CHAPTER 2

LITERATURE REVIEW

Although many refer to bananas as a tree, in actuality it is not a tree but rather "a monocotyledonous, herbaceous, evergreen, perennial" (Robinson, 2010). Herbaceous refers to fact that it dies after its fruiting season while perennial indicates that new off-shoots, also known as suckers, which are derived from the same underground base, replace the mother plant. One stalk produces between 5-20 suckers in its lifetime of 12-14 months (Singh, 2011).

Figure 1: Banana morphology: (a) pseudostem, 'trunk' of banana plant; (b) leaf sheath, base part of the leaf, making up the pseudostem; (c) sword sucker, young plant produced through vegetative propagation; (d) rhizome/corm, underground food-storage stem; (e) banana mat, group of bananas with interconnected corms; (f) transition zone, area where leaf sheath connects to corm; and (g) apical meristem, central growing point.

Morphologically, plants develop two types of suckers: ‘sword suckers’ or ‘water suckers’ (Fig. 2). They can be distinguished from each other most easily by the size of their leaves while in early development stages. While water suckers, from early on, have broad leaves, the leaves of sword suckers are very narrow, bract-like structures. As the sucker grows, the leaves begin to widen, enabling the sucker to produce more of its own food through photosynthesis, lowering dependence on the mother plant for nutrients (Blomme, 2008). Sword suckers have a strong, wide rhizome connection to the mother plant, making it unnecessary to produce leaves while young, hence the narrow, bract-like structures. Studies show complete independence from the mother stalk occurs when the first fully developed leaf is emitted (Robinson, 2010). Water suckers have a weaker connection to the mother plant, preventing it from developing into a strong, vigorous plant (Robinson, 2010). It is generally believed that sword suckers develop from buds deeper on the mat, while water suckers develop from much shallower buds. However, when plants are damaged or pruning is practiced, broad-leafed suckers emerge (Simmonds, 1966). Older mats tend to produce more water suckers than younger mats. Sword suckers are more desirable for use in propagation so it is important for farmers to maintain their mats, replanting every 7-10 years (Robinson, 2010).

While technically bananas derive from a rhizome system, the term “corm” is more commonly used because of its implied erect underground stem rather than the horizontal growth that is often associated with rhizomes (Simmonds, 1966). Thus, for this study, the term “corm” is used. These corms are important storage units,

consisting of mainly starchy parenchyma, for supporting the growth of the fruit and developing suckers (Robinson, 2010).

(a)  (b) 

Figure 2: Early sucker development produces two morphologically different types of suckers: (a) sword suckers and (b) water suckers.

Genus *Musa* has been divided into sections, Eumusa and Australimusa. In the Eumusa section has been further divided in Eumusa 1 (*Musa acuminata*) and Eumusa 2 (*Musa balbisiana*) amongst others. Bananas in the Eumusa sections contain 22, 33, or 44 chromosomes. Thus cultivars are referred to as diploid, triploid or tetraploid. Of the known existing clones, about half are triploids (ex. cv. 'Gros Michel and 'Kluai Nam wa'), covering more than 100 times more planted area than diploids (ex. 'Pisang Amon Putih') to which most of the remaining clones belong. There are only a few known tetraploids (ex. cv. 'Goldfinger') mostly resulting from breeding. Knowing the ploidy is necessary for correct classification and is done through a cytological chromosome count. Morphologically, diploids and tetraploids are larger and more vigorous, with thicker leaves than diploids (Robinson, 2010).

Eumusa bananas are crosses between *M. acuminata* and *M. balbisiana*. Simmonds and Shepherd (1955) created a key using major morphological characteristics such as petiole form, as well as size and shape of male bud and fruit ovules, to distinguish between the two species, allowing them to determine which genes are dominant. The letters, 'A' and 'B' were given to represent the presence of these species.

'Kluai nam wa' is a cultivar grown throughout Thailand, known for its hardiness and vigor. It is a cross between *M. acuminata* x *M. balbisiana* hybrids and classified as a triploid (ABB) due to the predominance of the *M. balbisiana* genes. This cultivar can also be found in Northeastern India and Malaysia, where it is known as 'Pisang Awak', which is the name more commonly found in scientific literature (Singh, 2011), where it is also found. In other locations it has clearly been recently introduced.

In spite of this cultivar's widespread presence throughout the tropics, it has the greatest importance in Thailand, the only area known to produce multiple somatic mutations: 'Kluai nam wa daeng' which has pinkish flesh, 'Kluai nam wa khao' with more waxy fruit, and 'Kluai nam wa kom', a dwarfed mutant (Simmonds, 1966). Somatic mutations play an important role in the diversity of banana cultivars (Robinson, 2010). The cultivar is partially fertile, which means that some fruits may contain seeds, sometimes causing the fruit to be inedible if pollinated by wild diploids (Robinson, 2010). Altogether, the members of this group show significant

characteristics that are descended from its *M. balbisiana* parentage, such as high vigor and drought resistance, immunity to yellow Sigatoka (*Mycosphaerella musicola*) and starchy pulp (Simmonds, 1966). Drought tolerance, in particular, has enabled this cultivar to thrive in Northern Thailand during the long dry season.

Bananas are very adaptable plants. Geographically, bananas grow best between latitude 20ºN and 20ºS, where there are predominantly tropical conditions. For growth and flower production, optimal temperatures are between 22 ºC and 31 ºC with rainfall of 2000-2500 mm, spread evenly throughout the year (Robinson, 2010). Although these are ideal conditions, bananas can also grow in the subtropics, between 20º and 30º north or south of the equator with fluctuating temperatures, and low, poorly distributed rainfall.

Temperature is the determining factor for growth rate of bananas when water is not limited. It is generally agreed that 27 ºC is the ideal temperature for optimal growth and flower initiation while 31 ºC is optimal for leaf emergence. Below 16 ºC, new leaf emergence stops and below 14 ºC, growth stops (dry matter assimilation). Extreme high temperatures can also be a problem, with growth stopping above 38 ºC, causing heat stress (Robinson, 2010). Rainfall is the next limiting factor, with bananas having high water requirements, 2000-2500 mm distributed evenly throughout the year. In areas without regular rainfall, bananas benefit greatly from supplementary irrigation in order to maintain growth and development.

Northern Thailand is just south of 20ºN latitude, where the climate is just on the border between tropical and subtropical. Temperatures average around 26 ºC, but range from 11 ºC in the cold season (November through February) to 37 ºC in the hot season (March through May). Total rainfall is under 1300 mm per year, the majority of which falls during the wet season (May to October) while the dry season (November to May) yields very little rain. Although Northern Thailand does not have ideal conditions for growing bananas, especially as few farmers can afford irrigation systems, using appropriate cultivars, like 'Kluai nam wa', with its drought resistance, makes it possible.

In addition to temperature and water, pests and diseases have been a major production constraint of bananas in Southeast Asia. The most common banana production system in Southeast Asia is the backyard garden system (Molina, 1998) which has served as a "breeding foci for pests and diseases, some of which will easily find their way into the subsistence systems with which they usually share boundaries" (Karamura, 1998). Fusarium wilt (*F. oxysporum* f. sp. *Cubense*), also called Panama disease, is considered the most important banana disease in Thailand. It is a soil-born fungus, that enters through wounded tissue when the plant is cut. It spreads through the xylem system, blocking the vessels and causing symptoms similar to drought. Currently, there are no economically practical means of controlling the disease once a field has been infected. Banana weevil borers (Curculionidae) are significant pests, able to cause loss in yields of up to 90% or more (Azam, 2010; Sadik, 2010). One specied of weevil borers, *Cosmopolites sordidus* Germar, causes damage mainly by boring into the banana corm while another, *Odoiporus longicollis* Olivier, is a stem

borer. They both cause the most damage during the larval stages while adults emerge and live in the soil. Methods of control include cultural methods such as destroying the sheltering and feeding places of the adult weevils, cutting and scattering cut pseudostems to allow for rapid drying, good weed control and planting unifested material (Simmonds, 1966) and injecting pesticides, organophosphates such as monocrotophus, into stems. Nematodes also cause significant damage. 'Kluai nam wa' is particularly susceptible to the endoparasite *Pratylenchus coffeae*, a lesion nematode that causes damage to roots. Some cultural practices are used to control nematodes, using organic mulching (Robinson, 2010), as well as nematicides are sometime used. The best way to protect bananas from pests and diseases is to have access to disease and pest-free planting material, reducing infection to only what is introduced from outside. The demand from farmers and development organizations for disease and pest free material and cultivars with improved resistance continues to increase.

Throughout the developing world, as well as much of the developed world, bananas have traditionally been propagated vegetatively through the removal of side sword suckers from the base of the main stem, which are then transplanted into the field. Although this method is inexpensive, it is not ideal for a number of reasons. First, the time it takes for the banana clump to grow large enough to produce suckers and reach its full production level limits the rate at which suckers can be transplanted. Due to apical dominance of the mother plant, natural regeneration is very slow. In a stalk's lifetime of 12-14 months, it will only produce 5-20 suckers (Singh, 2011). Secondly, vegetative propagation relies on seasonality. During the wet and warm

seasons, bananas grow much faster than during the dry and cold seasons, making the warm season an ideal time to propagate. Thirdly, there is a lack of uniformity of the suckers for a homogenous and predictable fruiting period. Lastly, vegetative planting often results in the spread of disease inherent in the suckers (IAEA, 2004).

Because of the problems with vegetative propagation, commercial banana multiplication is primarily done through tissue culture. "Plant tissue culture refers to the growing and multiplication of cells, tissues and organs of plants on defined solid or liquid media under aseptic and controlled environment" (Ahloowalia, 2004). Tissue culture has many advantages, some of which include: pest and disease-free plantlets, uniform size, ensured variety, and fast, year-round multiplication. There are disadvantages, however, especially in areas of the developing world. Tissue culture requires capital, labor and energy. Although labor is cheap in many developing countries, trained personnel and equipment are not as easily available. In addition, energy, such as electricity and clean water, are expensive. Tissue culture technology depends on pre-determined day-temperature, day-length, and relative humidity, and each must be controlled throughout the propagation process (IAEA, 2004).

In contrast to tissue culture, there are a number of different methods of macropropagation being done around the world. Most macropropagation methods use some technique to break the apical dominance of the banana in order to promote axillary growth. Apical dominance influences the entire growth of the plant. One part of this influence is the hormonal inhibition of axillary buds, through the production of auxins. Disrupting the apical meristem suppresses the production of

auxin, thereby allowing the growth of the axillary buds (Arinaitwe, 1999). In other words, with regard to bananas, apical dominance from the mother stalk, while alive, influences control over suckers, regulating the number of sucker formation.

Much work, primarily with tissue culture, has been done using different plant growth regulators, natural and synthetic, to try to disrupt apical dominance, thereby releasing side shoots and axillary buds. One important class of growth regulators used is the cytokinins. Cytokinins are "substances which, in combination with auxin, stimulate cell division in plants and which interact with auxin in determining the direction which differentiation of cells takes" (Wareing, 1970), such as meristematic or undifferentiated cells. They are found naturally, but can also be created synthetically. One such synthetically produced cytokinin is 6-benzylaminopurine (BA) ($C_{12}H_{11}N_5$) (Fig. 3). "Cytokinins such as benzylaminopurine are generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana" (Jafari, 2011). Cytokinins have been reported to have greater effectiveness than other growth regulators in inducing shoot tip cultures in different banana cultivars (Buah, 2010).

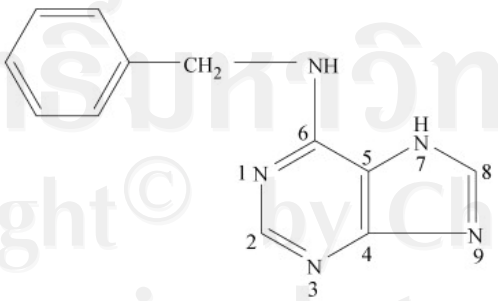

Figure 3: Molecular structure of 6-benzylaminopurine (Li,2009).

Another naturally occurring cytokinin can be found in coconut water, which has long been used in tissue culture work and much research has been done to analyze the chemical properties contained therein. Water from mature green coconuts has been said to contain a more effective stimulant in plant media than from ripe fruits; however this is not fully agreed upon (Thorpe, 2008). Due to the considerable variability of the chemical composition in coconuts, it is difficult to determine the presence and quantities of cytokinins. Research suggests that there are cytokinins such as *trans*-zeatin-O-glucoside ($C_{16}H_{23}N_5O_6$) and dihydrozeatin-O-glucoside ($C_{16}H_{25}N_5O_6$) found in young, green coconuts (Yong, 2009). Other studies have found these cytokins in mature coconuts as well (Ge, 2004).

A variety of research has been done with the macropropagation of bananas, using different mechanical and chemical methods to block or disrupt apical dominance. In Cameroon, CARBAP (Centre Africain de Recherches sur Bananiers et Plantains) has experimented with "plants produced from stem fragments" (PIF - Plants Issus des Fragments de tige). PIF is a technique in which a crosswise incision through the apical meristem of a corm, 5 to 40 cm in height, was made in order to break apical dominance. After a period of drying, to further stress the corm and help induce lateral growth, the corm was placed in a moist bed of sawdust until plantlets emerged. Typically, this method could produce 20-100 plantlets in 4-5 months and has been used to successfully propagate more than 40 cultivars of bananas (Boss, 2008). In Ghana, Dr. J. K. Osei of the Kade Agricultural Research Station at the University of Ghana in Legon experimented with a rapid field multiplication (in situ) of plantain using BA and coconut water injected into the base of young sword

suckers, 25 to 45 cm, and then sprouted in moist sawdust. Osei found comparable results for numbers of plantlets produced using either BA or coconut water. This technique resulted in an average of 10-15 plantlets produced from one corm in 18 weeks (Osei, 2005). Another commonly used technique is the split-corm method, in which the corm is cut into 4 sections, dipped into fungicide, and placed into sawdust. This technique has resulted in four new plantlets from one corm (Dzomeku, 2000). In San Pedro Sula, Honduras, Dr. Phil Rowe experimented with an in-field technique for banana multiplication. Mature banana plants (cultivars were not specified) were bent over at the top, the leaves were removed, a thin, flat stake was driven into the center of the pseudostem, and fertilizer scattered around the base. Within four months, 10-15 suckers were produced per plant (Price, 1999). In Columbia, Manzur Macias, a professor in Manizales, experimented with a technique similar to the PIF technique but performed in the field. In this technique, young suckers, cv. 'FHIA 20' plantain, were exposed, the pseudostem removed above the rhizome collar, and the apical meristem cut out. The remaining fragment was then cut with crosswise incisions, filled with BA and covered with compost. Taken to the fourth generation in 8 months, this technique is estimated to attain 156 plantlets from one corm (Macias, 2001).

Sawdust has typically been the material used for the initiation media in the planting beds, but other materials have also been evaluated. In a study done by Baiyeri (2005), rice husks and sawdust were evaluated as *Musa* sucker plant corm initiation media using five genotypes. The study looked at date of plantlet emergence, root growth of plantlets, number of plantlets excised, and survival rate of excised

plantlets. The study found that second and third plantlets emerged significantly earlier in rice husks, but there was higher survival rates in the sawdust. The study concluded that either rice husks or sawdust could be used with comparable results for generating plantlets from the corms of *Musa* species.

Singh (2011) writes that vegetatively propagated crops have not received much support on a national level, although there have been national seed policies created in most countries in order to advance quality seed production. He says that crops like bananas are often "marginalized in terms of support for quality production, farmer awareness and education", arguing the importance of creating a better awareness, for both farmers and industry, of quality vegetatively propagated plants (suckers, macropropagated plantlets and tissue culture plants). Even in areas with large-scale production of banana planting material, a good understanding of quality standards, healthy, pest and disease-free plants, is still lacking.

Although a number of macropropagation experiments have been performed for *Musa* species, there are few that have compared several different methods for obtaining the maximum number of plantlets per corm. In addition, little to no research using macropropagation methods has been done in Northern Thailand to determine appropriate methods and materials, and other studies have focused primarily on the propagation of plantain and commercial desert bananas. No literature was found evaluating the cultivar 'Kluai Nam Wa' for its adaptability to be used in macropropagation methods.