PROPAGATION AND MANAGEMENT OF VETIVER NURSERY

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ABSTRACT

The Vetiver Network International promotes the use of sterile vetiver cultivar to avoid it becomes a weed in the new environment. The vetiver cultivar used around the world is originally from the oil producing cultivar from southern India, which is genetically identical to Sunshine in the US or Monto in Australia.

The three commonly used methods for large scale propagation of vetiver are:

- *Vegetative Propagation*: Using various parts of a mother Vetiver plant
- *Bud multiplication:* In vitro micro propagation
- *Tissue culture*: Using a small part of the plant.

1. Vegetative Propagation

The splitting of tillers and cutting from a vetiver mature clump produce at least four kinds of planting materials:

1. ***Bare root slips***: A planting slip is defined as a stem, root, twig, etc. cut or broken off a plant and used for planting or grafting.
2. ***Culm slips*** A culm is defined as a stem, stalk of various grasses. The culm of the vetiver grass is strong, hard, and lignified, having prominent nodes with lateral buds that can form roots and shoots upon exposure to moist condition
3. ***Polybags or tubestock***. When the bare root or culm slips are ready, they are put in small pots or small plastic bags containing half soil and half potting mix
4. ***Planting strip.*** Planting strips are a modified form of polybags, instead of individual bags, bare root slips or culm slips are planted in specially prepared long strip medium, which would facilitate transportation and planting

2. Bud Multiplication

Lê Van Bé *et al*. (2006) of Can Tho University, Vietnam has developed a very practical and simple method to multiply buds. His protocol consists of four micro-propagation stages, all in liquid medium:

3. Tissue Culture

Tissue culture is another method of large scale propagation of vetiver planting materials. Instead of using a large part of the mother clumps, tillers and culms, tissue culture uses only some special tissues of the vetiver plant, such as shoot tips, young flower inflorescences or nodal buds. The procedure is fairly routine in the horticultural industry around the world.

The tissue culture methods varies with the individual laboratories, but basically it involves the use of a very small bit of tissue, growing it in a special agar medium under aseptic conditions, plant the very small plantlets out to appropriate medium until fully developed into a small plant.

3. Vetiver Nursery

Nursery is needed to provide stock materials for vegetative propagation of vetiver grass. The following are criteria needed to plan and establish a productive and easily managed vetiver nursery:

- Soil type
- Harvesting method
- Irrigation method
- Training of operational staf:
- Mechanical planter
- Availability of farm machinery

Keywords: Propagation, bud multiplication, tissue culture, nursery, planting materials

1. INTRODUCTION

Since most major applications require a large number of plants, the quality of the planting material is important the successful application of the Vetiver System. This requires nurseries capable of producing large quantities of high quality, low cost plant materials. The Vetiver Network International promotes the exclusive use of sterile Vetiver cultivars (*C. zizanioides*) to avoid it becomes a weed in the new environment. The vetiver cultivar used around the world is originally from the oil producing cultivar from southern India, which is genetically identical to Sunshine in the US or Monto in Australia. Because of its sterility, vetiver grass has to be propagated vegetatively.

2. METHODS OF PROPAGATION

The four common ways to propagate Vetiver are:

- Splitting mature tillers from Vetiver clump or mother plants, which yields bare root slips for immediate planting or propagating in polybags.
- Using various parts of a mother Vetiver plant
- Bud multiplication or in vitro micro propagation for large scale propagation
- Tissue culture, using a small part of the plant to propagate on a large scale.

2.1 Splitting Mature Plants to Produce Bare Root Slips

Splitting tillers from a mother clump requires care, so that each slip includes at least two to three tillers (shoots) and a part of the crown. After separation, the slips should be cut back to 20 cm length (Figure 1). The resulting bare root slips can be dipped in various treatments, including rooting hormones, manure slurry (cow or horse tea), clay mud, or simple shallow water pools, until new roots appear. For faster growth the slips should be kept in wet and sunny conditions until planting out.

Figure 1: How to split Vetiver slips

2.2 Propagating Vetiver from Plant Parts

Three parts of the Vetiver plant are used for propagation:

Vetiver tillers: (Figure 2)

- Select mature tillers with at least three or four well-developed leaves
- Separate tillers carefully, and be sure to include the bases and some roots.

Figure 2: Bare root slips ready for planting out (left); being dipped in clay mud or manure slurry (cow tea) (right)

Vetiver culms: A culm is the stem or stalk of a grass. The Vetiver culm is solid, stiff, and hard; it has prominent nodes with lateral buds that can form roots and shoots when exposed to moist conditions. Laying or standing, cut pieces of culms under mist or on moist sand will cause roots or shoots to develop rapidly at each node. Select old culms, which have more mature buds and more nodes than young ones. Cut culms in 30-50mm (1-2'') lengths, including 10-20mm (4-8'') below the nodes, and strip off the old leaf covers. Expect new shoots to emerge about one week after planting. (Figures 3 & 4).

Vetiver crown or corms: The crown (corm) is the base of a mature Vetiver plant from which new shoots sprout. Use only the top part of the mature crown.

Le Van Du, Agro-Forestry University, Ho Chi Minh City, developed the following four-step method of propagating Vetiver from cuttings: (Du *pers.com*. and Truong *et al*, 2008)

- Prepare of Vetiver cuttings
- Spray the cuttings with a 10% water hyacinth solution
- Use plastic bags to cover the cuttings completely, and leave it alone for 24 hours, and
- Dip clay mud or manure slurry, and plant in a good bed.

2.2.1 Preparing Vetiver cuttings

Figure 3: Old tillers (left) and young tillers (right)

Figure 4: Vetiver crown or corms (left) and pieces of Vetiver culms with nodes (right)

2.2.2 Preparing water hyacinth solution (Figures 5).

Water Hyacinth solution contains many hormones and growth regulators, including gibberellic acid and many Indol-Acetic compounds (IAA). To prepare rooting hormone from Water Hyacinth:

- Remove Water Hyacinth plants from lakes or canals
- Put plants into 20 litre-plastic bag, and tie it closed
- Leave the bag for about one month until the plant material has decomposed
- Discard the solid parts and keep only the solution
- Strain the solution and maintain in a cool place until use.

Figure 5: Spraying cuttings with 10% water hyacinth solution (left) and cover cuttings completely with plastic bags, and leave them for 24 hours

2.2.3 Treatment and planting (Figures 6).

Figure 6: Plant with manure, in a good nursery bed

2.2.4 Advantages of using bare root slips and culm slips

Advantages:

- Efficient, economic, and a quick way to prepare the planting material
- Small volume results in lower transportation cost
- Easy to plant by hand
- Large numbers can be mechanically planted in large areas.

Disadvantages:

- Vulnerable to drying and extreme temperatures
- Limited on-site storage time
- Requires planting in moist soil
- Needs frequent irrigation in the first few weeks
- Recommended for good nursery sites with easy access to irrigation.

2.3 Bud Multiplication or Micro Propagation

Lê Van Bé *et al*. (2006) of Can Tho University, Vietnam has developed a very practical and simple method to multiply buds. His protocol consists of four micro-propagation stages, all in liquid medium:

- ***Inducing lateral bud development*:** A flowering culm was used, and the nodes at 1.2 m high in the culm were chosen to avoid infections. The 20cm segments with lateral buds were cultured into glass tubes containing Murashige and Skoog's medium (1962) [MS] after sterilization. This stage was carried out under growth chamber conditions (24±1^{o}C, 12-h photoperiod provided by fluorescent tubes with a photosynthetic active radiation (PAR) of about 30 µmol.m^{-2}.s^{-1}.
- ***Multiplying new shoots**:* Materials of proliferation were the young shoots collected from Stage I, with 5-6 leaves and about 4 cm high. The young shoots were subcultured in jars of the same MS medium with different hormone BAl^{-1} (benzyl adenine) supplements.
- ***Promoting root development on new shoots*** Clump from above contained average 7-9 shoots, it was subdivided into smaller clump (4-5 shoots per clump), those with shoots longer than 4 cm, were used for root initiation. The liquid MS medium were supplemented with two levels of sucrose (3% and 4%), and modified NAA (naphthalene acetic acid) with 0 and 1 mgl^{-1}.
- ***Promoting growth in shade house or glasshouse***. Clump of shoots from above was acclimatized in the nursery in trays containing soil with high organic content. These clumps were kept in 130 - 140 µmolm^{-2} s^{-1}, 70-80% relative humidity of air. The cluster of shoots survived with a high ratio (over 95%) and it developed well after 10 weeks weaning. It can be concluded that the micro-propagated plantlets of vetiver well adapted under the natural conditions of nursery.

2.4 Tissue culture (Namwongprom and Nanakorn, 1992; Chomchalow2000).

Tissue culture is another way to propagate Vetiver planting materials in quantity, using special tissues (root tip, young flower inflorescence, nodal bud tissues) of the Vetiver plant. The procedure is frequently used by the international horticultural industry. Although the protocols of individual laboratories differ, tissue culture involves a very small bit of tissue, growing it in a special medium under aseptic conditions, and planting the resulting small plantlets in appropriate media until they

fully developed into small plants. More details are found in Truong (2006).

One method of tissue culture is described below by Dr.Malee Nanakorn, Botany Department, Kasetsart University, Bangkok, Thailand.

Shoot induction from young inflorescence method

This method can be accomplished in 5 steps:

Step I: Surface sterilization

- Wiping young inflorescence that still enclosed in the flag leaf, 10-15 cm in length, with 75% alcohol.
- Spraying with (or dipping into) 75% alcohol and flame.
- Cutting sterilized inflorescence into 10-mm pieces.

Step II Callus induction

- Culturing sterilized explants on Murashige and Skoog medium supplemented with 15 μmol/l 2,4-D for 30-45 days.
- Transferring the callus to MS medium supplemented with 10 μmol/l 2,4-D for 30-45 days to proliferate the callus.

Step III Shoot induction

- Selecting compact callus with creamy color and culturing on hormone free MS medium for 45-60 days. Plants or plantlets with roots are ready for transferring to greenhouse conditions.

Step IV Shoot proliferation (multiplication)

- Separating plantlets into single shoot.
- Transferring single shoot to MS medium supplemented with 10 μmol/l BA to proliferate more shoots.
- Subculturing every 30 days.

Step V Root induction

- Separating plantlets into single shoot.
- Transferring shoot to MS medium supplemented with 5 μmol/l IBA for 15 days.

Transplanting

- Keeping the culture bottles of rooted-shoots outside the culture room for a few days to acclimatize these plantlets.
- Transplanting the plantlets to well-drained growing medium in high relative humidity condition.
- Gradually lowering the humidity within 1-2 weeks

2.5 Advantages and Disadvantages of Tissue Culture and Bud Muktiplication Methods

The advantages of this method of propagation are:

- A very large number of plant can be produced very quickly
- No need for a large scale nursery
- Smaller volume and weight for transportation
- Free from pest and pathogen in nursery

The disadvantages of this method of propagation are:

- The need to set up a small laboratory, which can be expensive for a small nursery
- The need for a well trained technician and other skilled staff
- The need for more manual labour to transfer the seedling to different size pots during its growing period.
- It takes longer to get the plantlets ready for planting
- More susceptible to pest and disease on site and adverse conditions.

3. PREPARING PLANTING MATERIAL (Truong, 2006).

To increase the establishment rate under hostile conditions, when the plantlets produced by the above methods are mature enough or bare root slips are ready, they can be prepared for planting out by:

- polybags or tubestock
- planting strip.

3.1 Polybags or Tube Stock (Figure 7)

Plantlets and bare root slips are planted in small pots or small plastic bags containing half soil and half potting mix and maintained in the containers for three to six weeks, depending on the temperature. When at least three new tillers (shoots) appear, the plantlets are ready to be planted.

Figure 7: Bare root slips and tube stock (left), putting plants into polybags (middle) and polybagged plants ready for planting (right)

3.2 Planting strip (Figure 8)

Planting strips are a modified form of polybags. Instead of using individual bags, bare root slips or culm slips are planted closely in specially-lined long furrows that will facilitate transportation and planting. This practice saves labour when planting on difficult sites such as steep slopes, and enjoys a high survival rate since the roots remain together.

Figure 8: Planting strips (left) in containers and removed from containers (middle), and ready to be planted (right)

3.3 Advantages of polybags and planting strips

Advantages:

- Plants are hardy and unaffected by exposure to high temperature and low moisture
- Lower irrigation frequency after planting
- Faster establishment and growth after planting
- Can remain on site for longer before being planted
- Recommended for harsh and hostile conditions.

Disadvantages:

- More expensive to produce
- Preparation requires a longer period to prepare, four to five weeks or longer
- Transporting large volume and increased weight is expensive
- Increased maintenance cost following delivery, if not planted within a week.

4. VETIVER NURSERY

Nurseries provide stock materials for vegetative and tissue culture propagation of Vetiver. The following are criteria will facilitate the establishment of productive, easily managed Vetiver nurseries (Figure 9):

- ***Soil type****:* Sandy loam nursery beds ensure easy harvesting and minimal damage to plant crowns and roots. Although clay loam is acceptable, heavy clay is not.
- ***Topography****:* Slightly sloping land avoids water-logging in case of over watering. Flat site is acceptable, but watering must be monitored to avoid water-logging, which will stunt the growth of young plantlets. Mature Vetiver, however, thrives under waterlogged conditions.

- ***Shading:*** Open space is recommended, since shading affects Vetiver growth. Partially shaded areas are acceptable. Vetiver is a C plant and likes plenty of sun.

- ***Planting layout****:* Vetiver should be planted in long, neat rows across the slope for easy mechanical harvesting.
- ***Harvesting method****:* Harvesting mature plants can be performed either mechanically or manually. A machine should uproot the mature stock 20-25cm (8-10'') below ground. To avoid damaging the plant crown use a single blade mouldboard plough or a disc plough with special adjustment.
- ***Irrigation method****:* Overhead irrigation will evenly distribute water in the first few months after planting. More mature plants welcome flood irrigation.
- ***Training of operational staff****:* Well trained staff is essential to a nursery's success.
- ***Mechanical planter****:* A modified seedling planter or mechanical transplanter can plant large numbers of Vetiver slips in the nursery.
- *Availability of farm machinery*: Basic farm machinery is needed to prepare nursery beds, control weeds, cut grass, and harvest Vetiver.

Figure 9: Left: Machine planting; right: manual planting

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