

2009; Kang, 2007). As a result of high costs and lacking access of seed potato production, there is a high need for effective methods to produce high quality seed potato (Ojha, 1999; Ojha et al., 2001; Otazu, 2010). The conventional way of basic seed potato production is to multiply disease-free in vitro plantlets and microtubers in the greenhouse. This method usually produces low tuber yield, is time consuming and very laborious. For example, to obtain sufficient tubers for commercial planting of a new cultivar it is necessary to carry out 5-7 field generations. Alternatively, potato microtubers could be important for reducing the number of field generations of seed potato production in different parts of the world (Alix et al., 2001; Jimenez et al., 1999; Yu et al., 2000). Despite crop isolation and careful inspections, the yield of seed potato frequently deteriorates by about 10% yearly in the field. One of the difficulties in the production of good quality seed potato is the presence of soil-borne diseases and pests (van Loon, 2007). Thus, even when starting with high quality plant material, a major decline is apparent in the yield before a commercial crop can occur. Minituber production could reduce the number of generations required to produce commercial seed potato (Nichols, 2005). Moreover, using aeroponic system for minituber production has the advantage of eliminating soil-borne diseases and pests resulting in high quality tubers.

### **1.3. Why high quality seed potato?**

Seed potato are produced everywhere potatoes are grown. The use of high quality plant material is one of the most important factors leading to the production of a profitable potato crop. An outstanding problem in seed potato production is the relatively high degeneration rate of initially healthy tubers (van Loon, 2007). Soon after the introduction of the potato to Europe, potato crops started to suffer from degeneration due to diseases, especially those caused by viruses (Salaman and Hawkes, 1985). With the conventional method of vegetative propagation, potatoes are often prone to pathogens such as fungi, bacteria and viruses; thereby resulting in poor quality and yield. Seed potato can accumulate and transmit many reducing yield diseases such as potato virus X, potato virus Y, potato virus S, potato leaf roll virus, potato spindle tuber viroid, blackleg, bacterial ring rot, Fusarium wilt, Verticillium wilt. Some of these diseases may reduce the yields up to 90% varying by cultivar, virus strain and the length of growing season (Crissman, 1989; Mellor and Stace-Smith, 1977; Salazar, 2003; Simakov et al., 2008; Winch, 2006). Potato leaf roll virus, potato virus Y frequently reduce tuber yield by 50-80% (de Bokx, 1972). Singh et al. (1971) reported that 64% yield reduction can be caused by potato spindle tuber.

Degeneration of the seed tubers is reduced or prevented by regularly injecting new disease-free plant material in seed potato production (Rolot and Seutin, 1999). In seed potato programmes depending on clonal selection, 8-10 years of production are required before commercial quantities are available. In many potato-growing countries it is not possible to keep the seed potatoes healthy for such a long period due to the phytosanitary conditions. In such countries, quality seed potato is produced by making use of rapid multiplication methods allowing the production of commercial quantities in 3-4 years, or by importing high seed potato quality (van Loon, 2007). The seed potato quality can be influenced itself by production method and individual farmers. Thus, high quality seed potato production is still the main constraint matter in most potato growing countries and major efforts should be made to produce high quality seed tubers (Ojha et al., 2000; Salazar, 2003; Struik and Wiersema, 1999).

#### **1.4. Limitations facing seed potato production**

The certified seed potato is the most expensive input accounting for 40-50% of the total production costs (Kaguongo et al., 2008). Thus, most farmers in developing countries depend on non-certified seed potato sources including farm-saved and local markets (Muthoni et al., 2010). This leads to the use of poor quality seed potato and often accelerates the spread of seed-borne diseases (Mbiyu et al., 2012). Recently, much attention has been paid to rapid multiplication systems to provide large quantities of plantlets, microtubers and minitubers of high quality (Avila et al., 1996; Otroshy and Struik, 2006). Involving tissue culture techniques in seed potato production will allow the growers to produce disease-free plants for further propagation in less space, low costs and higher flexibility of production scheduling (Roca et al., 1978; Struik and Wiersema, 1999; Wang and Hu, 1982). Moreover, improvement of the culture systems to further shoot multiplication and microtuber production will be useful in this respect. Although tissue culture techniques have been improved, the availability of disease-free stocks production, the current seed potato production methods are only able to supply a limited number of microtubers and minitubers per plant. Strategies involving the mass production of high quality plantlets and transferring them into the field for seed tuber production are required to make them successful. This is due to the logistical difficulties of producing the required number of plants, the weakness of transplants during field establishment, labour intensive procedures and low productivity to produce the first generation of tubers.

In vitro, several types of semi-solid media in various cultivation vessels have been used for microtuber production (Donnelly et al., 2003). In addition, liquid cultures (Estrada et al., 1986) and different temporary immersion techniques (Kämäräinen-Karppinen et al., 2010) are used as well. Most systems currently used for plantlets and microtuber productions are still less competitive and not economically viable when compared with in vitro rapid shoot multiplication. Furthermore, in vitro propagation suffers from the following main problems (Dobranszki et al., 2008; Lommen and Struik, 1995; Nhut et al., 2006; Piao et al., 2003; Struik and Lommen, 1990; Struik and Wiersema, 1999):

- Production limitations associated with the components of the culture environment and culture systems
- Current production systems require a large number of small containers, use semi-solid media and aseptic division of plant tissues by hand.
- In vitro plantlets should be transferred to fresh media after subcultures of 4-6 weeks, due to exhaustion of the nutrients in the medium and continuous tissue growth, which is rapidly limited by the size of the culture container.
- Very low multiplication rate for microtuber, one microtuber or less than one per plantlet
- Less uniform and small microtuber size (less than 1 g in fresh mass) that limits the direct transplanting to the field conditions.
- High production costs generally limit the commercial use of the in vitro plantlets and microtubers.

Minituber production is applied in substrate culture as the traditional production method of the first ex vitro generation and, for the past 20 years, has been applied in aeroponics as well. Ritter et al. (2001) reported that an aeroponic system has the advantage to harvest the tubers when they have the appropriate size, hereby improving the minituber production. Despite more than 20 years of seed potato production using an aeroponic system, only little scientific information is available. Optimization of seed potato production using an aeroponic system is still required. In greenhouses, the aeroponic culture system has the potential to raise plant quality and multiplication rate of the first field generation (Nichols, 2003). The current minituber production methods are also not efficient as a result to the following weaknesses (Nichols, 2005)

- The low propagation rate using the traditional methods. One potato plant will generate from 4 to 8 minitubers after a 3-4 months growing period in peat moss.
- The weakness of the transplants during field establishment
- Production methods of the first generation seed potatoes are based on high-density plantations stimulate exposure the crop to disease. This results in low quality, highly labour intensive and high costs.
- High susceptibility of potato to viral, bacterial and fungal diseases. Moreover, the risk of these diseases increases with each multiplication in the field. This results with frequently deteriorates of yield tuber production potential.
- The cost of greenhouse production of minitubers from whole in vitro plantlets limits the amount of per-basic stock increases.
- The minitubers produced are smaller than ideal to be propagated in the field
- Limitation of the suitable seed producing areas availability regarding seed potato production regulations (Wattimena et al., 1983)

## **2. Literature review**

### **2.1. Potato - botanical characteristic and growing conditions**

The potato's story, from gathering wild plants to cultivation, started early in the human colonization of the Americas about 8000 years ago, at 3800 m above sea level in the Andes mountain, on the border between Bolivia and Peru (Bradshaw, 2010; FAO, 2008; Moseley, 2001; Salaman and Hawkes, 1985). Potato were probably first introduced from South America into the Canary Islands around 1562 and from there to mainland Europe. The first documentation of cultivated potatoes was in Spain in 1573 in the archives of the Hospital de La Sangre in Seville (Hawkes and Francisco-Ortega, 1992). In the late 17th century, they were transported from Europe to Asia. The second part of the 18th century witnessed more widely spread of potato cultivation in the world (AgroAtlas, 2012; Schwanitz, 1966). In fact, what we know as "the potato" contains just a section of the genetic diversity found in the seven known potato species all still grown in the Andes today (Hawkes, 1990). Furthermore, the European Cultivated Potato Database (Europlant, 2013) currently has informations of about 4136 cultivars from more than 102 countries worldwide stored in its databasae (Bradshaw, 2010; Pieterse and Hils, 2009).

### 2.1.1. Botanical description

The potato is an herbaceous annual that grows up to 100 cm tall. Plants from in vitro or true potato seeds usually have only one stem (Figure 1). Throughout plant growth, the plant leaves manufacture starch that is transferred to the ends of its underground stems called stolons to form a few or as many as 20 tubers close to the soil surface. At the end of the growing season, the leaves and stems die. The tubers then serve as later plant re-growth and reproduction. Each tuber has from 2 to 10 buds or eyes which generate shoots when conditions are once more favourable. Eyes are concentrated near the apical end of the tuber, with fewer near the stolon or basal end. Eye number and distribution are characteristic to the cultivar. On some late maturing cultivars, stolon formation and growth may extend well beyond flowering, but late forming tubers seldom reach marketable size. Below the tuber skin is the cortex, followed by the vascular system. The storage cells are contained within the vascular system which increases in number and size causing the tuber to become larger (Bains, 2003; Hoque, 2010; Huaman, 1986; Plissey, 2003).

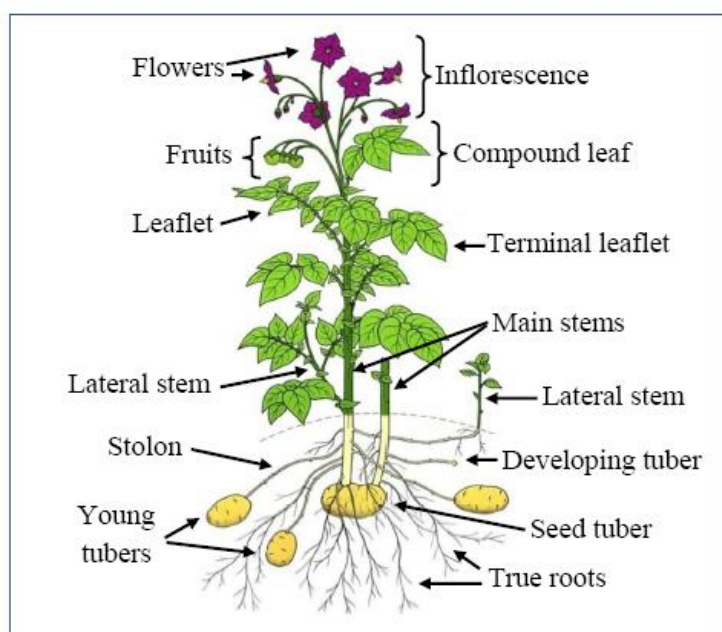


Figure 1. Morphology of potato plant developing from seed tuber, adapted from (CIP, 2012; Struik and Wiersema, 1999)

### 2.1.2. Growing conditions and growth stages

Growth and quality of potatoes are influenced by uncontrollable and controllable factors. The uncontrollable factors are length of growing season, air and soil temperatures,

light intensity and duration, humidity and wind. Other factors can be controlled by the grower as cultivar, size of mother seed tubers, seed-piece types, planting operation, plant density, moisture, nutrition, pest management, planting and harvest date. Only when all factors are at optimum levels can the most profitable yields of quality potatoes be attained. Potato is grown as a major crop in countries with very large populations, in diverse climatologically zones including temperate, tropical and sub-tropical regions, lowlands as well as highlands (Govindakrishnan and Haverkort, 2006; Struik and Wiersema, 1999).

For most cultivars, the maximum altitude is about 3000 m, though some Andean cultivars grow best at 2000-4000 m (Hawkes and Francisco-Ortega, 1992; Seabrook, 2005; Tarn et al., 2010; Winch, 2006). Short days generally induce tubers in potatoes, although many modern cultivars can initiate tuberization in the long days of north temperate regions. The higher the temperature, the more the aerial parts develop at the expense of tuber development (Winch, 2006). The potato is grown anywhere it is neither too hot nor too cold with ideally average daily temperature between 5-21°C and sufficient water from rain or irrigation. Very little sprout elongation occurs at 6°C and is maximized at about 18°C. The optimum soil temperature for initiating tubers is 16-19°C. Yields are highest when average day temperature is about 21°C. At lower night temperatures, respiration is slowed which enhances storage of starch in the tubers.

Whereas some cultivars can produce a small yield with only precipitation of 350 mm, the optimum precipitation is about 250 mm per week in the growing season. Drought can be disastrous for potatoes, especially if this occurs when the tubers should be bulking up (Winch, 2006). The amount of water needed by potatoes varies by soil type, temperature, humidity, air movement, plant density, cultivar and cultivation practices. The number of tubers that achieve maturity is related to available moisture and nutrient levels. Maintaining the available soil water capacity above 65% has been shown to produce high tuber set when compared to lower moisture levels (Bains, 2003).

Potato tubers are normally dormant for at least six weeks, and up to about 10 weeks. Sometimes after this they may begin to develop sprouts indicating that they are ready for planting. They are moderately susceptible to saline soils. About 2.5-4.5 tonnes of seed potato are required to cultivate 1 hectare with a planting distance of 20-30 cm between plants and 70-120 cm between rows and cultivation depth of 5-15 cm depending on seed tuber size. Potato plants should be earthed up or ridged up as they grown to improve the tuber

development and to prevent the upper tubers from turning green (solanine) when exposed to light and also to reduce the build-up of blight disease (Winch, 2006). Soil should be well drained with an optimum pH of 5.5-6. Potato plants do not normally need lime, but do need a good supply of nutrients, manure being particularly beneficial. The amount of nutrients required for the potato crop is determined by yield potential, plant spacing, soil type, irrigation and length of growing season. Fertiliser is commonly used, up to 220, 250 and 300 kg per ha NPK, respectively (Winch, 2006). If any mineral element becomes limiting, major yield capacity can be decreased (Plissey, 2003; Winch, 2006).

Researchers have identified potato plant growth into five stages. Knowledge of these growth stages helps the grower to understand the management actions that improves yield and quality potential (Bains, 2003; Plissey, 2003):

- **Sprout development and emergence:** Sprouts develop on seed tubers and grow upward to emerge. Development lasts from 20 to 30 days depending on environment and varietal characteristics. The seedling plant is very susceptible to soil and seed tuber-borne pathogens and environmental stresses at this stage. Seed treatment and ideal soil conditions encourage rapid emergence and protect the seedling plant.
- **Vegetative growth:** All vegetative parts of the plants are formed at this stage which lasts until tubers start to develop at the stolons tips. The stage lasts from 30-70 days depending on planting date, soil temperature and other environmental factors, tuber physiological age, and cultivars. Environmental stress during this stage can severely affect tuber initiation, final yield and quality potential and also may encourage pathogens.
- **Tuber initiation:** Tubers start to develop at the tips of stolons, but are not yet rapidly enlarging. The initiation of tubers is controlled by growth regulating hormones produced by the plant and is characterized by active cell division. Most tubers of marketable size are initiated during this period. This growth stage lasts from 10-15 days, generally starts 45-65 days after planting and usually ends with the onset of early flowering.
- **Tuber bulking:** During this stage, tuber cells expand as the result of accumulation of water, nutrients and carbohydrates through the plant supply system. If none of the