use them with the guarantee that they are free of viruses. In general, they anticipate fruit maturation and are not recommended for spur cultivars.

Rootstocks classified as very dwarfing have 15 to 20% of the vigor established for seed plants. They induce production precocity and advance maturation. For being recent, commercial plantations are few, and it has not been planted in Brazil. It may in the coming years be able to show interest in plantings at planting densities higher than those currently used.

It should be considered that the vigor of the apple tree may show variations, since other factors such as soil fertility, solar radiation, vigor and vegetative habit of the cultivar, fruit load, climatic factors and the rootstock vigor, which will determine the final vigor of apple trees.

Although rootstocks of M and MM series are the most widespread at international level and also in Brazil, other series have been developed, although only few have been widely disseminated at commercial level to the present moment. Table 1 shows the different series of apple tree rootstocks obtained and with greater potential for commercial use in the future.

Table 2. Apple tree rootstocks of several series of major importance worldwide.

Series	Most important clones	Origin
M	M-27, M-9, M-26, M-7, M-25	England
MM	MM-106, MM-111	England
MAC	Mark-9, Mark-24	United States
P	P-1, P-2, P-18, P-22, P-16	Poland
Budagovski	Bud-9, Bud-490, Bud491, Bud-29	Russia
C.G.	Novole, CG10, CG24, CG44, CG 60, CG 80	United States
Ottawa	Ottawa 3, Ottava 8	Canada
Jork	Jork 9	
JM	JM 7, JM 8	Japan
MI	MI 793	England
Clones M-9	M-9 EMLA	Unknown
Marubakaido	Maruba	Japan
Others	Nicolai 29 (NIC 29)	France
	Bemali	Switzerland
	Pajan 1	France
	Pajan 2	France

Some of the most important current releases of apple tree rootstocks come from Cornell University in Geneva, United States. In 1968, James Cummins and Herb Aldwinckle began a cross-breeding program and selection of rootstocks resistant to bacterial fire and root rot. These selections of rootstocks were then given the series name 'CG' (Cornell-Geneva). Once commercially launched, rootstocks were renamed only to Geneva^{Tm} (G) (e.g., G.202; G.41).

Currently, the main characteristics of most of the Geneva series rootstocks are resistance to diseases, such as bacterial fire, root rot (*Phytophora*), replanting diseases, wooly apple aphid, reduction of vigor and resistance to chilling (FAZIO et al., 2015).

One of the biggest challenges in relation to the Geneva series has been the form of propagation due to their poor rooting performance. Despite the high cost, tissue culture has been used with great success, especially for nursery owners wishing to establish new mother plants. Several nurseries in the United States are paying more attention to propagation by semilenous / vegetative cuttings, with satisfactory results.

The Geneva rootstocks best known in the American and international markets are, in increasing order of vigor: G.65 - vigor compared to M.27; G.11, G.41, G, 16, G.213, G.214 and G.814 – vigor compared to M.9; G.935, G.222 and G.202 - vigor compared to M.26, and G.969, G.30, G.210 and G. 890 - vigor compared to M.7 and MM 106 (FAZIO et al. , 2013). Among these, G.202, G.213, G. 214 and G.814 rootstocks are being evaluated in Brazil.

4. Canopy cultivars - description, current situation and trends

The apple tree culture has undergone a profound technological renewal worldwide with regard to the topic apple tree cultivars. Traditional cultivars of the Delicious and Golden group declined progressively, although they still dominate large part of the international market. For being a relatively new crop in Brazil, cultivation is based on Gala and Fuji cultivars. Extensive descriptions and origin of apple cultivars have been published and in the last years a great number of cultivars from different countries have been introduced in the world market, but their implantation in the world market is slow and often does not reach the desired expectation. Genetic breeding works are intense and new cultivars are being launched. It is common in apple tree crops the frequent occurrence of somatic mutations that are selected by the color of fruits, compact growth habit and time of maturation of fruits. These are introduced to the market more easily.

Due to the fact that the main apple producing regions in Brazil are characterized by the accumulation of chilling insufficient for the natural overcoming of dormancy, characteristics related to the adaptation of plants to the growing conditions are extremely important to obtain high yields over the years. In addition to the intrinsic characteristics of plants, it is important that the fruits produced have characteristics for high post-harvest storage capacity, resistance to handling and transportation, and that have organoleptic characteristics demanded by the consumer, and with a better red coating on fruits.

Considering that the Brazilian apple production system is based on the cultivation of apple clones from Gala and Fuji groups, the main clones are described in terms of fruit coloring (Table 2, Table 3 and 4).

Table 3. Main clones of Gala cultivar and respective characteristics of fruit skin coloration.

Clone	Origin	Fruit skin coloration
Royal Gala	New Zealand	Striped red with greater red area compared to standart cultivar
Imperial Gala	New Zealand	Striped red with darker red compared to Royal Gala and less visible striped red
Galaxy	New Zealand	Striped red
Mondial Gala	New Zealand	Striped red, but stripe not clearly visible
Baigent	New Zealand	Striped red, red color appear early and stripe is marked
Tenroy	New Zealand	Striped red, similar to Royal Gala, but more uniform
Regal Gala	New Zealand	Solid red, no stripes
Gala Must	New Zealand	Striped red
Lisgala	Brazil	Striped red
Red Gala	Brazil	Striped red

Table 4. Main clones of Fuji cultivar and respective characteristics of fruit skin coloration.

Clone	Origin	Fruit skin coloration
Fuji Suprema	Brazil	Dark red, little striped
Kiku 8	Italy	Striped red
Fuji 1	Japan	Not striped red, flat form
Nagafu 2	Japan	Not striped red, flat form
Nagafu 6	Japan	Striped red
Nagafu 12	Japan	Semi-striped red
Akifu 1	Japan	Striped red
Akifu 4	Japan	Striped red
Fuji Irradiada	Japan	Striped light red
Mori Hofu 1	Japan	
Red Fuji	USA	Semi-striped red
Yataka	Japan	Striped red, early maturation
Fuji Spur	Japan	Not striped, fruits of flat form and plant with spur habit

5. Production of apple tree seedlings

For the commercial production of apple seedlings, it is important to have propagation material of morphological and phytosanitary quality in order to enhance the propagation efficiency, as well as the final quality of seedlings produced. For that, the selection and formation of mother plants is one of the main stages in the commercial propagation of apple trees.

Plants from which the rootstock and the grafting are removed for vegetative propagation are called mother plants and should be plants that maintain the genetic characteristics of the cultivar and plant health, mainly regarding the absence of viruses, soil fungi and pests. Mother plants must be registered with the surveillance organ and preferably be originated from an official institution. Mother plants must be renewed in order to maintain quality, and above all to maintain their genetic identity and varietal purity.

The propagation material should be collected from identified mother plants, collected from lignified branches, eliminating the upper middle third of branches. Rootstocks should be removed from mother plants in late fall and early winter. All propagation material must be kept out of the sun, avoiding desiccation. It is recommended to keep the grafting material (rootstocks and branches of the canopy cultivar) in refrigerated environment (4 to 6ºC, relative humidity higher than 80%) for a period of 15 to 30 days to favor the budding and growth of seedlings in order to meet the physiological needs in cold to overcome the dormancy of buds, characteristic of temperate fruit trees. In addition to the quality of the propagation material, care should be taken as to where the production of seedlings will occur. The nursery should be located far from apple orchards in order to minimize risks of contamination and dissemination of pests and diseases.

As for the type of seedlings of apple tree produced, these can be classified into 'smooth' and 'preformed' type seedlings. 'Smooth' type seedlings are plants that do not develop lateral branches in the nursery, consisting of a single stem. Normally, this modality of seedlings have maximum of 18 months for budding grafting and 14 months for cleft grafting, with minimum diameter of 1.2 to 1.5 cm measured at 5 cm from the grafting point, height of 1.2 to 1.5 m, depending on the rootstock, with rooted stem of about 10 to 15 cm.

'Preformed' type seedlings are plants that already have 4 to 8 lateral branches at the moment of planting (Figure 3). The main advantage of 'preformed' seedlings is the anticipation in fruiting and formation of the productive structure of plants, facilitating bending operations of branches after the installation of the orchard. However, the induction and formation of lateral branches in the nursery does not occur naturally, requiring the use of cultural practices such as ring incisions and the use of phytoregulators, in addition to being extremely influenced by the rootstock and canopy cultivar propagated.

The use of phytorregulators has been the main strategy used in the formation of preformed seedlings. Both cytokinins and gibberellins can induce branching as a result of the spur growth stimulus in apple tree seedlings (GREENE et al., 2016). Benzyladenine, a cytokinin-based regulator commonly used in the chemical treatment of pear and apple trees (MILIĆ et al., 2012; SCHRÖDER et al., 2013; BOUND et al., 2015), being used alone or in combination with gibberellins (GA_3 and GA_{4+7}), in multiple applications, increases the formation of lateral branches (GREENE; MILLER, 1984; ROBINSON et al., 2014; GREENE et al., 2016).

The number of branches formed and their angle is associated with the type and frequency of growth regulator used (Jacyna, 2002). It is emphasized that the quality of seedlings is the result of the number, length, insertion angle, distribution of branches and their diameter. With these attributes of a seedling, the orchard could have its first production already in the second year, with significant economic benefits from the use of preformed seedlings.

Figure 3. Commercial plantation of apple trees using preformed seedlings.

In the production of preformed apple seedlings, Promalin (benzilandenin + GA $_{4+7}$) or 250-500 mg L^{-1} Maxcel (benzyladenine) is used. Phytorregulators are applied at the desired height of the emission of branches, and the treatment should be repeated as new growth occurs and new lateral branches are desired. Thus, it is possible to form seedling with 4 to 8 lateral branches. This practice can also be used in the first and second year after planting, when lateral branches have from 5 to 10 cm in length, aiming at the anticipation of the emission of lateral branches and consequently reduction in the apical dominance of branches, anticipating the entry in the fruiting stage. Elfving and Visser (2005) and Sazo and Robinson (2011) indicate the possibility of using cyclanilides in apple trees, which interferes with the auxin transport, favoring the development of lateral branches in growing branches in the nursery or in the orchard.

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