کشت و صنعت رعنـــــا [سهامی خاص]

RANA AGRO- INDUSTRY CORP.

Tissue Cultured Walnut After-Sales Manual

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INTRODUCTION:

Thank you for purchasing RANA Agro-Industry Corporation's tissue cultured walnut plants! Walnut plants propagated through tissue culture technology carry distinct advantages as compared to those produced through conventional seed or grafting propagation methods. A number of these distinct advantages are outlined below:

- Free from pathogenic bacteria and fungi as well as key pathogens such as the CLRV (Cherry Leaf Role Virus).
- Excellent field establishment rates (above 97%)
- Excellent field growth (1.5 – 2 meters under optimal conditions within the first year of planting with exponential growth experienced during subsequent years)
- Exceptionally high yields under optimal orchard management conditions.
- Self-rooted therefore, no need for grafting
- Possibility for the mass production of elite walnut varieties
- Excellent orchard uniformity
- No unwanted somaclonal variation
- Lack of grafting wound site avoids risk of pathogenic infection

The genus *Juglans* spp. includes species that are known to exhibit difficulties during vegetative propagation. Up to now, cuttings have been impossible to produce at a commercial level, while the grafting techniques exhibit several inconveniences:

- The Grafting process is prone to failure and is difficult to establish (as compared to many other woody plants). This has contributed towards the lack of plant availability in the market, and higher prices as compared to other plant species.
- Lack of credible propagation methods for walnut rootstocks warrants the usage of seedlings as rootstocks, which can heavily influence the development of the scion. This can greatly compromise the performance of a certain variety if the genetics of the seedling rootstock proves to be inferior.
- Experience suggests that in walnut trees, the graft union reduces tree growth vigour. The graft union injury site limits the exchange of nutrients and sap. Also, Self-rooted walnuts are more resistant to leaf chlorosis in soil conditions that affect grafted trees (e.g. basic pH or high active calcium soils, where availability of some nutrients is restricted).
- The lack of a graft union site in self-rooted walnuts avoids the possibility of disease transfer that can be transmitted throughout a nursery, e.g. crown gall disease (caused by *Agrobacterium tumefaciens*, which is a major phytosanitary problem and highly prevalent in Californian walnut nurseries). Also, other diseases, including the *black line disease,* which can affect grafted walnuts (particularly when *J. regia* scions are grafted onto different species).

This document aims to provide the orchard manager with certain guidelines regarding optimal planting of tissue cultured walnut trees, their irrigation, fertigation as well as the establishment of certain pesticide regimes. This document is by no means exhaustive as local orchard conditions may warrant deviations from such

guidelines. For the establishment of a highly commercially viable tissue cultured walnut orchard, RANA Agro-Industry Corp. strongly recommends the provision of consultancy services of tissue cultured walnut orchard management experts. Such experts can provide full consultancy advice in the areas of initial orchard feasibility assessments, orchard establishment and maintenance. RANA Agro-Industry Corp. can facilitate the contact between orchard owners and such experts, should the interest for such services arise through the customer.

1. SOIL ASSESSMENT:

The orchard's soil assessment is a highly recommended step that must be taken for the establishment of an optimised and commercially viable tissue cultured walnut orchard. The reasons for carrying out a soil assessment include the following:

1.1 Avoidance of Soil-borne Pathogens:

Certain soil-borne pathogens can severely limit the growth or threaten the viability of the trees. Some key pathogens that must be considered include *Phytophtora* or *Armillaria* spp.

1.2 Avoidance of Waterlogged soils:

Certain heavy soils have a tendency to waterlog with water-bearing strata shallower than 1.5 m from the soil surface most likely to create such a problem.

1.3 Assessment of Soil Depth and Structure: Physical Description of the Soil

Gaining an understanding of the layer structure of the soil will provide substantial information regarding soil depth, impermeable strata and their depth, possible water-bearing layers, and other details. This information is then used to design the soil preparation methodology as well as the ideal irrigation system to be used in the orchard. Such assessment is called the *physical description* of the soil.

1.4 Chemical Composition Analysis

The Chemical composition tests must include the establishment of the contents of the major and minor elements, pH, texture, soil fertility, Ca and K vs. Mg relationships, salinity, organic material content and all possible relevant or specific information related to the particular conditions of the orchard.

Such information can help in the design of fertigation regimes, supplementation of major and minor element deficits and avoidance of areas within the orchard that are deemed unsuitable for walnut growth. Chemical composition analysis can also reveal the level of soil salinity and the possible avoidance of orchard establishment if soil salinity proves to be too high.

The methodology used for soil preparation is closely related to the information obtained after carrying out the above soil assessment exercises. Local horticultural experts can carry out the interpretation of such information. However, ideally RANA can recommend and facilitate contact between the orchard owner and tissue culture

walnut orchard management experts who can assist in this process. Please contact RANA directly for further information.

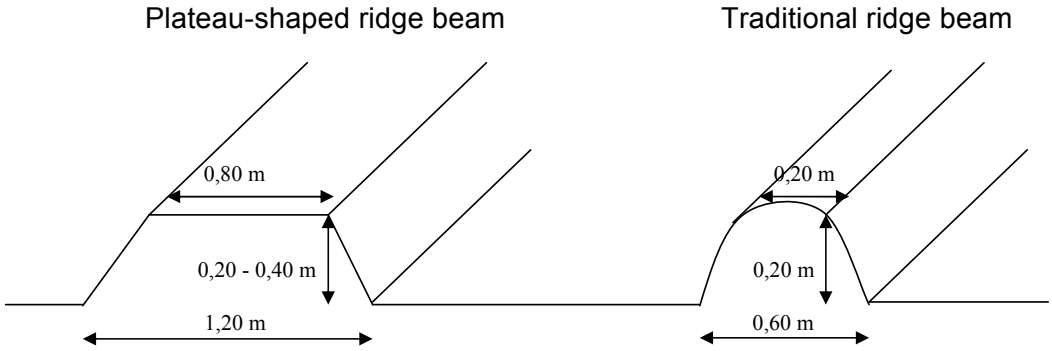
2. SOIL PREPARATION ACCORDING TO SOIL TYPE:

The first step in carrying out soil preparation is the performance of deep tillage, in order to establish the soil uniformity as best as possible. Deep tillage can be divided into methods:

1. Deep ripping and slip ploughing 1 to 2 m deep in order to break up naturally restricting layers in the subsoil.

2. Intermediate to shallow ripping: 50 cm to 1 m deep, in order to loosen farming-induced soil compaction.

If the soil is salty, trends to waterlog at some time, or is too shallow then it is necessary to plant in ridge beams. Ridge beams can be traditional or plateau-shaped. The latter is more favourable for the stability of the soil at higher levels (where the tree is planted), and eases the placement of irrigation lines. This not only facilitates irrigation but also eases maintenance and harvesting work by avoiding breakages caused by tractors.
Ridge beam dimensions will depend on the soil type, irrigation system and other limiting factors. The diagram shows examples of both ridge beam types.



It is necessary to level the orchard to ease the movement of the machinery especially during the harvesting process. Particular attention must be given to avoid leaving insufficient soil in certain areas. It may be useful to perform the planting following the levelling of contours.
Once ripping is completed, manure is applied to the orchard. It is necessary to apply the manure 2-3 months prior to planting in order to avoid the chemical reactions during the rotting process of the manure, which may adversely affect the plants. The amount of manure to be applied depends on the level of organic material present in the soil as detected during soil analysis. Some examples include the following:

1. For a soil with OM (organic material) <1,0% apply up to 60,000 Kg/Ha of manure

2. For a soil with OM between 1.0% and 2.0% apply 40,000 Kg/Ha

3. For a soil with OM >2.0% apply 20,000 Kg/Ha

When active calcium levels are present at high levels in the soil, manure must be mixed and applied with $FeSO_4$ in a ratio of 9:1 (manure:$FeSO_4$). This will reduce the effect of ferric chlorosis on the plants.

In case the soil is compact or tough (clay soil) it is convenient to plant horticultural crops like rape during the year(s) prior to walnut orchard establishment, in order to considerably crack the soil and incorporate the rest of the plants as an organic fertilizer. This process can also be carried out after walnut planting, in between the rows of trees.

3. ORCHARD DESIGN

A suitable design for a walnut orchard, which includes the selection of the most suitable walnut variety(s) based on the orchard's environmental conditions, can contribute up to 70-80% of the project's success rate. Prior to planting, the aim of the orchard manager must be the elimination and or avoidance of potential risks that can compromise the success or even the feasibility of the project.

A suitable design of the orchard is dependant upon the following information:

- Maximum Summer temperatures
- Minimum Winter temperatures
- Typical dates for the occurrence of late spring frosts, early autumn frosts and chilling
- Soil assessment information: including the soil structure, texture, % of active calcium, % of organic material, salinity and etc.

It is highly recommended to hire the consultancy of a walnut orchard management consultant at this stage, particularly if the grower is unfamiliar with the most recent technologies available for walnut orchard management.

3.1 Orchard Orientation:

The best orientation for the orchard is by following the East-West lines, for better sunlight procurement. Rows must be as longest as possible to reduce the number of interruptions for the machinery's movements towards the end of the row. The final

orientation will provide the shape of the orchard for planting as, for instance, in hill zones the planting will be carried out following levelling contours to avoid soil erosion.

3.2 Tree Spacing and Positioning:

Stakeout is then carried out, which consists of marking the position of each tree with small stakes. Previously, these positions are determined by strips or by GPS. Carrying out a stakeout is very important to establish good tree alignment and distribution.
The choice of the tree spacing directly depends on the variety, its bearing habit, vigour and soil fertility. In general the table below summarises the most commonly used spacing regimes:

Tree Spacing	Advantages	Limitations	Varieties
EXTENSIVE 12m x 12 m 14m x 14m 10m x 12m	Lower initial cost (less trees= cheaper planting) Easier management	Delayed yield (compared to other training systems) Extensive tree training needed Low yield potential	Franquette Fernor Hartley
SEMI-INTENSIVE	INTERMEDIATE OPTION	INTERMEDIATE OPTION	Chandler Serr Vina
INTENSIVE 7m x 3.5m	Early yields Higher yield potential	Higher initial cost High levels of technical management Difficult tree training	Howard Lara Tulare

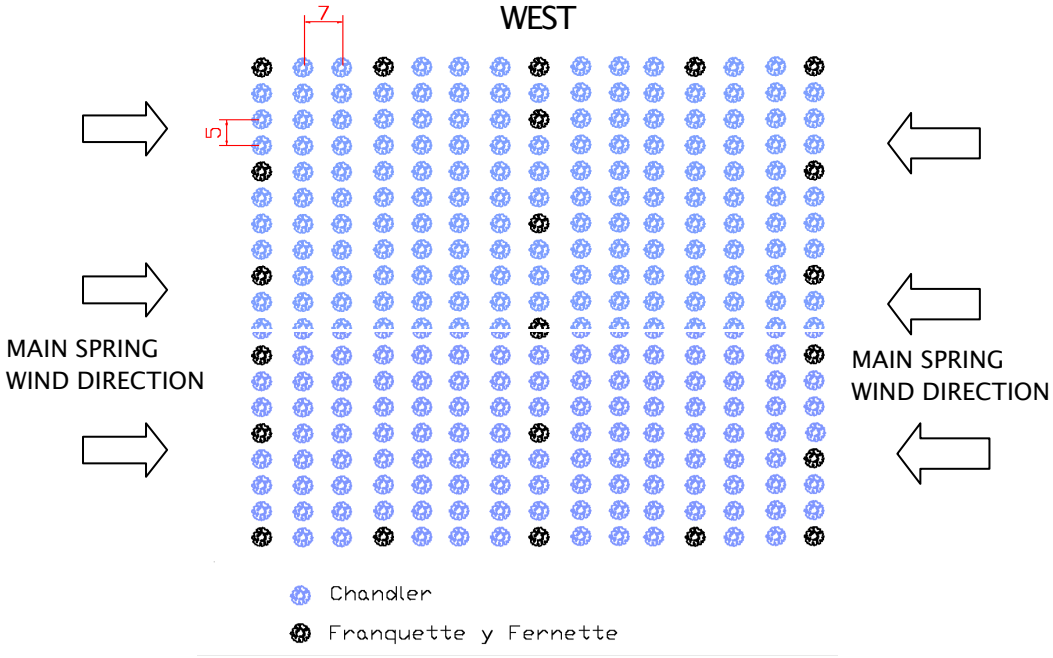
The most commonly used tree spacing currently include: 7 x 5 m (285 trees/Ha) e.g. for Chandler, and 7 x 3.5 m (408 trees/Ha) e.g. for Howard. The objectives are to obtain the earliest possible yields and the mechanization of the maximum number of tasks (pruning, harvesting and etc.).
With such high densities, the training system used is called the Modified Central Leader. It is suitable for varieties of high lateral bearing habit (Tulare, Serr, Chandler and Howard). The idea is to obtain a hedgerow to mechanically perform pruning of bearing trees when necessary. Mechanical pruning is very fast and much cheaper than manual pruning, and provokes new growth in bearing trees every year. This maximizes the sunlight exposure within the branches of the tree, which is necessary for female flower induction.

3.3 Pollinators and their distribution

A very important factor at the time of design of a walnut orchard is the amount and distribution of pollinator trees. It must be considered that:

- All walnut species are monoecious: the pollen-bearing flowers (male reproductive units) and the nut producing flowers (female reproductive units) are borne separately in the same tree.
- Walnut pollination is 100% anemophilous, i.e. wind-mediated. Insects do not take part in this process.

- The most common rule is that pollinators tend to be late leafing varieties as compared to the yielding trees. Therefore, the pollinator's catkins (groups of male flowers) are active (producing pollen) while the female (pistillate) flowers are receptive.
- Wind transports pollen over long distances (sometimes longer than 100 m). It is convenient to plant complete rows with pollinators, if possible. If the rows are too long, or the distance between them is too wide, the pollinators can be alternated with bearing trees in the same row. A block shape will have an influence in this distribution.
- Pollinators are mainly planted in the periphery of the orchard, and perpendicularly placed to the predominant wind directions present during the pollination time (mid-late Spring).
- Rate of pollinators to place in an orchard may vary depending on the variety and other factors, such as climatic conditions (Spring frosts, rainfalls, strong winds and etc.)
- For self rooted walnuts, the pollinator rate is around 5-10%, but is typically observed at 7%. The recommended varieties to use include Fernor and Ronde de Montignac. Pollinators are also bearing trees, and in most cases, their nuts can also be sold with the main bearing variety.
- When selecting the pollinator variety, it is convenient to consider that it is not susceptible to bacterial blight (*Xanthomonas juglandis*. var Campestris), as contaminated pollinators can transmit this disease to the bearing trees during pollination.
- It is also very important to take into account that self-rooted walnut trees are late catkin producers, as compared to grafted trees. This is because the injury caused for the establishment of the graft union stresses the tree and this advances catkin production. Therefore, in effect, this makes grafted trees much earlier catkin producers than self-rooted trees. The practical consequence of this is that grafted trees are convenient pollinators; otherwise yielding will be delayed in the orchard due to the lack of pollen.
- The opposite situation to the above mentioned is a physiological problem caused by the presence of excess pollen (in regions with a large quantity of walnuts orchards). The physiological issue is referred to as PFA (Pistillate Flower Abortion). This occurs when more than one pollen grain reaches a stigmata. This causes the pollen tube to break the style and the flower aborts. In some varieties (e.g. Serr) and yield losses exceed 80% under certain conditions. Nowadays, this disease is controlled by limiting the number of pollinators used. For example, in most of the new Californian orchards that exist in highly populated walnut regions, pollinators are not used at all.
- In the following diagram, 8% of the 1 Ha orchard consists of pollinators. The highest possible number of pollinators is placed perpendicularly to the two main late spring wind directions, as well as covering the entire periphery of the orchard.

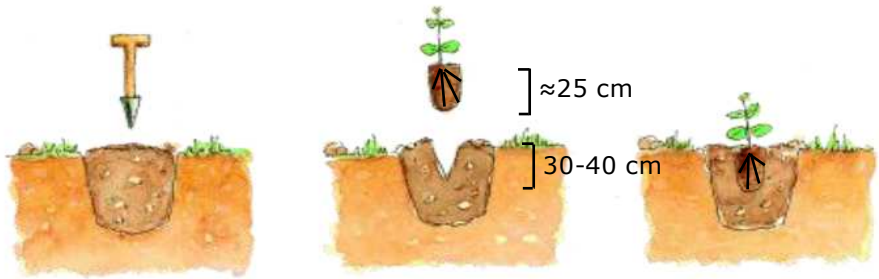


4. TREE PLANTING

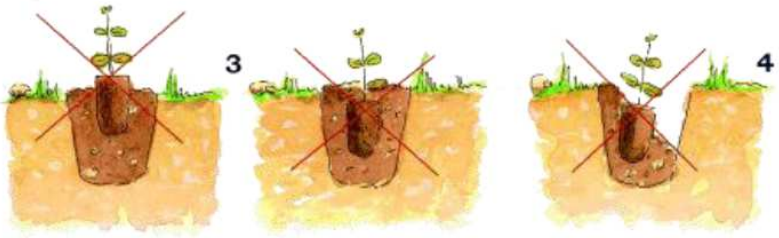
The best seasons for planting are from February-March to May (before the summer's peak temperatures) and September-October (before the winter's cold arrives). September planting using RANA's potted walnut plants will result in the vigorous growth and development of their rooting systems, which will be complemented by the same process that occurs naturally prior to the Spring-leafing season. This makes leafing extremely vigorous. Furthermore, the tree is progressively acclimatised to temperature conditions of the planting region, which avoids frost damage. Please note that RANA does not recommend planting later than the 15 OCTOBER, or even earlier if the region is cooler. This may cause loses due to frost damage and other physiological problems related to incomplete adaptation of the trees to the weather conditions of the planting region.

February – March planting can be carried out while the plants are dormant. Plants planted during May, however will be full leaf potted plants. This provides an opportunity for those growers who have missed the previous planting season (autumn-winter) to plant their trees without having to wait until the following year. Additionally, this may lead to some extra growth as compared to a later planting date).

When planting, it is necessary to prepare a generous hole. Pots are about 25 cm in height, therefore it is recommended to dig 30 – 40 cm hole in a well prepared soil (see soil preparation above):



- Plants should be planted up to (not above and not below) the stem-shoot junction (crown). Do not leave root balls uncovered and do not cover the trees above the crown.



The plastic pot can be used as a protector of newly planted trees by removing the base and placing the pot upside down on top of the plant. This can provide protection for the newly planted tree as well as adding protection when using herbicides (glyophosate is recommended).

If the planting is carried out during the growth season (March – end of summer) it is advisable to leaf-spray amino acids (there are several brands available on the market) no later than one week after planting. This will aid the plants to recover quicker from the stress caused by planting. This is not necessary if the trees are planted dormant.

During the growth period, it is recommended to continue with the amino acid application every 2 weeks. In alternate weeks, this application can be complemented with an organic fertiliser called Agriful that can be applied through drip irrigation. The combination of Agriful with an inorganic N fertiliser (supplying up to 90 g of N per tree) during the first growth period will allow an excellent development of the trees.

Once the planting is completed, it is very important to irrigate accurately to avoid the presence of air bubbles in the soil as well as to enhance a good mixing between the soil and the pot substrate.

In regions where herbivorous animals are present (e.g. rabbits) it is necessary to place protectors, preferably net-shaped around the plants. Avoid using closed-tube protectors, as they create a microclimate at the base of the stem, which, produces high temperatures during the summer, while during winter, the closed tube prevents the trunk from hardening.

5. IRRIGATION:

Evapotranspiration in the region may provoke a water consumption of 5,000 m^3 when using drip irrigation, and 6,000 m^3 with the use of sprinklers. In practical terms, to compensate for this, it is recommended to have a minimum water flow rate of 0.6-0.7 l/sec per Ha available for irrigation apart from water reservoirs, as irrigation requirements are not equally distributed along the year or between different years. The most efficient drip irrigation strategy consists of wetting 60% of the soil, covering 100% of ETo (Evapotranspiration). An example of irrigation requirements is displayed in the table below. Please note that this is just an example, as ETo rates may greatly vary irrigation requirements.

PERIOD	IRRIGATION REQUIREMENTS (m^3/ha and year)
Year 0	800
Year 1-5	1,500
Year 6-10	3,000
Year 11-25	5,000

The most efficient irrigation system is drip both in terms of water management and its facilitation of fertigation (combining irrigation with fertilisation), as well as avoiding the spread of diseases, which is often observed with conventional flood irrigation methods. This system also allows for a higher number of short volume irrigation schedules when the trees are recently planted and contain very shallow roots. The irrigation frequency will be increased and spread out over time as the trees grow older and their roots grow deeper. It is advisable to irrigate almost daily during the first growing season.

The best results are obtained by placing two irrigation lines per planting row with two emitters per irrigation line and tree (4 total emitters per tree). Emitter water flow settings are strongly dependant on the soil permeability and evapotranspiration in the zone. However, a guideline suggestion is around 4 litre/hour, which is a commonly used rate.

Drip irrigation for a dormant plant

Drip irrigation for a fully leafed plant

Please note that the pot substrate does not absorb water from the surrounding soil no matter how wet the surrounding soil is. For this reason, it is recommended that during the first two months of irrigation, the zone of the root ball is kept wet. If drip irrigation is used a useful approach is to place the irrigation pipes close to the trunk once planting, so the emitters remain close to the tree. After the first two months of irrigation as in the case of autumn planting, the root of the plants would have grown

from the root ball to the planting soil. For this reason, the irrigation line must then be separated such that the emitters are placed about 40 cm from the trunk of the trees.

6. RECOMMENDED PREVENTATIVE APPLICATIONS:

Aliette or Fosbel 80WP or TecnoPhyt PK (use with TecnoPhyt Ph) (300g/hL) once a month: Fungicide anti-Phytophthora treatment.

Carbendazim (0.05%) once a month: Broad-spectrum fungicide.

It is necessary to be aware of possible fungal and bacterial diseases. Disease risks of high concern during the first year include *anthracnosis* caused by the fungi *Gnomonia leptostyla*. It is able to defoliate the whole tree, which will compromise its growth (and may even kill it under certain circumstances). The disease can be detected in the shape of small polygonal dots on the larger leaves. The product Miclobutanil (trade mark: Systhane, from Dow Agrosciences) at 20 cc/hL must be applied once the first disease symptoms are detected. For further information on the management of pests and diseases, please contact your local Plant Health Organisation representative.

For further details and explanations please contact RANA Agro-Industry Corporation:

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