Introduction

Green manure is a type of crop that is grown to be turned under to add nutrients and organic matter to the soil. Green manure usually performs multiple functions, that include soil improvement and soil protection. Green manure increases the organic matter in the soil, thereby improves water retention, aeration and soil characteristics. More over, leguminous crop residues serve as a rich source of nitrogen because they contain nitrogen-fixing symbiotic bacteria, rhizobium, in root nodules that fix atmospheric nitrogen in a form that plant can use. The nitrogen benefit from green manure to the following crops is well documented and has been realized for decades. Several leguminous plants have been used to maintain soil fertility in areas where chemical fertilizers are costly or to substitute chemical fertilizers in organic farming. They provide large quantities of nitrogen and add many tons of organic matter to the soil (Bunch,1998). The amount of nitrogen and total biomass available from legumes depends on the species of legumes grown in appropriate areas. Conditions that encourage good biomass production include optimum soil nutrient levels, soil pH, good nodulation and adequate soil moisture. The portion of nitrogen in green manure plant available to a following crop is usually about 40-60% of total amont contained in the legumes (http://attra.ncat. org/attra-pub/cove crop.htm). The effectiveness of green manure as a source of nitrogen depends upon good synchronization of nitrogen release with nitrogen uptake by the subsequent crop. Sunn hemp (*Crotolaria juncea*) has been widely accepted as a versatile green manure, especially in semi-arid agricultural area. It can be planted in rotation with vegetables, ornamentals and others to add nitrogen, organic matter, suppress weeds, control erosion and reduce root-knot nematode. In 60 days, it can produce 145 pounds of nitrogen and 3 tons of dry matter per acre (Bunch,1998). Jackbean (*Canavalia ensiformis*), a bushy growth variety, can be used as green manure, intercropped with corn, sorghum or millet and under orchard tree.

Lablab bean (*Lablab purpureus*) starts flowering after 3 months and provides 110 t/ha of above-ground organic matter (wet weight). It can be planted towards the end of agricultural cycle. *Sesbania rostrata* has been introduced as green manure crop in rainy season rice growing system. The legume is incorporated into the soil after 55-60 days of broadcasting with the biomass of 2-4 t/rai or 12-20 kg available nitrogen (http://www.ldd. go.th). Cowpea (*Vigna unguiculata*) has a great potential as green manure due to its rapid nitrogen accumulation and efficiency of N_2 fixation, it is widely grown in semi-arid areas for green manure crop and fodder crop. A 6-week old cowpea can accumulate 12-38 kg/rai of nitrogen and can be incorporated into the soil 30-45 days after sowing. These leguminous plant utilizations are not only for green manure but also for fodder. Two in one is actually found in species of legumes. After harvesting, grains are used for feed and the plant residues are utilized as green manure to maintain soil fertility. Of these, mungbean and soybean are excellent examples. After seed harvesting, plant residues left in the field are turned under as green manure. Mungbean production gave seed yield of 317.76 kg/rai (Ngo Thi Hong Lien,1992) and biomass yield of 1,440 kg/rai (Jeevananthan,1998). The quantity of upper-ground and under-ground residues which is able to improve soil quality is in the average of 17.92 kgN/rai. This amount is equivalent to 6.88 kgN/rai which is adequade for growth of subsequent rice crop (Sharma,1994). In the north of

Thailand, rice-soybean is the main sequential cropping system in the irrigated lowland paddy field. Traditionally, lowland farmers plant rainy season rice with minimum input for subsistance. The biological nitrogen fixing soybean provides average yield of 1.25 t/ha with is able to maintain rice yield at 2.8-3 t/ha without chemical fertilizer (http://www.agroecology.org/cases/ green manure.htm). For the other subsequent crops, addition of soybean green manure increased sugar yield at the average of 80%.

Vegetable soybean green manure can provide N to subsequent crops and organically-applied N is retained in the soil, but is not always available at the right time and in sufficient quantity for crop growth. The release of organic N usually occurs during the process of decomposition of organic matter. Hence, it is difficult to manage a system both for maintenance of N or increase of organic matter levels in the soil. Green manures can be a source of organic N, but have not been found to significantly increase the levels of organic matter in the soil (Macrae and Mehuys,1985). Boone(1990) measured green manure immobilization of 54 kgN/ha over the course of the season in a corn field, which received 235 kgN/ha in the form of chemical fertilizer. Mineralization can occur more readily from some portions of the OM than from the other. Organic matter is commonly separated into a light fraction and heavy fraction. The light fraction represents lower density material; it usually consists of plant matter that has yet to be completely broken down(Sollins *et al*.,1983). Light fraction is typically more responsive to changes in management such as additions of organic amendments or rotations which add greater quantities of crop residues to the soil (Barrios *et al*.,1996; Janzen *et al*.,1992). The heavy fraction is denser material that is often associated with clay mineral particles ; it tends to be less labile than the light fraction and is sometimes associated with aggregate stability (Roberson *et al*.,1995). Since the light fraction is the more labile portion of the organic matter, it would be expected that more N mineralization would occur from that portion. Nitrogen mineralization from green manures have led to renewed interest in legumes as a N source for most of the crops. The techniques have been used to assess the impact of the N contained by the legume on subsequent crops, i.e., measurement of plant N uptake by applying isotopically-labelled legume residues and measuring the level of that isotope in the crop. These techniques have the adventage of measuring directly the N from the green manure which is available to the crop. Norman et al.,(1990) applied ^{15}N-labelled soybean residues to plots cropped with rice. They found that 52% of residue N was mineralized from soybean and 11% of residue N was taken up by the rice. Mahler and Hemamda(1993) measured soil inorganic N by addition of varying levels of Austrian pea residue. They found that sufficient N was released by mineralization of the highest rate of pea residue (30 kg/ha) to provide 51-63 kg $ha^{-1}$of N for the subsequent wheat crop.

Vegetable production systems in the tropics and elsewhere are mostly intensive, because vegetables are high-value cash crops. High fertilizer rates are commonly applied to maximize yields. There is an urgent need for the implementation of alternative methods to reduce excessive use of mineral fertilizers and to improve soil fertility and vegetable quality (Mahler and Hemamda,1993). Therefore, this study focused on the quantity of ^{15}N releasing from legume after amendment and decomposition process, and the rate of ^{15}N uptake by kale.

The objectives of this research were : 1) to analyse the quantities and properties of nitrogen composition of vegetable soybean from air, chemical fertilizer, and soil; 2) to monitor the quantity of NO_3^--N and NH_4^+-N release by vegetable soybean green manure during decomposition; 3) to find out the quantity of nitrogen uptake in kale by using isotope ^{15}N dillution technique and 4) to find out the appropriate quantity of vegetable soybean biomass incorporated into the soil and the decomposition of nitrogen available for the growth and development of kale.

This research work consists of three experiments 1) Experiment 1, deals with the study of the efficiency of N_2-fixation in vegetable soybean; 2) Experiment 2, deals with the effects of vegetable soybean decomposition on the quantity of N-uptake by kale and 3) Experiment 3, deals with the effects of vegetable soybean decomposition and the suitable rate of soybean biomass on the yield of kale.

Literature Review

The origin of vegetable soybeans

Vegetable soybean is believed to be prevailed in East Asia such as China, Manchuria, Japan and Korea (Mongkonsil, 2004). Also, vegetable soybean is grown in most parts of Thailand, particularly in the north such as Chiang Mai, Chiang Rai and Lampang. The popular cultivars in Thailand consist of Chiang Mai 1, AGS 292 or Khampangsaen 292, and variety #75 (number 75) which was used in this study. The agronomic characters of variety #75 contain white flower, white hair, 28-32 days to the first flowering and the crop can be harvested at 65-68 days after sowing or between the R6 and R7 of growth stage (Shanmugasundaram,1979). In Thailand, 7,962 rai of vegetable soybean were grown in 2001, with the production of 10,306.05 tons (DOAE, 2002). The vegetable soybean can be exported to Japan in the form of quick-frozen green pods. Thailand ranked 3rd as vegetable soybean exporter to Japan after China and Taiwan, respectively. In 2003, Japan imported 11,285 tons of vegetable soybean from Thailand which was accounted for 784 million Baht (Sriwatanapong , 2004). The average yield of vegetable soybeans was about 750 kg./rai, and the marketable price of the fresh green pods was 13-13.50 Baht/kg. (Chiang Mai Frozen Food Co, 2005) or equivalent to 9,750 -10,125 Baht/rai. Vegetable soybean No.75 was imported from Taiwan. The importance of soybean has been realized throughout the world and its production is increasing from 1974 to 1978. World production increased by 8% per year, whereas the world demand increased by 8 % per year (Singh,1983). Soybean can fix between 14 and 300 kgN/ha depending on the yield potential and availability of N and genetic interaction between the host genotype and the Rhizobium japonicum strain. However, nodulation and symbiotic N fixation are highly influenced by the N level present in the soil (Cassman *et al*.,1981; La Rue *et al*.,1981; Singleton *et al*.,1983).

Symbiotic Nitrogen Fixation in Vegetable Soybeans and Nodulation

Rhizobium is a kind of bacteria with rodlike shape, having 0.5-0.9 x 1.2-3.0 µ in lenght. Also, rhizobium can appear in club shape and many other shapes like X-, Y- and star (Waksman, 1952; Alexander, 1977). The life cycle of rhizobium can be concluded as follows:

1. Non-motile circular cell which prompts to transform itself to swim which can be found in neutral solution.

2. The non-motile circular cell expands itself, which can be found in the atmosphere of phosphate and some kinds of carbohydrate.

3. The cell reforms itself into an oval shape and establishing flagellum. At this stage, it is classified as swimmer which can move quickly.

4. The cell expands itself again into a small rod shape and moves slowly.

5. The cell appears in a long rod shape, establishing infection thread and enters the plant cells.

6. The cell splits itself by chromatin into round shape and then transforms itself as preswimmer.

The classifications of rhizobium can be done in several methods; morphological and physiological classification - classify by the forms and the amount of flagellas (Breed *et al*., 1957); classify by the production of acid or alkaline and the growth rates - the fast -

growing group tends to produce acid while the slow-growing group will produce alkaline (Norris,1965); numerical technique classification, it is the comparisons between the characters and qualifications of rhizobiums and then calculate the similarity percentage of the characters and the qualifications of those microorganisms (Graham, 1964); DNA classification (Deley and Russel, 1965; Gerald, 1971), and the most popular method is cross-inoculation group classification - the grouping of soybean species are classified by the capability of rhizobium by inducing N-fixation on the nodules of soybean roots (Burton, 1965). Soon after a legume begins to grow, special N-fixing bacteria that reside in the soil invade the tiny root hairs and multiply in large numbers. The legume roots, in reaction to this infection, form tumor-like swellings called nodules on the root surface. Bacteria inside the nodules absorb air from the soil and convert (fix) gaseous N into ammonia (NH_3). The association between the legume host plant and the nodule bacteria is mutually beneficial (symbiotic). The plant furnishes the necessary energy that enables the bacteria to fix gaseous N from the atmosphere and pass it on to the plant for use in producing protein. This partnership is known as symbiotic N fixation. Most, but not all, legumes have the capacity to fix N. The quantity of N fixed depends on several factors, such as (1) the kind of legume, (2) the effectiveness of the N-fixing bacteria, (3) the soil conditions including pH and N fertilizer, and (4) availability of necessary plant food such as carbohydrates, phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), molybdenum (Mo), copper (Cu), and boron (B), the greatest N_2 fixation is obtained in soils low in available N. Bacterium specificity and inoculum selection in vegetable soybeans legume plants only grow vigorously if they have functioning nodules, and this depends upon their roots encountering the appropriate bacteria strains in the soil. Bacteria involved in nodule formation and symbiotic N fixation belong to the genera Rhizobium and Bradyrhizobium. Rhizobium species are fast-growing, acid-producing, N-fixing bacteria associated with many temperate pasture legumes such as soybean. Bradyrhizobium species are slower-growing, alkaline-producing bacteria. Each legume selected for planting must be matched with sufficient quantity of compatible N-fixing bacteria in order to achieve an effective symbiotic N-fixing relationship. Closely-related legume species can be grouped according to their N-fixing-bacteria compatibility into cross-inoculation groups. In some instances, these groups have been used to designate the scientific names for the N-fixing bacteria; e.g., bacteria that nodulate soybeans by *Bradyrhizobium japonicum*. However, within some cross-inoculation groups, only certain bacteria strains are compatible with the particular legume being grown. Therefore, a producer must select the specific strain (inoculum type) that is compatible with the legume being grown. The study of nodulation that affected the capability of soybean N-fixation revealed that, the vegetative growth of the plant would have been stopped immediately once the plant approached R1 stage (determined from the first flowering at soybean nodes) (Pookpakdi , 2003). Effective nodulation takes place within four weeks of planting. Nodule size and shape vary among the different legumes. Visual sampling of the number of nodules and interior nodule color can indicate the status of N fixation in a legume plant. Nitrogen fixation in legumes is highly correlated with the content of a reddish pink pigment inside the nodules called leghemoglobin. A cross section of a functional nodule made with a pocket knife should reveal a pink to dark-red coloration, whereas a greenish color may indicate ineffective nodulation. Effective nodulation is generally indicated by vigorous growth of the legume itself and by improved growth and green color of grasses associated with forage legumes (Adjei *et al* .,2006). Legumes release compounds called flavonoids from their roots, which trigger the production of nod factors by the bacteria. When the nod factor is sensed by the root, a number of biochemical and morphological changes occurs: cell division is triggered in the root to create the nodule, and the root hair growth is redirected to wind around the bacteria multiple times until it fully encapsulates 1 or more bacteria. The bacteria encapsulated divide multiple times, forming a microcolony. From this microcolony, the bacteria enter the developing nodule through a structure called an infection thread, which grows through the root hair into the basal part of the epidermis cell, and onwards into the root cortex; they are then surrounded by a plant-derived membrane and differentiate into bacteroids that fix nitrogen. Nodulation is controlled by a variety of processes, both external (heat, acidic soils, drought, nitrate) and internal (autoregulation of nodulation, ethylene). Autoregulation of nodulation controls nodule numbers per plant through a systemic process involving the leaf. Leaf tissue senses the early nodulation events in the root through an unknown chemical signal, then restricts further nodule development in newly-developing root tissues. However, the proceed of nodulated crucial point was that, the amount of rhizobium had to be increased rapidly around the root hair of soybeans before entering the roots. The plant then released tryptophan so as to convert rhizobium to IAA substance. Then, the pubescence roots were bent by IAA reactions in order to form soybean nodules. Normally, there are 2 types of nodule formation; infection thread formation and non-infection thread formation. Providing infection thread formation, the pubescence roots will be shrunk and modified themselves as tubes covered by plasma membrane where rhizobium will be able to enter to the cortex layer. The rhizobium then pierces through the cell in order to constitute another cell envelope. Plasma lemma and the envelope then connect themselves to endoplasmic recticulum inside cytoplasm. For the second formation, the bacteria enters the plant roots via the nexuses of intercellular infection cells without forming infection thread. After entering the cortex layer, the bacteria then releases its enzyme to dissolve the cell wall and reproduce itself inside that cell and transform itself into bigger size, which is called "bacteroid". The inside cell contains a vacuole while mitochondria and amyloplast are resided around the cell. Then the endoplasmic reticulum will be split into many short membranes and vesicles. By the same time, the plant then establishes leghaemoglobin substance inside the envelope and cell membrane of bacteroid in order to control the amount of O_2 while the N-fixation is in process (Choonluchanon, 1998). The research of the effect of N-fertilizer rates together with rhizobium applications on soybean nodule weight disclosed that, the more applications of N-fertilizer ranging from 3 kgN/rai to 24 kgN/rai, the less nodule weight obtained ranging from 1.6 g/plant to 1.3 g/plant, (Land Development Regional Khon Kaen,1974). According to the study of the application of chemical fertilizer combined with rhizobium on soybean variety SJ.5, there was no significant difference of the yield between the application of rhizobium together with P-fertilizer at 9 kg.P_2O_5 and K-fertilizer at 6 kg.K_2O, and the combination of N-fertilizer at 12 kgN, P-fertilizer at 9 kg.P_2O_5 and K-fertilizer at 6 kg.K_2O. The yields of both treatments were 158 kg/rai, (DOA ,1992). The result showed that rhizobium could substitute N-fertilizer by 12 kgN.

Green manure

For vegetable soybean green manures to be considered as effective nitrogen source for horticultural crops, they must supply sufficient nitrogen and their nitrogen release must be in synchrony with vegetable's nitrogen demand. Green manure decomposition and subsequent nitrogen release depend largely on residue quality and quantity, soil moisture, temperature and specific soil factors such as texture, mineralogy and acidity, biological activity and the presence of other nutrients (Myers *et al*.,1994). Legume biomass and composition of plants of the same age varied between location and growing season (Thonnissen,1996), making it difficult to predict their decomposition when grown under different conditions. Residue decomposition can be governed to some extent by green manure placement incorporation with soil (Wilson and Haargrove,1986). In the southeast of the USA , the greatest nitrogen release from decomposing legumes occurred 2 to 5 weeks after cover crop killing in spring (Sarrantonio and Scott,1988), and early decline of mineral nitrogen level over the growing season (Ebelhar *et al*.,1984) leads to poor synchronization between nitrogen release and vegetable's nitrogen demand.

Vegetable production systems in the tropics and elsewhere are mostly intensive, because vegetables are high-value cash crops. High fertilizer rates are commonly applied to maximize yields. There is an urgent need for the implementation of alternative methods to reduce excessive use of mineral fertilizers and to improve soil fertility and vegetable quality (Thonnissen *et al*.,2000). Therefore, this study focused on the quantity of ^{15}N releasing from legume after amendment and decomposition process, and the rate of ^{15}N uptake of kale.

Laboratory studies that use ^{15}N labelled plant residues can trace N from residues and distinguish it from N that is mineralized from organic N already present in the soil samples. Ladd and Amato (1980) mixed medic material that had been labelled with ^{15}N into soil samples and allowed the material to decompose over the course of 130 days. At 34 days, 67% of the plant N was still in inorganic form. After that point, decomposition slowed. Norman *et al.*(1990) applied ^{15}N-labelled soybean residues to plot cropped with rice. They found that 38% of residue N was mineralized from soybean and 11% of residue N was taken up by the rice. Conversely, several studies have found N contribution from green manue without a consequent yield increase. Sarrantonio and Scott (1988) measured the soil nitrate status as well as the total N uptake by corn plants after a hairy vetch winter cover crop. They found that corn grown after hairy vetch had a higher rate of N uptake, but no higher yield. They attributed this lack of yield effect to N release from the vetch residue that occurred after the time when addition N would increase the plant N yield. Griffin and Hesterman (1991) tested the effect of several green manue treatments on subsequent potato crops. They concluded that the N from the residue was released too late in the development of the potatoes to provide any yield benefit.

Factors Affecting N Availability from Legume Green Manures.

There are a number of factors that affect the availability of green manure N to the subsequent crop:

1. Lower C:N ratio of green manure material is usually correlated with higher rates and total quantities of N mineralization. Norman *et al*.,(1990) found an inverse correlation between C:N ratio and mineralization in their study of soybean residue application to rice. At C:N ratios over a certain threshold (usually 25), N becomes immobilized rather than mineralized. Aulakh *et.al*.,(1991) found that vech residue (C:N 8) caused mineralization of N where soybean residues (C:N 43,39 and 82 respectively) all caused immobilization when incubated in soil samples.

2. Tillage regime and depth of incorporation also affect availability of green manure N to the following crop. Typically, more tillage and deeper incorporation cause higher rates and/or levels of N mineralization from the green manure. Aulakh *et al*.(1991) found an average of 51% of N in various crop residues mineralized when incorporated into soil samples, as compared with an average of 31% of residues left on the surface of the sample.

3. The timing of incorporation can also be quite important in determining the timing and level of N release from green manure. Wagger (1989) found that the benefits of later spring desiccation of a winter cover crop (allowing more time for spring growth) were not sufficient to justify the delayed corn planting that would be entailed.

4. The moisture content of the soil is a factor which affects green manure and which also has an effect on the mineralization of N from green manure. Aulakh *et al*., (1991) found that various plant residues decomposed more slowly in a wetter (90% water-filled pore space) soil pot than in a dry (60% water-filled pore space) treatment.

5. Soil texture can also affect the availability of N from green manure to the crop. Ladd (1981) applied ^{15}N-labelled medic material to wheat grown on three different soils. Recovery rate of the ^{15}N by the wheat and soil was 93.1% and 92.3 for a sandy loam and a sandy soil, respectively, compared to 87.7% in a heavy clay soil.

Vegetable green manures incorporated into the soil for organic matter decompose very quickly if the soil temperature is sufficiently high. Under normal summer weather conditions, 70% of the nitrogen present in organic form in crop residues becomes available for absorption by the next crop for a period of 10 weeks following incorporation. However, legumes release more mineral nitrogen than other crops when they decompose-an average of 5 kg/tonne of fresh biomass. The quantitative nitrogen requirements of vegetable crops consist of : 1) the amount of nitrogen that will actually be taken up by the plant and integrated into its biomass, and 2) a quantity of nitrogen that must nevertheless be present in the soil in order for the crop to achieve its full potential yield. Several factors affect the rate and amount of nitrogen uptake by crop plants. Nitrogen uptake is enhanced by a warm, sunny climate because photosynthesis rates are high under these conditions. While a plant's nitrogen requirement when it is small is low, the nitrogen supply at this time is critically important. Approximate nitrogen uptake per tonne of yield of Chinese cabbage was 3.5 kgN/ha, average yield was 70 t/ha and nitrogen uptake for average yield was 250 kgN/ha (Nicolas *et al*., 2001).

Nitrogen Isotope

Nitrogen isotope is the nucleide that contains the same atomic number but induces different mass number. Atomic number refers to the total amount of proton in nucleus or the total amount of electron in nucleus cycle using Z as the symbol. M stands for the symbol of mass number or atomic weight. Mass number is the sum of the amount of protons and neutrons, it is the index of atomic masses. Isotope of the same element must contain the same chemical qualifications – radioactive isotope and stable isotope. The half-life cycle of radioactive isotope of ^{12}N, ^{13}N, ^{16}N and ^{17}N is very short and cannot be used to monitor the transformation process of nitrogen. Instead, the stable isotope of ^{14}N and ^{15}N have replaced. Naturally, the mass concentration of ^{14}N is 99.634 atom % and 0.366 atom % for ^{15}N, and it is the constant ratio (Moungprasert and Masena ,1991).

The analysis of nitrogen isotope has to be conducted by mass spectrometer. The mass spectrometer is an instrument used for measuring the amount of ion which is caused by the break of N_2 molecules in terms of masses, such as 28(^{14}N), 29(^{14}N ^{15}N) and 30(^{15}N). Generally, N_2 emerges from the reactions between NH_4 oxidation (using Kjeldahl method) and Na or Li-hypobromite. The said reactions have to be done in vacuum container. Once N_2 exists, then insert the element into the mass spectrometer for measurement. Yet, N_2 can be located by the oxidation of the plant tissues and then measured by the mass spectrometer (Choonluchanon , 1998). In order to measure the amount of ^{15}N conducted by Kjeldahl-Rittenberg oxidation method, the sample must be decomposed by concentrated sulphuric acid together with the application of hydrogen peroxide in order to dissolve the microbes to NH_4^+. The NH_4^+ is then transformed into N_2 by Rittenberg method by using alkaline sodiumhypobromite solution (NaOBr) as oxidiser. According to Dumas-drycombustion method, the sample must be burned at the temperature higher than 450^{o}c inside the non-nitrogen burner together with the application of CuO and CaO. The sample was transformed into nitrogen after the treatment with a specific ratio between ^{14}N and ^{15}N. Finally, the amount of ^{15}N was measured by mass spectrometer (Chairin,2002). The comparison showed that the accuracy of the mass spectrometer is better than the emission spectrometer (Hardson ,1990).

Another indirect method is N-dilution technique. The principle of the method is that the dilution of ^{15}N of the sample plant which is caused by N-fixation is more severe than the standard plant. Normally, the comparison should be conducted under the same species and the same variety of the plant. If not, it should be done upon the plant in which the roots system is similar with the N-fixing plant. Also, the root depth, the physiological maturity stage, the growth rate in each plants' life cycle and the reactions to the environment must be the same as the N-fixation plants (Impituk ,1981). Yet, the application of ^{15}N fertilizer must be achieved in order to establish the level of ^{15}N ^{14}N in the soil to be higher than the air. One of the research using ^{15}N isotope dilution technique showed that the application of rhizobium combined with N-fertilizer at 25kg/ha provided the weight of dry roots and pods at 9,658 kgN/ha. The amount of nitrogen derived from fertilizer was 13.82 kgN/ha, 187.25 kgN/ha from soil, and 106.07 kgN/ha was derived from air (Cao Ngoc Diep *et al.*,2002). The research calculated only the pods and stems of the soybeans, while the calculation of vegetable soybeans consisted of the weight of the stems, leaves and roots without the seed weight.

There is another method that can be used to measure the amount of N fixation as well. But it is the method of measuring only the effectiveness of N- fixation at a specific time, which is called Acetylene Reduction Assay method (ARA). Providing that the capability of nitrogenase enzyme is not only reducing N_2 into NH_3, but it can also reduce other substances. From this advantage point, some compound substances have been applied in order to evaluate the activities of nitrogenase enzyme and then convert the result into the effectiveness of microorganisms' N-fixation. The most popular compound substance to be used is acetylene (C_2H_2) because of its availability. Nitrogenase enzyme can reduce acetylene into ethylene (C_2H_4) and then measure the volume of ethylene by gas chromatography, using flame ionization detector (FID) as an analyzer. The crucial part of gas chromatography, in analyzing C_2H_2 which derives from nitrogenase enzyme, is the column. The column is made of stainless steel with 3 m. in length and contains prorapak. Prorapak is another property of polymer, it is constructed by the combination of ethylvinyl benzene and divinyl benzene which caused multiple holes with various sizes. The flowing rate of 100-200 mesh prorapak N, by using N_2 as carrier gas, is 50 ml./min, while the flowing rate of hydrogen and air is 300 ml./min. The purity of acetylene being used in the analysis is 99% which can be purchased elsewhere in the markets.

The measurement of the standard volume of ethylene by gas chromatography.

To measure the volume of ethylene deriving from nitrogenase enzyme activities, the crucial point was to locate the standard volume of ethylene by the injection of a specific amount of ethylene sample into gas chromatography. The sample was analyzed by the machine, and then passed the analyte on to the recorder. The recorder then interpreted the analyte in terms of the height or the peak area of the sample. The repetitions of the treatment with various levels of ethylene could provide the standard volume that revealed the relationships between the height or the peak area activated by the recorder (Choonluchanon ,1998).

The study of nitrogen components from various parts of soybeans disclosed that the contribution of N-fixation from air accounted for 72-78% (Yathaphutanon *et al*.,1998) equivalent to 1,250 kg./rai of the fresh weight of soybean biomass (Chotiyanvong ,2005). The study conducted by Ledgard *et al*.,(1985) using *Trifolium subterraneum* L. Woogenellup compared with *Lolium rigidum* L.cv. Wimmera by applying ^{15}N sodium nitrate fertilizer revealed that there was 95% of nitrogen derived from air. The similar study which was conducted by Renne *et.al*., (1982) reported that the application of Rhizobium japonicum strain GIA 101 caused the amount of nitrogen derived from air at 38-70%. In this study, the analytes of soybean N-fixation have to be recalculated.

Kale

Kale is the main leafy vegetable crop grown in south-east Asian countries, especially in Thailand (Sagwansupyakorn,1994). The Brassica species originated in China, however, different cultivars of them can be grown successfully in a wide range of climatic conditions including cool winters, warm summers and even the hot and humid conditions(Issarakraisila *et al*.,2007). The optimum pH should be around 6.0-6.5 while the temperature is around 20-25^{0}C. (DOA,2001).

Kale is a kind of vegetables that can be planted in every region of Thailand. The good growing conditions depend on soil that is nourished with fertilizers, good drainage system and appropriate aeration. According to the ratio of fertilizer applications, 20-10-10 chemical fertilizer at a rate of 25-30 kg./rai should be applied before kale is transplanted. After kale has reached 20 days, then apply another 20-10-10 chemical fertilizer at a rate of 25-30 kg/rai mixed with urea 46-0-0 chemical fertilizer at a rate of 10-20 kg/rai. As a consequence, there are 2 times of N fertilizer application which is equal to 21.2 kgN/rai. Kales are ready to be harvested in 45-50 days after planting. When the time comes, cut them at ground level while their leaves show a white-green color (DOA,2002.). Yields of kale 2 kg per square metre can be obtained (Huxley,1992). However, yield of kale as reported by Jaturong (2005) was at 16 kg/18 m^2 at Chiang Mai University. In addition, Seok-In Yun *et al*.,(2006) studied the growth of Chinese cabbage when applied with 10 gN/1 kg of compost (made from pig manure mixed with sawdust) which gave dry matter yield of 7.4, 29.7 and 65.8 g/plant and uptake of nitrogen at 388, 656 and 700 mgN/plant at 20, 40 and 60 days after transplanting.