Nitrogen Assimilation-Metabolism in Relation to Potassium Use in Cauliflower (*Brassica oleracea* var. *botrytis*)

by

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Abstract

A field trial and a greenhouse experiment were conducted to investigate the impacts of N and K nutrients on plant N/K assimilation, nitrate reductase activity (NRA), curd yield and quality of cauliflower cv. 'Minuteman'. In the field study the treatments consisted of five N rates (0, 55, 110, 165 and 220 kg/ha) and three K rates (0, 25 and 50 kg/ha). In the greenhouse study the treatments were five levels of N (0, 16.5, 33, 50 and 66.5 mg/plant/day) using a modified Hoagland nutrition solution. In the field study the interaction of N/K rates was significant in cauliflower whole plant N/K uptake. Head N/K accumulation was 32-35% of plant total N/K uptake (8.0 g/plant), which was significantly correlated with head yield and size ($P<0.05$). Cauliflower NRA was associated with leaf/head sap NO_3-N concentrations. It is suggested that nitrogen and potassium translocation is an important factor of cauliflower yield and quality.

List of Abbreviations and Symbols Used

Abbreviation formatting	Description
N_2	Dinitrogen
NO_3^-	Nitrate
NO_2^-	Nitrite
NH_4^+	Ammonium
N	Nitrogen
K	Potassium
P	Phosphorus
kg/ha	Kilogram Per Hectare
NR	Nitrate Reductase
NiR	Nitrite Reductase
NRA	Nitrate Reductase Activity
KNO_3	Potassium Nitrate
NS	Nova Scotia
DAT	Days After Transplanting/Treatments
A	Photosynthesis
E	Transpiration Rate
Gs	Stomatal Conductance
Ci	Intercellular CO_2 Concentration
A/Ci	Instantaneous Carboxylation Efficiency
WUE	Water Use Efficiency
AAS	Absorption Spectrophotometer
ppm	Parts Per Million
M	Morlarity
mM	Milimolar Per Litre
µM	Micromolar Per Litre
TFW	Total Fresh Weight
TDW	Total Dry Weight
L, S &R	Leaf, Stem &Root
WP	Whole Plant
Nmin	Available N
TDS	Total Dissolved Solid

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Chapter I

Introduction

Nitrogen is essential to plant growth. It is a necessary constitute of amino acids, proteins, enzymes, chlorophyll and growth hormones (Ruiz et al., 2000). Although 78% of the atmosphere is made of N_2, only a small percent of N_2 is available for plant use through fixation. Most plants absorb nitrogen in the form of nitrate via root systems. The nitrate assimilated by the plant is reduced to nitrite, converted to ammonia via nitrite, and finally incorporated into nitrogenous organic compounds (Sivasankar and Oaks, 1996; Migge and Becker, 1996).

In modern agriculture fertilizers have often been over-applied to maximize crop production. Environmental issues associated with overuse of fertilizer N are concerned. Fertilizer N not recovered by the crop is lost from the soil by leaching or denitrification with NO/N_2O emissions. NO_3^- levels in groundwater close to farms is a major environmental and social concern and can be impacted by N application (Li et al., 2003).

Cauliflower is a cool season crop that demands high levels of nitrogen to maximize yields. N fertilization affects cauliflower development in terms of yield, quality and aspects of N metabolism. Kaniszewski and Rumpel (1998) reported that cauliflower curd yield and foliar Nitrate-N increased with N applications. An increased N rate (80 to 120 kg N/ha) increased the nitrate level (by 33%), whereas it decreased the vitamin C content in cauliflower (by 7%) (Lisiewska and Kmiecik, 1996). Moreover, N fertilization showed a limited effect on storage loss of head

(Toivenen et al., 1994). Research conducted in Nova Scotia found that cauliflower inflorescence and head quality were related to N rate (Li et al., 2007).

Cauliflower is highly dependent upon N fertilizer inputs and excessive N applications rarely negatively affect cauliflower (Stivers et al., 1993). However, crop production is not consistently increased with N rates. Fertilizer N not taken up by crops is subject to loss processes and pollution of ground water (Li et al., 2003). Everaarts (2000) reported low fertilizer N recovery was related to high N rates. In addition, N uptake and nitrate accumulation in plants is another subject of concern for human health, as high NO_3^- concentration in edible parts has been implicated in causing gastric cancer (Cardenas-Navarro et al., 1999). In order to achieve higher crop yields and quality and minimize pollution, there is an urgent need to increase the use efficiency of fertilizer N with low N application rates. Understanding the processes of N uptake and mechanism of nitrogen assimilation are the key to increase fertilizer N recovery.

Additionally, it is reported that Brassica vegetables such as cauliflower and broccoli are short-season crops that mature 6 to 9 weeks after transplanting (Bowen et al., 1998). During a short growing season, N supply is very critical for production of high quality cauliflowers (Bowen et al., 1998). Therefore, more efficient use of N fertilizer becomes particularly important. In Nova Scotia, little is known about the relations between N fertilizer application and the production and quality of broccoli and cauliflower. Understanding the N uptake and distribution within crops is of major importance with respect to both the crop quality and environmental concerns (Gastal and Lemaire, 2002). There have been many research studies on the effects of fertilization on cauliflower yield, however, most work has focused on single nutrient factors (Yang et al., 1994). Only a few studies were conducted on interaction of

multifactor fertilizations on N metabolism, growth and yield in cauliflower. In addition to N fertilization, K application enhanced the N, P, and Ca uptake by cauliflower (Guo et al., 2007). Potassium deficiency resulted in significantly lower concentrations of starch and protein N in the laminae of cauliflower (Sharma and Singh, 1992). Moreover, K also plays a role in N metabolism in plants. Yang et al. (1994) found that higher K rates increased nitrate reductase activity when a lower N rate was applied to cauliflower.

In Nova Scotia, little information is available on the effects of K application on cauliflower growth, yield and quality. The objectives of this study were as follows:

1. Investigate the effects and interaction of N and K applications on N uptake and assimilation (NRA), cauliflower plant development, curd yield and quality;
2. Determine the optimal N and K rates to maximize the transformation of NO_3^- into NO_2^- for producing high quality of cauliflowers;
3. Deliver a research-based recommendation to the horticultural industry for N/K management planning that will enhance cauliflower production in Nova Scotia.

Chapter II

Literature Review

2.1 Physiological Development of Cauliflower

Cauliflower (*Brassica oleracea* var. *botrytis*) is a vegetable in the Brassicaceae family. Cauliflower and other Brassica plants such as broccoli and cabbage are cool season crops and they grow poorly in hot weather. Cauliflower is rich in fibre, vitamin C and vitamin B, which are beneficial to human health. It also has a high content of anti-cancer compounds, such glucosinolates and sulforaphane (Kirsh et al., 2007). Cauliflower is an annual crop that reproduces by seed and is grown for the head or curd which is an inflorescence primordium. Cauliflower inflorescence development involves proliferation of the meristem resulting in a highly ramified inflorescence. Developmental arrest due to high temperature can occur during floral bud development and internode elongation (Carr and Irish, 1997).

There are five developmental stages of the inflorescence: vegetative, straightened, bowed, crowned and headed stage (Bjorkman and Pearson, 1998). Overall, cauliflower plants go through vegetative, shoot-tip straightened, curd initiation and heading stages. Physiological descriptions regarding two main growth stages of cauliflower are presented in detail as shown in Table 2.1.

For cauliflower plants the two key growth stages are juvenile and mature vegetative. During the juvenile stage, curd initiation does not occur and cannot be initiated. This stage ends when the plant has developed 6-19 leaves, depending on the cultivar. It appears to be correlated with the development of the number of leaves with

a minimum of 6-8 expanded leaves. This range can be increased under certain conditions such as temperature, as hot temperatures can delay curd initiation.

With the completion of a juvenile stage, the plant reaches a mature vegetative phase when curd initiation can occur or be induced. The length of time the plant remains in a mature vegetative phase before curd initiation varies, as well as the time required to produce harvestable curd after initiation. Both are cultivar and temperature dependent, as hot temperature can delay curd initiation and the response of a cultivar to temperature affects curd development.

Table 2.1 Description of cauliflower growth stages

Primary stage	Secondary stage	Description (old growth stages)	Comments
1 Vegetative stage			Leaf development
	1.3	(1) Early establishment	3rd true leaf unfolded
	1.6		6th true leaf unfolded
	1.7	(2) Vegetative	7th true leaf unfolded
	1.9		9th true leaf unfolded
4 Head development			Development of harvestable Vegetative plant parts
	4.3	(3) Early head	30% of the expected head diameter reached
	4.5		50% of the expected head diameter reached
	4.7	(4) Late head	70% of the expected head diameter reached
	4.8		80% of the expected head diameter reached
	4.9	(5) Harvest	Typical size and form reached; head tightly closed

(Source: Serve-Ag, 2004)

2.2 Nitrogen Nutrition and Plant Relations

2.2.1 Biochemistry of Nitrogen

Nitrogen is a nonmetal chemical element. Nitrogen gas (N_2) is colourless, odourless and tasteless under standard conditions and it makes up 78.1% of the air by volume. Molecular nitrogen has a very strong triple bond, therefore, it is chemically very unreactive and this bond is difficult to break in order to convert it into other nitrogenous compounds for plant and organism use (Fowden, 1997).

Nitrogen is present in all living organisms. It is a fundamental constituent of amino acids that are building blocks of protein. Proteins can act as enzyme catalysts in metabolic pathways, as structural elements of cytoplasm and membranes and as carriers in transport functions (Novoa and Loomis, 1981). In addition, nitrogen is also a major component of nucleic acids, which allows cells to grow and reproduce. Nucleic acids provide the means of codification, storage and translation of genetic information.

Furthermore, nitrogen is a component of energy-transfer compounds such as ATP. Plant chlorophyll contains four atoms of N and serves as a photoreceptor to allow the process of photosynthesis to happen. Thereby N is a significant component of chlorophyll and essential to plant growth (Fowden, 1977).

2.2.2 Nitrogen Cycling Processes in Plant and Soil Systems

The nitrogen cycle describes the transformations of its organic and inorganic forms in nature. There are several processes that are involved in biological nitrogen cycling. N_2 in the atmosphere can be transformed by N-fixing bacteria into ammonia. Organisms that possess the capacity of N fixation are categorized into free-living

bacteria and symbiotic bacteria. One typical example of symbiotic systems is the association between leguminous plants and species of *Rhizobium*.

Ammonia can be directly taken up by plants. However, in most instances nitrate is the preferred form absorbed by plants (Bray, 1983). Ammonia can be converted to nitrate in the process of nitrification. Under soil conditions of poor aeration, nitrification is inhibited with limited oxygen supply and plants absorb nitrogen mainly as ammonia. Plants take up nitrate via root hairs. Nitrate in the plant cell is reduced to nitrite by nitrate reductase. Nitrite is then converted to ammonium by nitrite reductase and subsequently incorporated into N-containing compounds.

Nitrate and nitrite in the soil can be converted to N_2 by denitrification under anaerobic conditions. The process uses inorganic ions as a substitute for oxygen during respiration (Bray, 1983). Animals that use plants as a food source usually contain a large amount of organic N and they also secrete nitrogenous waste products. When plants or animals die or decay, bacteria or fungi in the soil can convert the dead organisms or their excretory N-containing wastes to ammonium in a process known as ammonification.

2.2.3 Principle and Mechanism of Nitrogen Uptake

Plants can take up nitrogen in both forms of nitrate (NO_3^-) and ammonium (NH_4^+). Soil temperature plays a major role in the N forms available to plants. The uptake of NH_4^+ predominates when nitrification is limited by low soil temperature in the winter or early spring. As the soil becomes warmer and nitrification proceeds, plants tend to absorb N mainly as NO_3^- (Olson and Kurtz, 1982). However, NO_3^- is generally the principal form that most plants uptake. The uptake process of nitrate is influenced by many factors including temperature, pH, external nitrate concentration,

transpiration and the presence of ammonium (Novoa and Loomis, 1981). As pH increases, nitrate uptake tends to decrease while ammonium uptake increases. There are three stages that occur in the process of nitrogen uptake as follows:

(1) Step 1: Ionic N movement to plant root surface

Nitrate is very soluble in water and mobile in the soil. It can be readily transported to the root surface by mass flow, as water is taken up in response to transpiration in the above-ground portion of the plant. Mass flow thereby contributes most of the N uptake of plants. The process of N entering the plant by mass flow is a passive process.

When N uptake is in excess of the N supply from the mass flow, a depletion zone of N is created adjacent to the root (Olson and Kurtz, 1982). Consequently, the N concentration is lowered at the root surface and the process of diffusion then occurs. Nitrogen nutrient from a zone of high concentration diffuses through the soil solution to the low concentration zone close to the root for plant uptake. This process depends on plant demand for nitrogen and is believed to be controlled by the plant (Timlin et al., 2006).

(2) Step 2: Ionic N enters the plant root cell for absorption

With nitrogen moving to the root surface, nitrogen is absorbed first into the free space of the roots (cell wall spaces) and then across membranes into plant cells (Novoa and Loomis, 1981). N reaches the protoplast of the root cells and enters the interior of the cell through the plasma membrane. The N uptake is induced by an N-specific permease as the plasma membrane can form a barrier to its uptake (Bray, 1983).

Two mechanisms are involved in membrane transport: passive and active. Passive uptake is subject to the membrane permeability and occurs by diffusion along an electrochemical gradient only when a fairly high N concentration is present in the soil solution and cell levels are low (Novoa and Loomis, 1981). Active uptake requires metabolic energy to overcome the unfavourable electrochemical gradient between the soil and the root. This process is probably accomplished by 'carriers' (Novoa and Loomis, 1981). Temperature plays a role in the active mechanism of nitrate uptake and maximum rate increases exponentially with increasing temperature. However, nitrogen can efflux back out of the root via a passive diffusion or a carrier-mediated process, either of which depends on the internal N concentration in the root (Pessarakli, 2002).

(3) Step 3: N transport or storage within the plant

Independent transformations occur after nitrogen absorption by roots (Olson and Kurtz, 1982). N can be accumulated in the root cells and reduced by root tissues for the biosynthesis of amino acids, which may either be stored or transported to the shoot. Plants may also transport N across root cells and deposit it in the xylem for translocation to other regions of the plant such as shoot, petiole and leaf. A schematic of N movements in higher plants is shown in Figure 2.1 (Novoa and Loomis, 1981). Some nitrate is transformed into amino acids in the roots, however most of the nitrate reduction takes place in leaves, where amino acids formed can move back to the roots or to other plant organs via phloem.

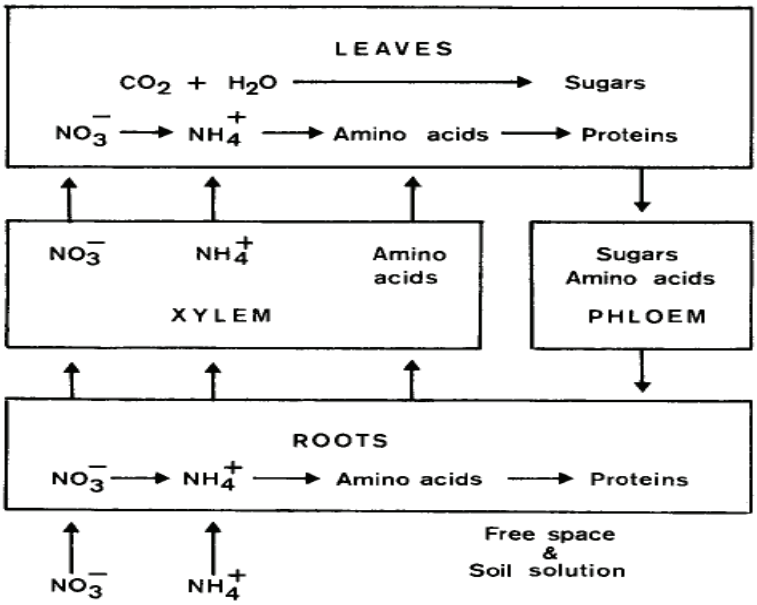

Figure 2.1 Basic schematic of N uptake and reduction and protein formation in higher plants (Novoa and Loomis, 1981)

2.2.4 Behaviour and Metabolism of Nitrogen in Plants

Plants take up N mainly as nitrate. The nitrate absorbed by plant roots is reduced to nitrite, which is converted to ammonia, and finally incorporated into organic nitrogenous compounds (Sivasankar and Oaks, 1996). In roots, both nitrate and nitrite reduction occurs in the cytosol; in leaves, nitrate is also reduced in the cytosol, but the conversion of nitrite to ammonium occurs in the chloroplast (Novoa and Loomis, 1981). Marschner (1995) reported that higher plants were capable of reducing nitrate in both roots and shoots. However, nitrate reduction is more efficient in leaves than in root because of the readily available reductants, energy and carbon skeletons produced by photosynthesis (Solomonson and Barber, 1990; Oaks, 1994). This also applies in most leafy vegetables, although it is dependent on plant species (Matraszek, 2008). Nitrate taken up by plants can be either stored or reduced in the vacuole, or transported through xylem transpiration stream to the leaf where the reduction occurs

(Chen et al., 2004). Moreover, most of nitrate is stored in the vacuole until it is released for nitrate reduction in the cytosol (Chen et al., 2004). Therefore, the vacuole is called the nitrate storage pool (SP), and cytosol is the nitrate metabolic pool (MP) (Ferrar et al., 1973; Miller and Smith, 1996). MP is a small one and accessible to the nitrate-reducing system while SP is relatively large but inaccessible where nitrate cannot be reduced to nitrite (Ferrar et al., 1973).

(1) Nitrate reductase

Nitrate reductase (NR) that catalyzes nitrate to nitrite is a key enzyme in nitrate assimilation. Nitrate reduction is the rate-limiting step for nitrate assimilation (Caba et al., 1995; Bussi et al., 1997) and NR is an inducible enzyme, therefore NR activity (NRA) is believed to be closely related with nitrate concentration in plants (Skrdleta et al., 1979). Moreover, NR exists in the cytosol that serves as nitrate metabolic pool. Cytosolic nitrate concentration must influence NRA and thereby determine the rate of nitrogen assimilation (Miller and Smith, 1996). Novoa and Loomis (1981) also reported that NR activity was affected only by the concentration of nitrate in the cytosol. The level and activity of NR can be induced by the substrate nitrate and ammonia. High soil nitrate concentrations increased nitrate uptake, mechanistically related with nitrate reduction (Fowden, 1977). In green algae, the synthesis of nitrate reductase was restrained when ammonia was added to the growth medium containing nitrate.

On the other hand, nitrate induces the expression of both the uptake and reduction systems (Sivasankar et al., 1997). Nitrate accumulation in plants is of concern in regards to human health and the environment. Several studies have stated that nitrate accumulation is highly sensitive to both endogenous and exogenous

factors and might be regulated by such factors as plant growth, endogenous nitrate, and nitrate uptake and reduction (Cárdenas-Navarro et al., 1999; Chen et al., 2004). Higher NRA would result in less nitrate accumulation in plants. Additionally, it is observed that nitrate content is variable between plant species, and even between cultivars of the same species (Blom-Zandstra, 1989). Tree and shrubs among higher plants have the lowest shoot nitrate content (Bussi et al., 1997; Cárdenas-Navarro et al., 1999). Therefore, there is a need for understanding the influence of nitrate supply on NRA in plants, especially in vegetable crops with respect to food quality and safety.

Photosynthesis is closely related to nitrate metabolism. Under low light intensities, nitrate tends to accumulate in the leaves. Fowden (1977) also stated that nitrate reduction relied on the availability of electron donors produced by light-dependent processes. In leaves, release of nitrate from vacuoles to the nitrate metabolic cytosol is stimulated by light, thus assuring the most of the reduction occurs during the photoperiod (Novoa and Loomis, 1981). In addition, deficiency of some essential nutrients in higher plants can influence nitrate metabolism. NR activity was inhibited due to S-starvation when sufficient nitrate was supplied (Prosser et al., 2001). Beevers (1976) also reported high concentrations of nitrate accumulated in molybdenum-deficient plants.

(2) Nitrite reductase

The enzyme nitrite reductase (NiR) is responsible for the reduction of nitrite to ammonia. Nitrite rarely accumulates since the nitrite reductase activity is invariably at a much higher level than nitrate reductase (Bray, 1983). NiR is often found in illuminated chloroplasts where the reduced ferredoxin that acts as an electron donor is

produced via coupling to cyclic photosynthetic electron transport (Beevers, 1976). Nitrite can be readily metabolized by roots where proplastids are involved in nitrite reduction (Bray, 1983). Anaerobiosis inhibited nitrite utilization *in vivo*, whereas nitrite utilization was rapid under aeration. This implies nitrite metabolism directly depends on aerobic metabolism (Beevers, 1976).

(3) Ammonia incorporation into organic compounds

Ammonia is the ultimate form of inorganic N in metabolism. It is then assimilated into amino acids, and subsequently into protein and other organic N compounds primarily via the synthesis of amino acids. The principal model of ammonia incorporation to organic compounds was believed to be through glutamine and glutamate in the presence of glutamine synthetase and glutamic dehydrogenase (Beevers, 1976).

2.2.5 Nitrogen Use by Plants

2.2.5.1 Nitrogen Use Efficiency

The efficient use of fertilizer N is important to the sustainability of world agricultural systems. Over application of fertilizer can negatively affect soil water quality and trace gas emissions into the air. Therefore, it is of importance to reduce the dependence on excessive fertilizer applications in agriculture for improved crop productivity. There are three main ways suggested by Lal (2003) to reduce the fertilizer use through 1) increasing the fertilizer use efficiency by a properly chosen fertilizer application rate and time, 2) reducing fertilizer loss from the soil caused by erosion, leaching and volatilization and 3) reinforcing nutrient recycling mechanism systems. Moreover, the growing world population results in an increasing demand for

crop production, therefore maximizing N use efficiency becomes increasingly important in order to achieve optimum yield.

It is reported that improved efficiency of N management in vegetable crops is possible if the fertilizer could be applied at the time of maximum crop need and placed for maximum contact with root system (Hart, 1992). Nitrogen use efficiency of crops can potentially be improved through manipulating the N use efficiency of the individual plants (Gastal and Lemaire, 2002). If an appropriate fertilizer placement can contribute to a higher availability of fertilizer N to the plant, high yields or similar yields can possibly be achieved with the same or lower fertilizer applications (Everaarts and De moel, 1995). In field trials conducted by Hart (1992), methods of fertilizer placement did not affect broccoli yield, while cauliflower yield and head size appeared higher with a banded application compared with broadcast. In the literature band placement of fertilizer can greatly increase the yield of Brassica crops (Everaarts, 1993). Furthermore, broadcast side-dressed N application was found to be superior to banded placement, although there was no significant difference between the two placement methods (Hart, 1992). Thereby sidedress application has been referred to as a suitable method of fertilizer placement for brassica crops.

In order to optimize N application rate for maximum yields, several factors would be considered such as climatic conditions, product quality and size requirements, and soil mineral N availability (Everaarts and Willigen, 1999). Understanding N uptake and assimilation is necessary to improve the N use efficiency of crops through the adaptation of N fertilization strategies (Gastal and Lemaire, 2002). Therefore, the effects of applied N rates on total N uptake and metabolism on vegetable plants were investigated in the present study and cauliflower plant in Brassica family was selected.

2.2.5.2 N Uptake Pattern in Cauliflower

Cauliflower is a heavy consumer of N and fertilizer N has more influence on the growth and yield of cauliflower than any other plant nutrient (Doerge et al., 1991). Doerge et al. (1991) investigated N uptake pattern for snowball-123 cauliflower. N uptake by cauliflower is very low prior to the 4 to 6- leaf stage at approximately 45-50 days after transplanting. The trend of increasing N uptake slows at the beginning of curd initiation and then continues to increase, and until harvest N uptake remains high as cauliflower is harvested before entering the reproductive growth stage.

2.2.5.3 N Effects on Crop Growth, Yield and Quality

Nitrogen is one of the essential nutrients required for plant growth. Among all the plant required nutrients, N is the most limiting factor affecting crop performance and also ranks the most frequently deficient nutrient in crop production (Havlin et al., 1999). It is reported that slight N deficiency can cause malfunctions in cell processes and inhibition of plant growth (Lincoln, 2002). N-deficient plants grow very slowly with spindly stalks and stems and chlorosis of older leaves, mature early and the yield and quality of crop are often reduced (Jones, 1998). In addition, N plays an important role in the use of carbohydrates within the plant (Brady and Weil, 2004). High N use efficiency can result in an increase in accumulations of leaf dry matter and final yield of the plant (McDonald et al., 1996; Lo´pez-Cantarero et al., 1997; Ruiz et al., 2000). Crop yield cannot be continuously increased with N application, while over-applied N could have disadvantageous effects on crop. Taghavi and Babalar (2007) reported that too much N can cause poor fruit quality, excessive plant growth and increasing runner numbers in strawberry.

Cauliflower is a cool weather crop. It is often heavily fertilized to maximize yields. Cauliflower grows initially slowly with little N uptake during the first 60 days of growth with 90% or more of total N uptake occurring during the final 50 to 60 days before harvest (Welch et al., 1987). Cauliflower is highly responsive to N fertilizer inputs and excessive N applications rarely negatively affect cauliflower development (Stivers et al., 1993; Thompson et al., 2000). However, under conditions conducive for head rot, high rates of N can promote the occurrence of head rot, thus resulting in reduced marketable yield (Everaarts and Willigen, 1999).

Cauliflower yield increased with increasing N rates up to 270 kg/ha (Hart, 1992). Application of N fertilizer as urea increased nitrate reductase activity and decreased leaf nitrate-N content (Yang et al., 1994). Nitrate-N in cauliflower leaf and head increased linearly with N fertilization (up to 600 kg N/ha) (Kaniszewski and Rumpel, 1998). Nitrogen application or application method did not consistently influence the number of cauliflower harvested, whereas the size of the marketable curd was affected by N application (Everaarts and De moel, 1995). Nitrogen fertilization rates did not significantly affect the storage loss and thus storage life of Brassica vegetables like broccoli and cabbage, as weight loss is the major determinant of shelf life (Freyman et al., 1991; Toivonen et al., 1994). Studies have agreed that the benefits of increasing N application on marketable yield can highly outweigh any negative effects such as weight loss during storage (Berard, 1990; Freyman et al., 1991; Everaarts and De Moel,1998).

Moreover, no effect was found on the quality of cauliflower with an increased N application from 150 to 300 kg N/ha (Nilsson, 1980). Everaarts and De moel (1995) found that N fertilizer did not affect the earliness of the harvest of cauliflower plants. Lisiewska and Kmiecik (1996) claimed that quality of harvested cauliflower was

significantly correlated to N rates. An increased amount of N fertilizer (80 to 120 kg N/ha) decreased the vitamin C content in cauliflower by 7%, accompanied with raising the level of nitrates by 33% (Lisiewska and Kmiecik, 1996).

2.3 Potassium Nutrition and Plant Relations

2.3.1 Biochemistry of Potassium

Potassium is a chemical element with an atomic weight of 39. It makes up about 1.5% of the earth's crust by weight and is the seventh most abundant element on earth. Potassium is one of the most reactive and electropositive metals and can readily react with water and oxidize in air. Additionally, potassium is an important nutrient in the human diet for neuron function. A deficiency of potassium in the body can result in a condition known as hypokalemia that is potentially fatal to humans. Potassium is a necessary nutrient required for plant growth and reproduction. The number given on the label of N-P-K fertilizer represents the percentage of K_2O in the fertilizer.

2.3.2 Effects of Potassium on Plant Development, Crop Yield and Crop Quality

Potassium is the third macronutrient essential for plant growth, after nitrogen (N) and phosphorus (P). Unlike N and P as a component of cell structure, K exists in a mobile ionic form and acts primarily as a catalyst (Wallingford, 1980). Thereby plants absorb potassium as K^+. K influences plant development since it is involved with over 60 enzyme systems in plants and plays a major role in enzyme activation, energy relations, assimilate translocation, and protein and starch synthesis (Wallingford, 1980). K deficiency results in a reduced growth rate initially with chlorosis and necrosis occurring in later stages (Mengel and Kirkby, 2001). Moreover, K also plays an important osmotic role in plants especially in arid environments (Fageria et al.,

1991). In general, effects of potassium on crops include 1) increased plant resistance to disease and drought stress; 2) improved crop quality and use efficiency of other nutrients; and 3) increased total yield (Armstrong, 1998).

Most field crops require a large amount of potassium to achieve maximum yield and K tissue concentration typically varies between 3 and 5% (DW) in plant (Errebhi et al., 2004). A significant positive correlation was obtained between leaf K concentration and the grain yield of spring barely (*Hordeum vulgare L.*) (Leigh and Johnston, 1983). Grant (1989) found that K concentration in barley tissue grown on K-deficient soils was increased by K application. However, varying rates of K applied to barley grown in an arid environment did not significantly affect forage yield (Errebhi et al., 2004).

Aygun and Algan (2000) also found that plant height, stem diameter and stem dry matter in kenaf plants were greatly increased by K application. Low potassium supply retarded flower development in tomato plants (Besford and Maw, 1975). It was reported by Habi et al. (1990) that more than 536 lbs K_2O/acre was removed by alfalfa, therefore a high amount of K fertilizer must be applied for plant uptake, especially during rapid growth.

Brassica crops such as broccoli responded significantly to major essential nutrients like N, P and K in respect of crop growth and yield, whereas K is the key element for yield and dry weight of broccoli (Islam et al., 2010). Broccoli yield and yield contributing characters were significantly affected by K application, and yield increased with the increasing K levels up to 200 kg/ha and then decreased (Islam et al., 2010). The number of days required for cauliflower curd initiation was shortened with increasing K fertilizer rates. Similarly to broccoli, application of K (90-270 kg/ha) increased cauliflower yield and resulted in increasing contents of protein and

ascorbic acid by 8.0-24 and 4.0-5.3%, respectively (Shi et al., 2004). A shortage of potassium resulted in lower dry matter in cauliflower plants and significantly decreased starch and protein N in the laminae of plants (Sharma and Singh, 1992).

K application increased the uptake of the nutrients N, P and Ca by cauliflower; but, it reduced the N content in all tissues and Mg content in side leaves (Guo et al., 2007). However, little is known of the effects of K application rates on cauliflower growth, yield and quality in Nova Scotia. When K was applied together with N or N and P, yield and vitamin C content of broccoli were increased (Ying et al., 1997). Ying et al. (1997) found that K applied with N and P had 110.8% higher yield than N alone. NK nutrition has demonstrated significant effects on the growth, yield and quality of crops such as broccoli and potato (Yang et al., 1994). Excessive N and K fertilization was believed to be disadvantageous to potato plant growth. Few investigations have looked at the interaction between N and K fertilization on cauliflower. Therefore, there is a need to study the influence of K fertilization individually or combined with other nutrients on cauliflower growth and yield.

2.4 Potassium, Nitrogen and N Metabolism Relations

2.4.1 Effects of K on Nitrogen Uptake and Metabolism

Potassium can play a role in nitrogen uptake and nitrogen metabolism in plants. Potassium acts as a transporter of photoassimilates from source leaves to the sinks and also co-transports NO_3^- in its uptake (Villora et al., 2003). Moreover, as K is involved in enzymatic activities, nitrate reducatase activity can be directly or indirectly related with plant tissue K. It was found K deficiency inhibited the accumulation and synthesis of nitrogen substances in pumpkin and pea seedlings (Fialova and Pichl, 1973). Higher levels of solution K increased the concentrations of N and soluble

protein in alfalfa roots (Li et al., 1997); nevertheless, protein concentration in shoot apices of tomato plants significantly increased with decreasing K supply (Besford, 1975).

Yang et al. (1994) found that increasing levels of K fertilizer promoted NR activity and reduced NO_3-N content in cauliflower. Nitrate content in cauliflower was decreased by K treatments by 12.4-36.1% as compared with the control (Shi et al., 2004). In the preliminary study on the relation between NK nutrition and N metabolism conducted by Yang et al. (1994), high K rates increased NRA and leaf nitrate-N when a low N rate was applied to cauliflower; as a result of this treatment (high K rate and low N rate), the best plant growth and highest curd yield were obtained. In contrast, NRA decreased while NO_3-N increased when increasing K rates at the high N application.

2.4.2 Effects of N on Nitrogen Uptake and Metabolism

Hoagland solution (Hoagland and Arnon, 1950) is used as a popular nutrient solution to grow plants in the greenhouse. Many studies have used Hoagland solution to cultivate plants such as alfalfa, peppers, and cauliflower in greenhouse experiments (Li et al., 1997; Drew and Bain, 1986; Ruiz et al., 2000).

It was reported that nitrate uptake rate increased in response to increasing external nitrate concentration (Darnell and Stutte, 2001). Additionally, Taghavi and Babalar (2007) studied the effect of NO_3^- concentrations supplied in modified Hoagland solution on nitrate uptake, NR activity and yield of strawberry. Results showed the increase in NO_3^- supply (less than 3.75 mM) increased nitrate uptake, while NO_3^- from 0 to 0.25 mM decreased leaf NR activity. Nitrate uptake rate

increased with increasing external NO_3^- concentrations (3.75-15 mM), whereas NR, growth and yield of strawberry were not affected by treatments.

In a greenhouse experiment studying the effect of Mo and B deficiency on cauliflower (Drew and Bain, 1986), cauliflower grown hydroponically with 15 mM N modified Hoagland solution received 210 mg N weekly to ensure nutrients except Mo and B were adequate. Following this instance, modified Hoagland solution was used to supply cauliflower plants adequate nutrients except for N which was added with various levels in the form of KNO_3. Chen et al. (2004) stated that most studies investigated nitrate effect on NR activity under two to three nitrate levels, or short term nitrate induction, or in hydroponics. Hydroponic nitrate levels varied between plant species, for examples, tomato plants grown in 3.0 mM NO_3^- or a limited nitrate nutrition of 0.2 mM; pea plants in 20 mM NO_3^-; and vicia faba in 12 mM NO_3^-. In the present work more N supply rates were carried out at a gradient to study N effect on cauliflower NR activity.

Chapter III

Interaction of Nitrogen and Potassium Fertility on N-K Nutrient Uptake, Curd Yield and Quality, and Growth of Cauliflower (*Brassica oleracea* var. *botrytis*)

3.1 Introduction

In Nova Scotia, there are nearly 100 hectares of cauliflower, and most of the Nova Scotia grown cauliflower is sold in the Maritimes (Growing Nova Scotia, 2008). Cauliflower is a crop that prefers cool weather and also is a heavy consumer of N. Nitrogen is the most limiting factor to plant development and crop yield. N applications affect N uptake and curd yield in cauliflower (Thompson et al. 2000). We have published a series of proceeding papers and conference abstracts on cauliflower N uptake and plant nutrition in different cauliflower cultivars under Atlantic conditions (Li et al., 2007, 2009a; Huang et al., 2010). However, no information related to N transformation and cauliflower quality is available.

There have been many research studies on the effects of fertilization on cauliflower yield, however, most work focused on single nutrient factors (Yang et al., 1994). Potassium, one of the essential nutrients for plant growth, can increase N uptake in cauliflower (Guo et al., 2007). K fertilization significantly affects the yield of broccoli, a Brassica crop (Islam et al., 2010). In Nova Scotia, little is known about the relationship between K application and the growth and production of cauliflower. Therefore, the objective of this study was to investigate the effects of different rates of nitrogen and potassium and their interaction on total N uptake, cauliflower growth, curd yield and quality under field conditions.

3.2 Materials and Methods

3.2.1 Site Description

A field experiment was carried out in a research plot at the Nova Scotia Agricultural College during the late growing season (July-September, 2009). The soil was loamy. Based on the soil test report conducted in April 2009, the soil in the 0-0.5 m layer had a pH of 6.7 and contained 3.5% organic matter, 625 kg/ha P_2O_5, 333 kg/ha K_2O, 3324 kg/ha Ca, 530 kg/ha Mg, and 34 kg/ha S. According to the soil test and rating, the regional recommendations of N, P and K for Cole crops in Nova Scotia were determined to be 220 kg/ha N, 110 kg/ha P_2O_5 (48 kg P/ha) and 60 kg /ha K_2O (50 kg K/ha), respectively (Soils & Crops Branch, 1994). As soil rating for P is high in the research plot, P fertilizer was not applied in order to minimize the interference effect of P application except main factors N and K applications.

3.2.2 Field Design and Treatments

The treatments of the experiment consisted of three rates of K (0, 25 and 50 kg K/ha) in the form of KCl and five rates of N (0, 55, 110, 165 and 220 kg N/ha) in the form of NH_4NO_3, for a total of 15 treatments. A split plot design was used to arrange treatments in a randomized complete block. Each treatment was replicated four times in plots of 4 m × 3 m wide. The main plot factor studied was K and three rates of K fertilizer were randomly applied to three main plots in each block. Nitrogen was the subplot factor and five N rates were assigned at random to the subplots within each main plot. The layout of treatments in the field is indicated in Table 3.1. There were a total of 12 (3 x 4) main plots and 60 (5 x3 x 4) subplots in this study.

Table 3.1 Layout of randomized experimental treatments in the field

Block 1

K25					K0					K50				
N 55	N 0	N 110	N 165	N 220	N 0	N 110	N 220	N 165	N 55	N 165	N 220	N 110	N 0	N 55

Block 2

K0					K50					K25				
N 0	N 55	N 165	N 110	N 220	N 0	N 220	N 110	N 55	N 165	N 55	N 220	N 0	N 165	N 110

Block 3

K50					K25					K0				
N 0	N 220	N 165	N 110	N 55	N 220	N 0	N 110	N 55	N 165	N 220	N 165	N 110	N 55	N 0

Block 4

K25					K50					K0				
N 55	N 165	N 110	N 220	N 0	N 0	N 165	N 110	N 55	N 220	N 55	N 0	N 220	N 110	N 165

K (0, 25 & 50): K rates in kg/ha; N (0, 55, 110, 165 & 220): N rates in kg/ha.

3.2.3 Field Planting

Cauliflower cultivar ‘Minuteman’ was grown and also studied by Dr. Hong Li in the Annapolis Valley of Nova Scotia in the previous years (Li et al., 2009). The same cultivar was used in the present study and seeds were purchased from AB Seed Company in USA. Seeds of cauliflower cultivar ‘Minuteman’ were sown in cell flats filled with pro-mix in late June, and maintained in the Plant Science Department greenhouse. The day/night temperature in the greenhouse was approximately 20/15^{o}C. Seedlings were watered as needed and fertilized weekly with liquid fertilizer of 20 mg N, 20 mg P and 20 mg K per litre.

In mid-July, about three weeks after sowing, the cauliflower seedlings at 4-5 leaf stage were transplanted into the research plot described above. The field was divided into four blocks with a 2.5 m pathway between blocks. In each block, 15 plots

were set up that corresponded with the 15 treatment combinations of N and K fertilizers. Each plot was 4 m × 3 m wide and contained 5 rows with 1 m between and 0.25 m plant spacing in row. There were 65 plants in each plot. In order for seedlings to adapt to the field conditions and to optimize use efficiency of NK fertilizers, fertilizer application was started approximately two weeks after transplanting. On August 2 2009, half N rates (0, 27.5, 55, 82.5 and 110 kg N/ha) combined with K rates (0, 25 and 50 kg K/ha) were side-dress applied to plots according to the treatment layout (Table 3.1) and incorporated to the soil. The other half N fertilizers were applied three weeks later.

3.2.4 Plant Sampling and Measurements

3.2.4.1 General Plant Growth Measurements

Cauliflower plants were sampled four times at the vegetative stage, curd initiation stage, early heading stage (30-50% of the expected head size) and maturity stage. Those stages occurred 30 days after transplant (DAT), and continued at approximately two weeks interval until harvest. At each sampling date, three cauliflower plants were randomly taken from each plot and total plant fresh weight of the three plants was then measured.

At 30 DAT (vegetative stage), three cauliflower plants were processed and separated into leaves and other parts including stems & roots for dry matter determinations. At 47 DAT (curd initiation stage), 60 DAT (early head stage) and 75 DAT (at harvest), of the three sampled cauliflower plants, only one representative plant was taken and processed in terms of plant height, total leaf number, fresh and dry weights of the whole plant. Cauliflower head size, yield and dry matter were obtained from all cauliflower samples to determine the means. Total dry matter of the

three cauliflower plants were estimated based on the fresh: dry weight ratio of the whole processed plant. Cauliflower samples were oven dried at 70°C for 36 hours, cooled and measured for dry weight determination. The main symbols used for plant measurement variables in the result section are presented in Table 3.2 below.

Total N was determined in cauliflower plant and head at each sampling time. Additionally, total P and K were measured in mature cauliflower heads at harvest, in order to study nutrient uptake and accumulation in the marketable portion of the cauliflower plants. Total nutrient uptake in the cauliflower plant and N harvest index was determined according to the equation described by Li et al. (2003) as follows:

N/P/K uptake (mg/plant) = DW (g/plant) x Plant N/P/K concentration (%) x 1000

Table 3.2 Main plant measurement variables and symbols used

TFW:	Total fresh weight (g)	DW:	Dry weight (g)
TDW:	Total dry weight (g)	FW:	Fresh weight (g)
Head size:	in cm	Height:	in cm
$N_{(0\text{-}220)}$:	N rate of 0, 55, 110, 165 & 220 kg/ha	$K_{(0\text{-}50)}$:	K rate of 0, 25 & 50 kg/ha
$N_{(0\text{-}220)}K_{(0\text{-}50)}$:	treatments of NK fertilization (kg/ha)		
N*K, N*time, K*time:	interaction between two variables		

3.2.4.2 Chlorophyll Measurement

During the curd initiation stage, three cauliflower plants were randomly selected in each plot for leaf chlorophyll measurement. The youngest mature leaf of each cauliflower plant was marked and measured for leaf chlorophyll content using a hand-held chlorophyll content meter 200 (CCM200). Chlorophyll readings were taken in the middle of cauliflower leaf blade. Starting at 45 DAT, leaf chlorophyll measurements were taken weekly four times on the same leaf marked until 66 DAT

(early head stage). For each date, three chlorophyll readings were obtained from each plot and averaged to generate one per plot.

3.2.4.3 Gas Exchange Measurements

Gas exchange measurements were taken from the youngest fully expanded leaves (about the 4th leaf counting from the centre shoot tip) of cauliflower by using an LCi portable photosynthesis system (ADC, Bioscientific, England). The measurements included photosynthesis (A), transpiration (E), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and instantaneous carboxylation efficiency (A/Ci). The water-use efficiency (WUE) was calculated as the ratio between A and transpiration rate (Ashraf et al., 2002). Around 60 days after transplant (DAT), gas measurements were taken between 2:00 pm and 4:30 pm, at leaf chamber temperature ranging from 24.3 to 27.8 ^{o}C and ambient CO_2 concentration ranging from 365 to 382 vpm (cm^3/m^3).

3.2.5 Soil Sampling

The soil of each plot was sampled on August 25 (midseason) and October 11 2009 (postharvest), which were respectively before the second split N application and after harvest. Within each plot, soil was collected to a depth of 0 -15 cm from three random spots near cauliflower plants using a soil hand probe (Li et al., 2003). Soil moisture was determined by drying a portion of the soil sample in an aluminum foil tray at an oven temperature 110^{o}C, in order to investigate whether soil water availability is possibly responsible for any difference in nutrients absorption and plant growth. The rest of the soil samples collected at each time were all air dried and sieved through a 2 mm mesh for soil chemical analysis. Soil available N including

nitrate (NO_3^-) and ammonium (NH_4^+) was analyzed for each soil sample to determine soil N availability and plant uptake relations.

3.2.6 Plant and Soil Analysis

Dry plant and cauliflower head tissue were all ground into 1 mm fine power using a mill in the Plant Science Department. To measure plant total N, P and K, ground plant tissues were digested with concentrated sulphuric acid (H_2SO_4) using the Labconco digestion unit in the Edible Horticulture Research Lab (Dr. Hong Li).

1. Acid digestion

Ground plant tissue samples of 0.5 g were weighed and added to 10 ml of concentrated sulphuric acid to a digestion tube, which was then put on a preheated digestion block at 300°C for 40 min in a fume hood. Tubes were removed from the block and cooled for 5 min. After cooling, 2 ml of 30% hydrogen peroxide (H_2O_2) was added and tubes were replaced on the digestion block for 15 min. The procedure was repeated until the digestion solution was colourless. Resulting solutions were transferred to 50 ml volumetric flasks and distilled water was added up to 50 ml. The final diluted digestion solution was used for chemical analysis of N, P and K.

2. Total N

Total N in the plant was determined by the Kjeldahl method (Bremner and Mulvaney, 1982; Li et al., 2003) using the Labconco distillate unit in the Edible Horticulture Research Lab (Dr. Hong Li). A 10 ml sample of the plant digestion solution was transferred into a distillation tube. The tube was then added to 10 ml of 10 N sodium hydroxide (or 40% NaOH solution) and put in the distillation unit for 6 min. A 125 ml Erlenmeyer flask was used to receive the distillate and 5 ml of 2%

boric acid (H_3BO_3) and 10 drops of mixed indicator (red methyl & green bromocresol) were added. Hydrochloric acid (0.01 N HCl) was used to tritrate N in the distillate until the colour changed from blue to pink.

3. Total P

Total P in the plant was determined spectrophotometrically using the method described by Bremner (1965) using the UV-visible Biochrom spectrophotometer in the Edible Horticulture Research Lab (Dr. Hong Li). A 1 ml sample of the decolourized digest was pipetted into a 50 ml volumetric flask, 5 drops of 0.25% p-nitrophenol was added, and subsequently neutralized to a yellow color endpoint with 10 N sodium hydroxide (NaOH). A mix reagent was prepared with a composition of 40% (v/v) 2.5 M H_2SO_4, 3.88 mM ammonium molybdate solution $\{(NH_4)_6Mo_7O_{24}\cdot 4H_2O\}$, 0.34 mM antimony tartrate solution ($KSbO\cdot C_4H_4O_6$) and 24 mM ascorbic acid (fresh prepared daily). Eight ml of the mix reagent was added to the 50 ml flask and made up to volume with distilled water. After 15 min of colour development, total P was measured at 880 nm wavelength using a spectrophotometer.

4. Total K

Plant total K determination was done using an atomic absorption spectrophotometer (AAS) in the Environmental Science Department. The standard range of K concentration for this instrument was from 0 – 10 ppm, therefore before starting K analysis, 1 ml of decolourized plant tissue digest was diluted 50 times into a 50 ml volumetric flask and stored in plastic vials for analysis. Prior to analyzing the sample solutions, standard solutions with K concentrations of 0, 2, 4, 6, 8 and 10 ppm were prepared using Melich II solution and run by AAS to observe absorbance. The K

concentration in the sample solution was then measured on AAS according to the standard curve.

5. Soil available N

Kjeldhal N analysis was used to determine soil NO_3-N and NH_4-N using distillation and titration procedures (Bremner, 1965; Li et al., 2003). Soil NH_4^+ and NO_3^- were analyzed using the Labconco distillate unit in the Edible Horticulture Research Lab (Dr. Hong Li). Prior to soil N analysis, 2 M KCl was used to extract both NO_3^- and NH_4^+ from the soil (Keeney and Nelson, 1982). 50 ml of 2 M KCl was added to 5 g of 2 mm sieved air dry soil in a 125 Erlenmeyer flask. The solution was placed on a shaker and shaken for 30 min at 200 oscillations/min. Whatman # 5 paper was then used to filter the soil suspension into 50 ml plastic vials.

For the analysis of soil NH_4-N, 10 ml of soil extract filtrate was transferred into a 50 ml distillation tube and 0.2 g MgO and 0.2 g $CaCl_2$ were added. Five ml of 2% boric acid (H_3BO_3) and 10 drops of mixed indicator (red methyl & green bromocresol) were added to a 125 ml receive flask. The tube was placed on the Labconco rapid distillation unit for 6 min. After distillation, the flask with distillate solution was removed from the unit and replaced with another one. For soil NO_3-N determination, 0.2 g devarda alloy was subsequently added to the distillation tube and distilled for 6 min. Hydrochloric acid (0.01 N HCl) was used to tritrate the distillate until a pink endpoint. Total soil available N was obtained by the sum of soil NH_4^+ and NO_3^- concentrations.

3.2.7 Statistical Analysis

Data obtained from this experiment were submitted for assumptions test including normality, constant variance and independence in Minitab v.14.

Assumptions for normality of data distribution and constant variance were tested using Minitab 15. Independence was assumed through randomization of treatments. After assumptions were validated, analysis of variance (ANOVA) was used to test the significance among treatment means using Proc GLM in SAS v8. Least Squares Means (LSmeans) method was conducted for means comparison if a significant difference was found among treatment means. Correlation and regression analysis were conducted to analyze the variables using Minitab. A 0.05 probability level of significance was tested for all the data analysis in this study.

3.2 Results

3.2.1 Effects of N and K on Cauliflower Plant Growth at the Vegetative Stage

At 30 DAT, cauliflower plants were at the vegetative growth stage. Cauliflower TFW was significantly affected by both N and K. However, N did not influence leaf DW, stem- root DW, and TDW of cauliflower plants. K rates significantly increased plant TFW and TDW by 26% and 12% as well as leaf DW by 12% (Table 3.3). The lower K rate (25 kg/ha) enhanced cauliflower growth and resulted in higher accumulation of cauliflower plant, leaf and stem-root DW. Compared to the control, N application significantly increased plant TFW. Plant TFW and leaf DW increased with increasing N rates above 55 kg/ha, although no significant difference was found among 110, 165 and 220 kg N /ha. In addition, there were no significant differences observed for the ratio of leaf DW to plant TDW, and the DW ratio of stem-root to the whole plant between N and K treatments. N applications of 110-220 kg/ha tended to have higher dry matter accumulation on leaf based on the higher ratio of leaf DW.

Table 3.3 ANOVA of N and K effects on cauliflower growth variables at 30 days after transplant (DAT)

Source	Rate (kg/ha)	plant TFW	plant TDW	leaf DW	stem-root DW	leaf ratio	stem-root ratio
N	0	447c	66a	35a	32a	0.52a	0.48a
	55	459bc	64a	33a	32a	0.51a	0.49a
	110	511ab	70a	37a	33a	0.53a	0.47a
	165	532a	72a	38a	33a	0.53a	0.47a
	220	522a	70a	37a	33a	0.53a	0.47a
K	0	435b	65b	34b	31b	0.52a	0.48a
	25	546a	73a	38a	35a	0.52a	0.48a
	50	501a	68ab	36ab	32ab	0.53a	0.47a
ANOVA[§]	d.f.						
Block	3	5.0**	3.4*	7.2**	5.9**	27.8**	27.9**
Block*K	6	2.7*	3.0*	2.5**	3.0*	2.1 ns	2.1 ns
N	4	3.3*	1.2 ns	2.1 ns	0.4 ns	1.4 ns	1.4 ns
K	2	11. 8**	3.78*	3.6*	3.1*	0.5 ns	0.5 ns
N*K	8	0.4ns	0.5 ns	0.8 ns	0.4 ns	1.0 ns	1.0 ns

[§] F values; ns: non-significant; * and ** significant at 5 and 1% probability level, respectively. Means with the same letter are not significant at $p<0.05$ using LSmeans.

The interaction between N and K on cauliflower development at the vegetative stage was not significant in terms of cauliflower growth variables (Table 3.3). At 30 DAT, the highest plant TFW was observed under the treatments of $N_{165}K_{25}$ and $N_{220}K_{25}$, however, no significant difference was found when compared to $N_{165}K_{50}$. Under all N treatments, K_{25} had the highest plant TFW and TDW. An increase K rate from 25 to 50 kg/ha decreased cauliflower growth variables with 2-16% decrease in plant TFW and 0.4 -17% in TDW. When low N rates of 55 kg /ha were applied, K_{50} resulted in a decline of 16 % and 13% in plant biomass and dry matter, respectively. Nitrogen rates had a significant impact on affecting cauliflower development during the vegetative stage. It became obvious that cauliflower growth increased as N application rose especially under the 25 kg/ha K treatment and to a less degree in the

50 kg/ha K treatment. Plant TFW, TDW and leaf DW were greatly increased with N rates under K_{25}.

3.2.2. Effects of N and K on Cauliflower Plant Growth and Curd Initiation

3.2.2.1 N and K Effects on Plant Growth Variables

Cauliflower curd initiation occurred at 47 days after transplant. N had significant effects on most of the measured variables. Increasing N application rates increased cauliflower growth in terms of plant TFW, leaf number and plant height (Table 3.4). There was a significant difference for those variables between N_0 and N treatments. Among N_{110}, N_{165} and N_{220}, no difference was observed on the growth of cauliflower plants, whereas N_{55} resulted in a significant low plant TFW. Cauliflower plants grew significantly slower with low N rates of 55 kg/ha than 220 kg/ha. No significant differences were found among N treatments, whereas plant TDW appeared to increase with increasing N rates and there was a maximum 13% increase from N_0 to N_{220} (Table 3.4). The main effect of K on cauliflower plant growth was more significant compared to N effect. K fertilization significantly influenced all the measured variable of cauliflower plants at 47 DAT (Table 3.4). Without K application, plant TFW, TDW and leaf number were significantly reduced. However, the higher K application (K_{50}) did not promote cauliflower growth variables compared to K_{25}, as no significant difference was observed.

Table 3.4 ANOVA of N and K effects on cauliflower growth variables at 47 DAT

Source	Rate (kg/ha)	plant TFW	plant TDW	leaf number	Plant height
N	0	1288c	179a	14b	46c
	55	1503b	186a	15bc	48bc
	110	1630ab	196a	15ab	50ab
	165	1626ab	197a	15ab	51ab
	220	1803a	203a	16a	53a
K	0	1372b	172b	14b	47b
	25	1670a	206a	15a	51a
	50	1668a	198a	15a	50a
ANOVA[§]	d.f.				
Block	3	0.4 ns	0.2 ns	2.1 ns	1.3 ns
Block*K	6	3.6**	2.9*	1.9 ns	4.4*
N	4	7.0**	0.9 ns	4.5*	5.9*
K	2	9.5**	5.4**	8.9**	5.5*
N*K	8	0.5 ns	1.1 ns	1.5 ns	1.0 ns

[§] F values; ns: non-significant; * and ** significant at 5 and 1% probability level, respectively. Means with the same letter are not significant at $p<0.05$ using LSmens.

3.2.2.2 Interaction of N and K on Plant Growth Variables

There was no significant interaction between N and K on cauliflower growth at 47 DAT. Regardless of K treatment, plant TFW, DW, and plant height were increased by high applications of N (data not presented). When lower N rates from 0 to 110 kg/ha were applied, K50 increased the growth of cauliflower plants on the measured variables. When increasing N applications from 110 kg/ha, an increased K rate from 25 to 50 kg/ha tended to decrease plant TFW and TDW; while total leaf number and plant height were increased. The treatment combination of $N_{220}K_{25}$ had the highest plant fresh biomass, dry matter and plant height. At 47 DAT, cauliflower leaf number varied from 13 to 17. For most NK treatments, the leaf number was around 15. The highest leaf number of 17 was obtained in $N_{220}K_{50}$, which had the

second highest plant TFW. In the control plots, N_0K_0 resulted in poor cauliflower growth on the basis of plant TFW, leaf number and plant height.

3.2.2.3 Effects of N and K on Head Initiation

N application did not influence the curd initiation of cauliflower plants, as N application of 110-220 kg/ha did not result in significant differences in head TFW and size when compared with the control (Table 3.5). However, cauliflower plants grown in N_{55} had the lowest head TFW and the smallest head size both of which significantly differed from N_{220}. Compared with the control K_0, head TFW and size were significantly increased by K_{25}, resulting in a 30 mm increase of the mean head size at 47 DAT. Additionally, N and K fertilizations did not have significant interaction effect on cauliflower curd initiation. At 47 DAT, cauliflower heading occurred and average head size ranged from 1.6 to 2.5 cm under the 15 treatments of NK (data not presented). The largest head size of 2.5 cm at 47 DAT was obtained in $N_{220}K_{50}$, which also had the highest head TFW. No significant difference was found between $N_{220}K_{25}$ and $N_{220}K_{50}$.

Table 3.5 N and K effect on cauliflower head total fresh weight and size at 47 DAT

Source	Rate (kg/ha)	head TFW (g)	head size (cm)
N	0	10ab	2.0ab
	55	8b	1.7b
	110	14a	2.1ab
	165	11ab	2.0ab
	220	15a	2.2a
K	0	9b	1.8b
	25	13a	2.1a
	50	12ab	2.0ab

Means with the same letter are not significantly different at 0.05 using LSmeans.

3.2.3 Effects of N and K on Cauliflower Plant and Head at Early Heading Stage

3.2.3.1 N and K Effects on Plant Growth Variables

The interaction effect of N and K was not significant on most of the measured plant growth variables in terms of whole plant TFW, leaf-stem-root DW, leaf number and plant height in cauliflower at 60 DAT (Table 3.6). However, there was a significant interaction of N and K found for whole plant TDW. The two factors of N and K have showed significant effects on cauliflower growth variables. In contrast to K, N appeared to have a greater effect on cauliflower on the basis of the number of growth variables at P=0.01 (Table 3.6).

Table 3.6 ANOVA of N and K effects on cauliflower plant growth variables at 60 DAT

Source	d.f.	plant TFW	plant TDW	L,S&R DW	leaf number	plant height
Block	3	7.7**	2.0 ns	3.5*	7.3**	2.8 ns
Block*K	6	2.2 ns	1.9 ns	1.5 ns	2.8*	1.0 ns
N	4	30.6**	9.4**	10.4**	28.1**	17.5**
K	2	17.3**	11.1**	6.7*	5.3*	4.0*
N*K	8	1.3 ns	2.4*	2.1 ns	0.8 ns	1.0 ns

[§] F values; ns: non-significant at P=0.05; * and ** significant at 5 and 1% probability level, respectively. plant: whole plant; L, S&R: leaf, stem & root.

Results indicated that N and K applications significantly increased cauliflower whole plant TFW, TDW, leaf-stem- root (plant) DW, leaf number and plant height (Table 3.7). N_0 and K_0 resulted in the lowest values for cauliflower growth variables from N and K treatments, respectively. There were no significant differences between applied K rates 25 and 50 kg/ha on cauliflower growth variables, but K_{25} tended to have a higher whole plant TFW and TDW. Whole plant TFW and TDW, leaf-stem-root DW, and leaf number increased as N application rate increased. Whole plant TFW increased by 35-79% as N rates increased from 55 to 220 kg/ha. An increase in

N application increased whole plant TDW, however, no significant difference was obtained among N_{110}, N_{165} and N_{220}. The trend of cauliflower plant growth under N treatments can be observed through plant height (Figure 3.1). With N application of 220 kg/ha, cauliflower grew significantly higher.

Table 3.7 N and K effects on cauliflower plant growth variables at 60 DAT

Source	Rate (kg/ha)	plant TFW	plant TDW	L,S&R DW	leaf number	plant height
N	0	2228d	303c	87c	15d	48d
	55	3006c	363b	101b	17c	54c
	110	3414b	379ab	103b	18b	57bc
	165	3549b	395ab	111b	19ab	58b
	220	3972a	414a	127a	19a	62a
K	0	2801b	330b	96b	17b	54b
	25	3534a	392a	108a	18a	57a
	50	3366a	391a	114a	18a	56ab

Means with the same letter are not significantly different at $p<0.05$ using LSmeans. Plant: whole plant; L, S&R: leaf, stem & root.

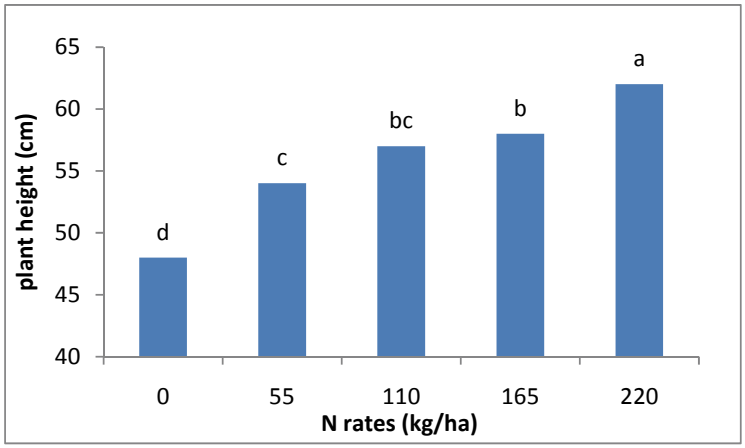

Figure 3.1 Plant height versus applied N rates at 60 DAT

3.2.3.2 Interaction of N and K on Plant Growth Variables

At 60 DAT, the cauliflower plants were at the early heading stage with a total leaf number of 15-17. As mentioned earlier the interaction between N and K did not

have a significant impact on cauliflower plants. $N_{220}K_{50}$ had the best cauliflower growth on the basis of whole plant TFW, TDW and leaf-stem-root DW. However, among treatments $N_{220}K_{50}$, $N_{220}K_{25}$ and $N_{165}K_{50}$, there were no significant differences in whole plant TFW, leaf number and plant height.

From the plot of the interaction NK fertilization on whole plant TFW (Figure 3.2), the highest cauliflower plant biomass was obtained with $N_{220}K_{50}$. Cauliflower whole plant TFW was increased by K application (0-25 kg/ha), but decreased by an increased K rate from 25-50 kg/ha at the N rates of 0, 55 and 110 kg/ha. With the N rate of 220 kg/ha, K rates above 25 kg/ha did not affect cauliflower growth on the basis of whole plant TFW. Cauliflower whole plant TFW increased with N rates only when K_{50} was applied. When K_{25} and K_0 were applied, whole plant biomass was not affected with N applications from 55 to 165 kg/ha but increased above 165 kg/ha.

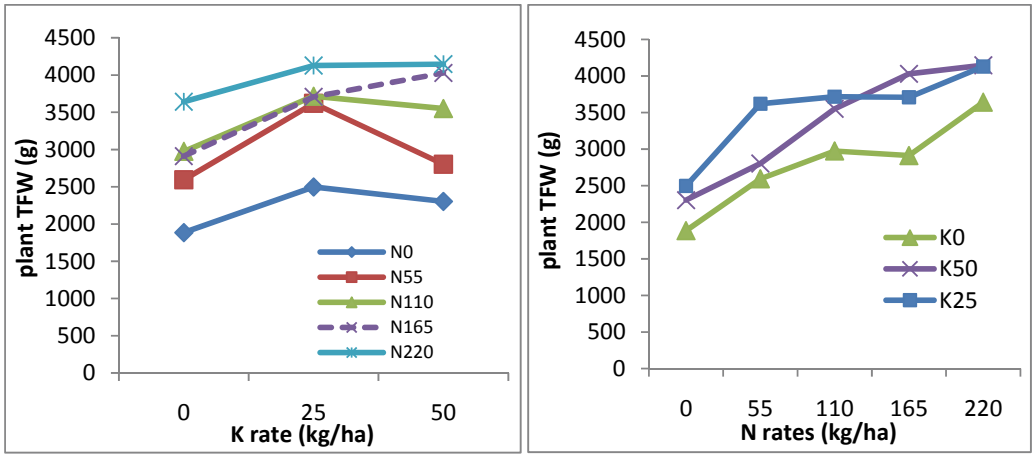

Figure 3.2 Interaction of N and K on cauliflower plant total fresh weight (TFW)

3.2.3.3 Effects of N and K on Early Head Development

N and K influenced cauliflower inflorescence development at 60 DAT. Cauliflower head TFW, TDW and head size were significantly increased by N

fertilizer input (Table 3.8). N and K fertilization affected head TDW and size the same way as head TFW. N_0 had the lowest head TFW and TDW as well as the smallest head size. No significant difference was obtained between the rest of the N rates (Table 3.8). In contrast to no K treatment, both K_{25} and K_{50} greatly increased head TFW, TDW and head size at 60 DAT. The higher K application significantly reduced curd yield and size of cauliflower compared to K_{25} treated cauliflower. The lower K rate produced the largest cauliflower head accompanied with the highest head fresh biomass and dry matter.

Table 3.8 N and K effects on cauliflower head yield and size at 60 DAT

Source	Rate (kg/ha)	Head TFW	Head TDW	Head size
N	0	282b	44b	7.1b
	55	418a	55a	8.1a
	110	450a	57a	8.3a
	165	424a	55a	8.0a
	220	454a	56a	8.4a
K	0	296c	44c	7.2c
	25	524a	64a	8.8a
	50	396b	52b	8.0b

Means with the same letter are not significantly different at $p<0.05$ using LSmeans.

The treatment combinations of N and K affected head development in cauliflower. Interaction of N and K application demonstrated similar effects on cauliflower curd yield, dry matter and head size as shown from the interaction plots in Figure 3.4. The interaction plot for cauliflower head TDW is not shown here. N application increased cauliflower head yield and size in the 25 kg/ha K application. However, a further increase in K rate (25-50 kg/ha) significantly reduced cauliflower yield and resulted in smaller head size at the N rates of 0, 55, 110 and 165 kg/ha. With N application of 220 kg/ha, head yield and size linearly increased with K rates applied. At 60 DAT, the maximum head size of cauliflower during early heading stage reached

9.6 cm under the treatment combination of N_{110} and K_{25}, in which the highest head TFW was obtained (Figure 3.4). There was no significant difference between $N_{110}K_{25}$ and $N_{55}K_{25}$. Interestingly, when K_{25} was applied, low N application (0-110) consistently increased cauliflower head biomass and size; whereas both greatly decreased with increasing N rates from 110 to 220 kg/ha. N_{110}, N_{55} and N_{165} applied with K_{25} produced the highest curd yield of cauliflower. It is suggested that K_{25} produced the best cauliflower production with respect to curd yield and head size.

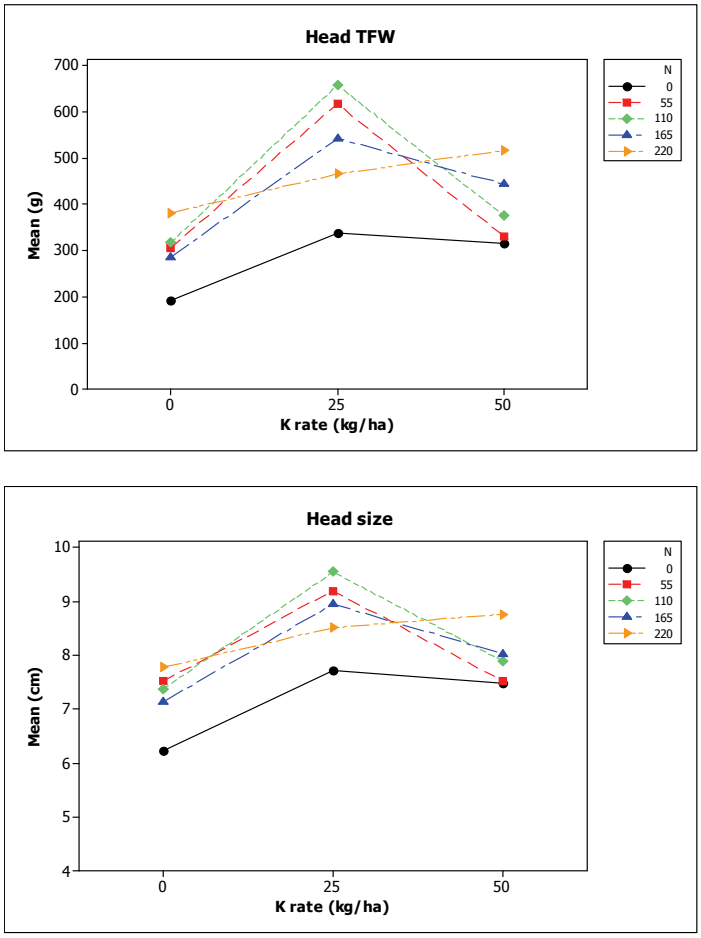

Figure 3.4 Interaction of N and K on head total fresh weight and size at 60 DAT

3.2.4 Effects of N and K on Cauliflower Yield and Quality at Head Maturity

3.2.4.1 N and K Effects on Cauliflower Plant and Head

Cauliflower plant and curd yield at harvest were affected by N application. Among N rates, differences were obtained on whole plant TFW and TDW, head yield and size in cauliflower (Table 3.9). N input significantly improved curd yield. Cauliflower head biomass and size increased with N rates. N application resulted in significantly higher amount of whole plant biomass and dry matter compared with no N treatment. Cauliflower whole plant biomass increased as N rates increased; however, there was no significant difference on whole plant TDW with increasing N rates. N_{220} produced the highest head size on average and head yield. Head TFW in N_{220} was significantly higher than N_{165}. Cauliflower head size varied from 18.3 to 20.1 cm among N application treatments. Cauliflower head fresh yield increased by 37% when increasing N application rate from 55 to 220 kg/ha, while head size increased by approximately 2 cm.

K application showed significant effects on increasing whole plant biomass and dry matter, curd yield, dry weight and size in cauliflower (Table 3.9). K_{50} decreased plant whole TFW and head TFW in cauliflower, although no statistically significant difference was found between K_{25} and K_{50}. The largest head size was obtained at 19.2 cm under the K_{25} treatment. Comparing main effects, N had a greater effect than K as N_{220} resulted in the largest head size of 20.1 cm.

Table 3.9 ANOVA of N and K effect on cauliflower plant, curd yield and size at harvest

Source	Rate (kg/ha)	plant TFW	plant TDW	head TFW	head TDW	head size
N	0	4573d	629b	2029d	217c	16.8d
	55	6573c	856a	3011c	309b	18.3c
	110	7138bc	857a	3146c	301b	18.5bc
	165	7869ab	856a	3691b	332b	19.3ab
	220	8742a	935a	4125a	375a	20.1a
K	0	6095b	719b	2829b	269b	17.8b
	25	7686a	905a	3507a	339a	19.2a
	50	7156a	856a	3265a	312a	18.8a
ANOVA[a]	d.f.					
Block	3	5.9**	4.0*	18.4**	18.0**	15.7**
Block*K	6	0.4 ns	1.2 ns	1.0 ns	2.0 ns	1.3 ns
N	4	21.5**	11.5**	31.5**	20.8**	17.7**
K	2	7.7**	10.5**	7.5**	8.2**	7.9**
N*K	8	0.3 ns	0.3 ns	0.7 ns	1.1 ns	1.2 ns
Contrast[b]						
0 vs K rates	1	15.8**	20.9**	17.9**	16.4**	15.8**
25 & 50	1	1.0 ns	0.4 ns	1.0 ns	0.9 ns	0.4 ns
0 vs N rates	1	60.7**	50.0**	83.2**	60.7**	45.1**
55 vs 110,165 &220	1	11.5**	0.2 ns	15.0**	2.3 ns	7.7*
110 vs 165 & 220	1	8.0*	1.1 ns	19.5**	11.8**	12.0**
165 vs 220	1	3.7 ns	3.5 ns	5.4*	7.2*	4.2 ns

[a, b] F values, ns: non-significant at P=0.05; * and ** significant at 5 and 1% probability level, respectively. Means with the same letter are not significantly different at $p<0.05$ using LSmeans.

3.2.4.2 Interaction between N and K on Cauliflower Plant and Head

The interaction effects of N and K fertilization on cauliflower whole plant fresh biomass and dry matter, curd yield and head size were presented in Table 3.10. Under all the K applications, cauliflower whole plant TFW tended to increase with increasing N rates (Figure 3.5). When increasing N rates from 110 to 220 kg/ha, the magnitude of increase in cauliflower whole plant TFW was significantly higher in K_{50} (38%) than in K_0 (16%) and K_{25} (16%). Regardless of N application rates, K_{25}

significantly increased whole plant TFW. The K application of 50 kg/ha decreased whole plant TFW under N applications below 220 kg/ha.

The interaction of N and K influenced cauliflower head yield the same way as cauliflower plant TFW. A similar trend was observed on the changes of cauliflower head TFW under NK treatment combinations. In addition, cauliflower in $K_{25}N_{220}$ produced the highest whole plant TFW and head fresh yield. $K_{25}N_{165}$ and $K_{50}N_{220}$ did not result in significant differences of whole plant TFW compared to $K_{25}N_{220}$. However, $K_{25}N_{220}$ resulted in a significantly high cauliflower head yield, which was approximately 12-14% higher than K25N165 and $K_{50}N_{220}$.

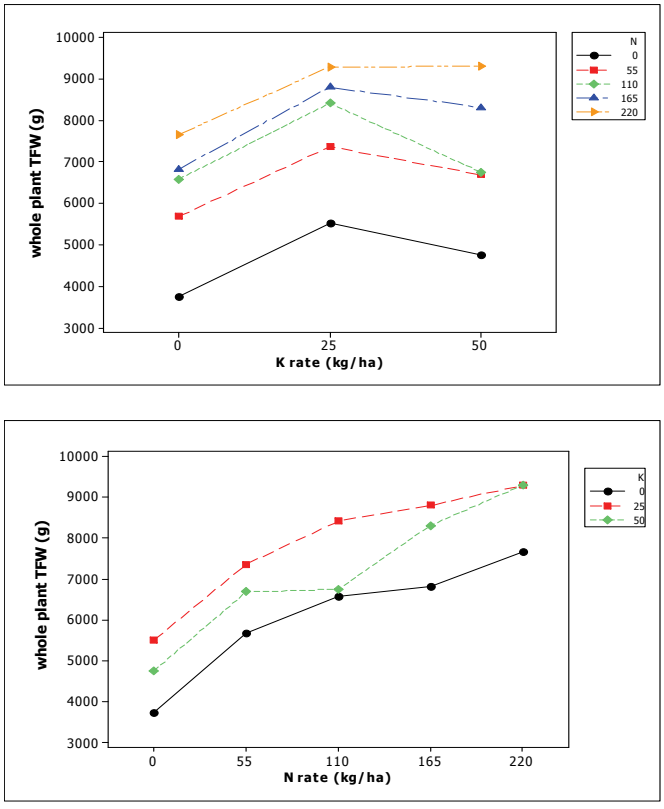

Figure 3.5 Interaction of N and K on cauliflower whole plant total fresh weight (TFW)

Table 3.10 Interaction of N and K effect on cauliflower plant, curd yield and size at harvest

K (kg/ha)	N (kg/ha)	plant TFW	plant TDW	head TFW	head TDW	head size
0	0	3747 f	535 f	1811 g	207 g	16.1 g
	55	5681 de	742 ef	2542 def	266 defg	17.1 efg
	110	6575 cd	772 cde	2994 cde	267 defg	18.5 cde
	165	6820 bcd	733 de	3242 bc	281 cdef	18.3 cde
	220	7654 bc	812 bcde	3555 bc	326 bcd	19.1 bcd
25	0	5209 def	667 ef	2284 efg	209 fg	17.6 defg
	55	7356 bc	951 abc	3320 bc	342 bc	18.9 bcd
	110	8096 abc	949 abc	3536 bc	351 abc	19.1 bcd
	165	8484 ab	935 abc	3968 ab	370 ab	19.7 abc
	220	9283 a	1037 a	4425 a	422 a	20.7 a
50	0	4765 ef	685 ef	1990 fg	235 efg	16.7 fg
	55	6682 cd	888 abcd	3171 cd	320b cd	18.9 bcd
	110	6744 cd	849 bcde	2907 cde	285 cde	17.8 def
	165	8302 ab	900 abcd	3864 ab	344 bc	20 ab
	220	9289 a	958 ab	4394 a	376 ab	20.7 a

Means with the same letter are not significantly different at $p<0.05$ using LSmeans

For dry matter accumulation of cauliflower whole plant and head, N and K treatments demonstrated similar effects. Cauliflower grown with K applications of 25 kg/ha had the greatest whole plant and head dry matter under all N treatments. In control N_0 plots, K application of 50 kg/ha did not differ in whole plant or head dry matter accumulation from that of the 25 kg/ha treatment (Figure 3.6). N had an important impact on plant and head dry matter accumulations, as dry matter accumulation in N_{220} treatments, irrespective of K application rates, were higher than other N rates. Total whole plant and head dry matter were not influenced by N applications from 55 to 165 kg/ha, but increased when N rates of 220 kg/ha were applied (Table 3.10).

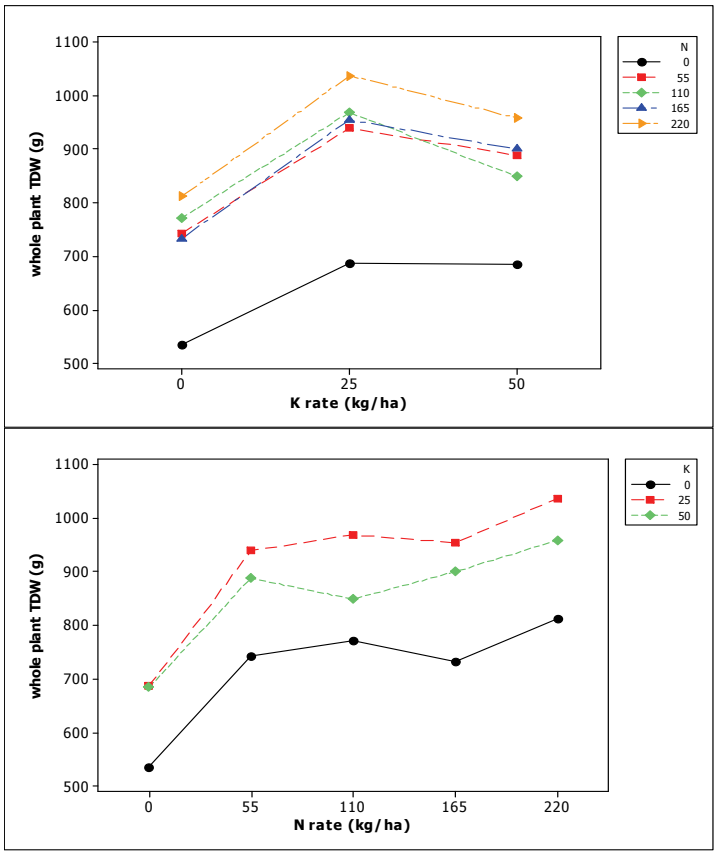

Figure 3.6 Interaction of N and K on cauliflower whole plant total dry weight (TDW)

3.2.4.3 Effects of N and K on Head Nutrient Concentration

K concentration in cauliflower heads was not affected by either N or K fertilizers (Table 3.11). K application did not influence N concentration in cauliflower heads. However, cauliflower head P concentration was significantly increased by K application compared to the control. Higher K rates slightly increased head P concentration whereas no significant difference was obtained between K_{25} and K_{50} (Table 3.11). N did not significantly impact nutrients K and P concentration in cauliflower heads. A medium N rate of 110 kg/ha resulted in the highest head K and P concentrations. In addition, head N concentrations increased linearly with increasing

N application rates. No significant difference in head N concentration was found between N_{165} and N_{220}.

Table 3.11 ANOVA of N and K effect on cauliflower head nutrient concentration (mg/gDW) at harvest

Source	Rate (kg/ha)	K conc. (mg/g)	P conc. (mg/g)	N conc. (mg/g)
N	0	36.0a	4.3a	21.4c
	55	35.7a	4.3a	24.5bc
	110	37.5a	4.6a	28.2b
	165	37.4a	4.5a	32.8a
	220	35.8a	4.5a	33.4a
K	0	35.9a	4.2b	28.9a
	25	36.5a	4.5a	27.5a
	50	37.0a	4.4ab	27.9a
ANOVA[§]	d.f.			
Block	3	0.3 ns	2.6 ns	1.6 ns
Block*K	6	2.4 ns	1.2 ns	4.7**
N	4	2.0 ns	1.2 ns	20.9**
K	2	0.2 ns	2.8*	1.4 ns
N*K	8	1.1 ns	0.2 ns	0.6 ns

[§]F values, ns: non-significant at P=0.05; * and ** significant at 5 and 1% probability level. Means with the same letter are not significantly different at $p<0.05$ using LSmeans.

3.2.5 Comparison of N/K Effects on Cauliflower at Different Growth Stages

3.2.5.1 Plant Biomass

Comparisons between N and K effects on cauliflower whole plant TFW at different growth stages through the life cycle were conducted. The interaction effect of N and K was not significant on cauliflower plant growth over time. N, K and date of sampling were the main significant factors affecting cauliflower whole plant biomass at 30, 47, 60 DAT and at harvest that are referred to as vegetative growth stage, curd initiation, early heading and head maturity. The interaction N* time and K* time were significant (P <0.05) on cauliflower whole plant TFW (Table 3.12).

Table 3.12 Fixed effects of applied N, K and sampling date on cauliflower whole plant biomass during the growing season

Source	DF	F value	P value
time	3	1304.4	<.0001
N	4	42.45	<.0001
time*N	12	15.29	<.0001
K	2	26.38	<.0001
time*K	6	7.03	<.0001
N*K	8	0.49	0.861
time*N*K	24	0.5	0.977

When looking at plant growth at different stages, whole plant biomass of cauliflower under the main factors of N and K increased exponentially as plants grew overtime. There was approximately a 3-fold increase in plant biomass from the vegetative growth stage (30 DAT) to head initiation at 47 DAT, a 2-fold increase from 47 DAT to the early heading stage at 60 DAT, and an additional 2-fold increase from 60 DAT to harvest (data not present). The effect of N on cauliflower plant growth at different growth stages is shown in Figure 3.7. Cauliflower whole plant biomass significantly increased with increased N rates. N effect on whole plant biomass became more significant starting from 60 DAT at the early head stage. N applications from 55 to 220 kg/ha produced significantly higher whole plant biomass when compared with N_0. Figure 3.8 shows cauliflower plant biomass under K applciaton treatments at different growth stages. In contrast to N application, K had less of an effect on the cauliflower plant. At each growth stage there was no sigfincant difference in whole plant biomass between K_{25} and K_{50}, while a marked reduction was seen with K_{50} which can be seen at the head maturity stage (Figure 3.8). However, whole plant biomass over time was signficantly enhanced by K applications at vegetative stage, head initiation, early heading and head maturity.

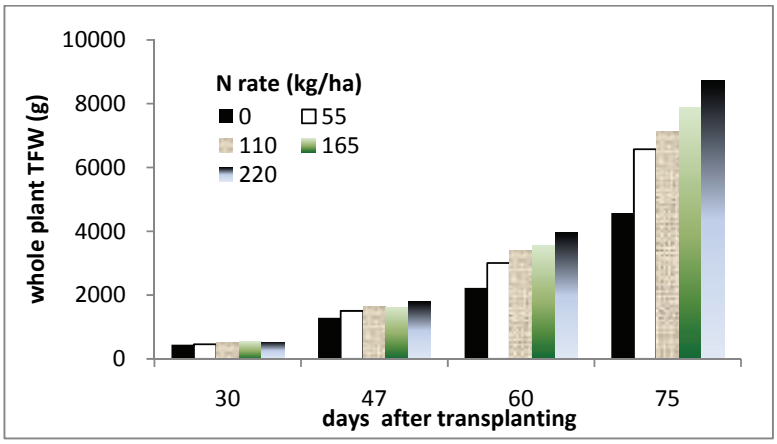

Figure 3.7 Whole plant total fresh weight versus growth stage under N treatments

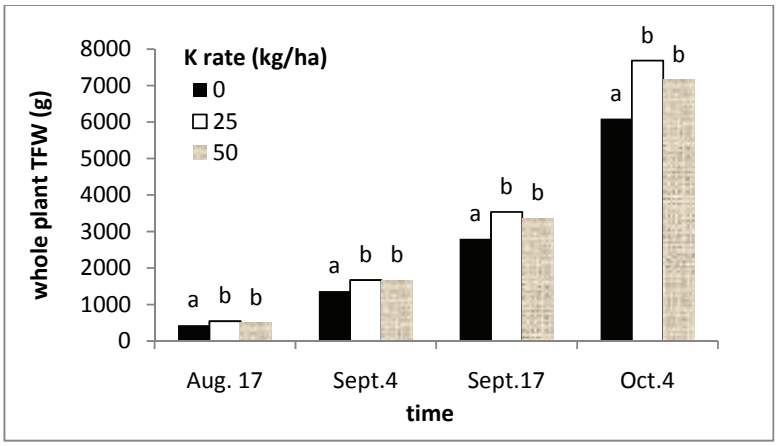

Figure 3.8 Whole plant total fresh weight versus growth stage under K treatments

3.2.5.2 Leaf Number

Cauliflower leaf number was affected by the sampling date, and N and K fertilization. The interactions between N *time, K * time, N * K, and N* K * time did not have significant impacts on cauliflower leaf number during the life cycle (Table 3.13). From head initiation (47 DAT) to the early heading (60 DAT) cauliflower leaf number significantly increased (P<0.001). N and K fertilization effects were both significant on leaf number (Table 3.14). Cauliflower plants with higher N rates tended to have more leaves. At head initiation, leaf number did not vary among N treatments

expect N_0, but varied at the early heading stage when N_{55} had significantly lower total leaf number than other N rates. This suggests N effects on cauliflower leaf number became significant during late growth stages. K fertilization promoted cauliflower plant growth as indicated by increased leaf number of cauliflower plants. However, higher levels of applied K_{50} did not produce more leaves than K_{25} at 47 DAT and 60 DAT during head development.

Table 3.13 Effects of applied N, K and sampling date on cauliflower leaf number

Source	DF	F value	P >F
time*N	4	1.76	0.1434
time*K	2	0.02	0.9824
N*K	8	1.19	0.3136
time*N*K	8	0.44	0.8912

Table 3.14 N and K effects on plant leaf number at different sampling date (SE)

Source (p-value)	Rate (kg/ha)	47 DAT	60 DAT	Average per SE
N	0	14b	15c	14
(<0.001)	55	15ab	17b	15
	110	15a	18a	16
	165	15ab	19a	17
	220	16a	19a	17
K	0	14b	17b	15
(<0.001)	25	15a	18a	16
	50	15a	18a	16
Date (<0.001)		15a	17b	N/D

Means with the same letter are not significant at p<0.05 using LSmeans.

3.2.5.3 Head Yield and Size

The effects of N and K on cauliflower head development with respect to head yield and head size were investigated as presented in Table 3.15. Cauliflower head yield and size were influenced by the main factors N, K and sampling date, which

were referred to as cauliflower head developmental stage. In contrast to K effect, N fertilization had a greater effect on cauliflower head yield (based on the higher F value), however, cauliflower head size was more affected by K fertilizer application than by N (Table 3.15). The interaction between N*time and K*time significantly affected cauliflower head yield and size. However, N*K and N*K*time did not show any significant effects on cauliflower head.

Table 3.15 Fixed effects of applied N, K and sampling date on cauliflower head fresh yield and size during the growing season

		Head yield		Head size	
Source	DF	F value	P >F	F value	P >F
time[§]	2	1502.59	<.0001	3354.25	<.0001
N	4	17.3	<.0001	8.83	<.0001
time*N	8	18.59	<.0001	3.79	0.0006
K	2	11.4	<.0001	16.95	<.0001
time*K	4	6.72	<.0001	2.51	0.0455
N*K	8	0.54	0.8211	0.60	0.7759
time*N*K	16	0.51	0.9356	0.62	0.8608

[§]time: 47 DAT, 60 DAT and at harvest

Cauliflower head initiation occurred around 47 DAT and heads achieved an overall width of 2 cm. From early heading at 60 DAT to head harvest, cauliflower plants developed the heads rapidly within approximately 2 weeks. There was an 8 fold increase in head fresh yield accompanied with a10-11 cm increase in head width (Table 3.16). N fertilization played an important role on cauliflower head development. Cauliflower head yield and size were significantly increased with increased N rates. In contrast to the control (N_0), N_{220} doubled cauliflower yield and produced 18.4% larger heads. Overall, there were nearly 34% and 10% increases in head yield and size when increasing N application from 55 to 220 kg/ha (Table 3.16). Throughout the head development of cauliflower N_{220} resulted in the highest cauliflower head fresh yield and largest head size. At head initiation (47 DAT) and

the early head stage (60 DAT), there were no significant differences in both cauliflower head fresh yield and size among N_{220}, N_{165} and N_{110}. However, at head maturity, increasing N rates increased harvested cauliflower head yield, and head TFW in N_{220} was significantly higher than N_{165} and N_{110} (Figure 3.9). Prior to head maturity, cauliflower head size did not vary among N treatments. At harvest, an average head size of 20 cm was obtained in N_{165} and N_{220} that was much larger than heads from plants treated with N rates below 165 kg/ha.

Table 3.16 Effects of applied N, K and sampling date on cauliflower head fresh yield and head size during the growing season

parameter		head yield (g)				head size (cm)			
Source (p-value)	Rate (kg/ha)	47 DAT	60 DAT	Harvest	Avg	47 DAT	60 DAT	Harvest	Avg
N	0	10	282	2029	798	2.0	7.1	16.8	8.7
(<0.01)	55	8	418	3011	1146	1.7	8.1	18.3	9.4
	110	14	450	3146	1227	2.1	8.3	18.5	9.7
	165	11	424	3691	1399	2.0	8	19.3	9.8
	220	15	454	4125	1531	2.2	8.4	20.1	10.3
K	0	9	296	2829	1045	1.8	7.2	17.8	8.9
(<0.01)	25	13	524	3507	1392	2.1	8.8	19.2	10.1
	50	12	396	3265	1223	2.1	8.0	18.8	9.6
Date (<0.01)		12	405	3245		2.0	8.0	18.7	

Contrast	d.f.	p-value	p-value
47 DAT vs 60 DAT & harvest	1	<.0001	<.0001
60 DAT vs harvest	1	<.0001	<.0001
0 vs K rates	1	<.0001	<.0001
25 & 50	1	0.0074	0.0097
0 vs N rates	1	<.0001	<.0001
55 vs 110, 165 & 220	1	0.0002	0.0120
110 vs 165 & 220	1	0.0007	0.0888
165 vs 220	1	0.0943	0.0990

At 47 DAT, the K effect on cauliflower head initiation was not obvious because of small variation on head fresh weight and head size. However, K application had a significant impact on increasing cauliflower head yield and size at early heading (60 DAT) and head maturity. Overtime there were significant (P= 0.01)

differences between K_{25} and K_{50} on affecting both head yield and size (Table 3.16). However, interesting results have been obtained. At 60 DAT, the 50 kg/ha K fertilizer rate significantly reduced fresh yield of early cauliflower head as well as head size by 32% and 10% respectively, when compared to the 25 kg/ha K application. At harvest, there was no significant difference between K_{25} and K_{50} on harvested cauliflower head. Throughout head development, K_{25} produced the highest head yield and largest head size (Table 3.16; Figure 3.9). Therefore, K_{25} is suggested to be the best K application for producing better cauliflower heads in terms of fresh weight and size.

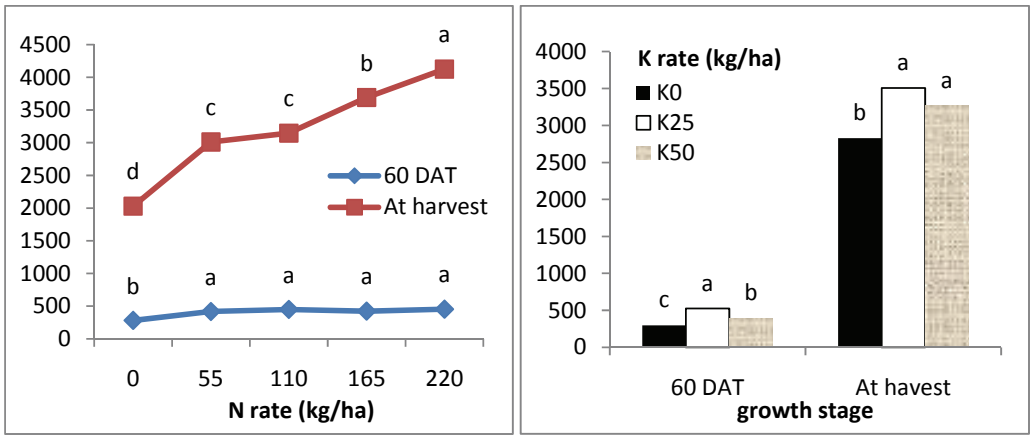

Figure 3.9 Cauliflower head fresh yield versus N rates and K rates at the early heading stage (60 DAT) and head maturity (at harvest).
Means with the same letter indicate non-significant differences at 0.05.

3.2.6 N Uptake in Cauliflower Plant and Head

Cauliflower leaves play a critical role as a N source to support cauliflower head development. At the vegetative stage (30 DAT), leaf N content was related with applied N. Leaf N concentration was significantly increased by N fertilization input (Figure 3.10). Higher N rate applied produced higher leaf N concentration and total N accumulation. N uptake was significantly high in cauliflower leaves with applied N

rate above 110 kg/ha. This may imply higher N source to transport into the head as the sink and more N accumulation. At the vegetative stage (30 DAT) leaf N concentration (% dry matter) did not vary among K treatments (Table 3.17). However, leaf total N (g/plant) was significantly enhanced by K application. There was no significant difference in leaf total N between K_{25} and K_{50} (Table 3.17). The interaction of N and K fertilizations did not show significant influence on either total N or N concentration in cauliflower leaves at the vegetative stage. Furthermore, during head initiation (47 DAT) K did not influence N concentration and total N uptake in cauliflower whole plant (Table 3.17).

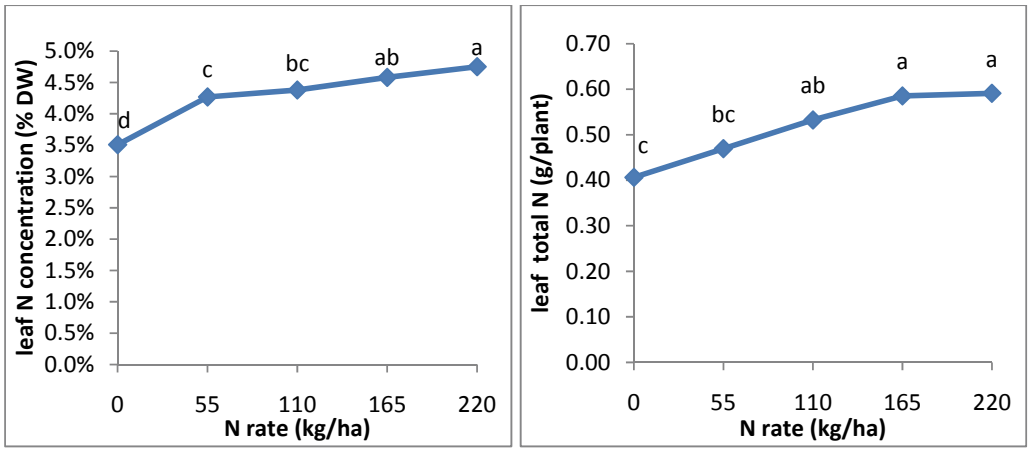

Figure 3.10 Effect of applied N on cauliflower leaf N concentration and accumulation at the vegetative stage (30 DAT).
Means with the same letter indicate non-significant differences at 0.05.

When it comes to total N uptake in cauliflower whole plant, N fertilization demonstrated similar main effects at the two heading stages of head initiation and maturity. Total N uptake was linearly correlated with applied N rates. The N effects at head maturity were significantly greater when compared with the head initiation stage (Table 3.17). Variation of total N uptake among the different N rates varied from 1.0

to 2.2 g/whole plant among NK treatments at 47 DAT during the head initiation stage (data not present here). In contrast, the range of total N uptake was from 3.6 to 10.3 g/whole plant at harvest (Table 3.17). Results indicated that total N uptake at head initiation accounted for 21.3-27.7% of that at harvest, which was around 4 weeks later. This suggests that cauliflower plants take up most of their N (72.3-78.7%) within the last 4 weeks of growth. Therefore, higher use efficiency of N fertilizer in the commercial cauliflower field can be achieved by proper N application management.

Table 3.17 N and K effects on N concentration and total uptake in cauliflower plant on the basis of dry weight during the growing season

Growth Stage		Vegetative stage		Head initiation		Head maturity
		30 DAT		47DAT		Harvest
Source	Rate (kg/ha)	Leaf N (%DW)	Leaf N (g/WP)	WP N (%DW)	N uptake (g/WP)	N uptake (g/WP)
N	0	3.5	0.41c	1.9	1.1d	3.9 e
	55	4.3	0.47bc	2.5	1.5c	6.2 d
	110	4.4	0.53ab	2.8	1.7bc	7.0 c
	165	4.6	0.59a	3.1	2.1a	8.2 b
	220	4.8	0.59a	3.2	2.0ab	9.4 a
K	0	4.2	0.47b	2.7	1.5a	6.5 b
	25	4.3	0.55a	2.6	1.8a	7.1 ab
	50	4.4	0.53a	2.7	1.8a	7.2 a
ANOVA[§]	d.f.					
N	4	27.9**	7.9**	28.3 ns	16.1**	55.10**
K	2	1.6 ns	3.7*	1.1 ns	2.6 ns	3.82*
N*K	8	0.5 ns	0.6ns	1.3 ns	0.7 ns	0.92 ns

[§]F values, ns: non-significant at P=0.05; * and ** significant at 5 and 1% probability level. Means with the same letter are not significantly different at p<0.05 using LSmeans. WP: whole plant.

Throughout the 2009 growing season, there were two dates of cauliflower sampling with harvested cauliflower head for yield determinations. They were respectively at 60 DAT and at harvest which correspond to the early heading stage and head maturity. Overall, the interactive effect of N and K fertilizations was not

statistically significant on N concentration and accumulation in the cauliflower head. N fertilization significantly affected head N concentration and total N in heads of cauliflower at both head development stages. At the early heading stage (60 DAT) K did not have an impact on cauliflower head N concentration (% DW), however, it significantly influenced total N accumulation in the head. A similar effect of K fertilization was obtained on head N concentration and accumulation in cauliflower at head maturity. Total N in the cauliflower head was significantly affected by K applications at harvest (Table 3.18).

Cauliflower head N content is significantly related to N fertilization input. Head N concentration and total N accumulation increased linearly with N application rates. The highest applied N rate of 220 kg/ha had the highest N concentration and accumulation in cauliflower head at 60 DAT and at harvest, which were significantly higher than other N application rates such as N_{55} and N_{110}. There was no difference observed between N_{165} and N_{220} on head N concentration and accumulation (Table 3.18). The main effects of both N and K on head N at 60 DAT were the same as that at harvest. This suggested that N and K effects on head N content were independent of the head maturity status (or head developmental stage) during cauliflower plant growth.

K played a role on N accumulation in cauliflower head, although it did not influence head N concentration. Head N concentration did not vary among K_0, K_{25} and K_{50}. Compared with K_0, K_{25} significantly enhanced N accumulation in cauliflower head at 60 DAT and at harvest, although no differences were found between K_{50} and K_0 at harvest. Increasing K application to 50 kg/ha reduced head N accumulation at both early heading stage and head maturity. K_{25} resulted in the highest N accumulation in cauliflower head throughout head growth. In addition,

cauliflower head N concentration decreased as the head developed. There was a 32.1% drop of head N concentration from early head stage (60 DAT) to head maturity at harvest, while head total N increased by 3.8 times as head dry matter increased (Table 3.18).

In addition to head N concentration, applied N also enhanced N concentration in the cauliflower whole plant at harvest (Table 3.19). The main effects of N and K were significant on N accumulations in both head (sink) and plant leaf-stem-root (source) as well as total N uptake in cauliflower whole plant at harvest. N fertilization positively increased cauliflower head N, plant leaf-stem-root N and total N uptake in whole plant with increasing N rates (Figure 3.11). N_{220} had significantly higher total N uptake in cauliflower whole plant as well as total N in plant leaf-stem-root, whereas it was not significantly difference from N_{165} with respect to total N in the cauliflower head.

Cauliflower plants with K applications appeared to have lower N concentration in both head and plant leaf-stem-root when compared with K_0 treatment (Table 3.19). However, K greatly promoted total N uptake in the cauliflower whole plant. K had less of an impact on cauliflower N uptake from N content variation among K_0, K_{25} and K_{50} (Figure 3.12). There was no difference between K_{25} and K_{50} on affecting N uptake in cauliflower partitions including head, leaf-stem-root and whole plant (Figure 3.12).

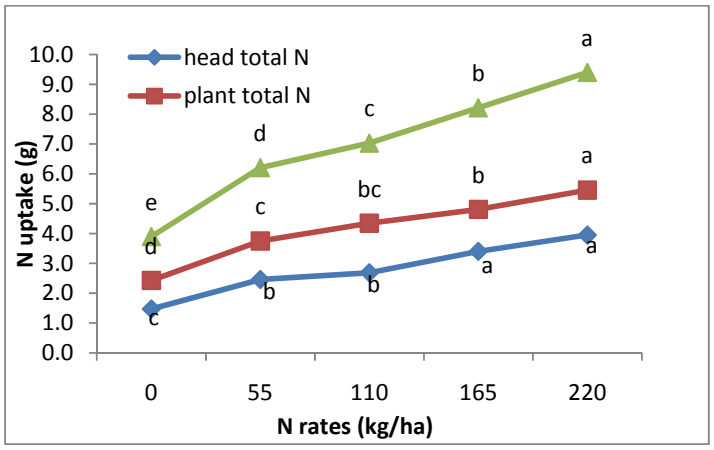

Figure 3.11 Effect of N rates on N uptake in cauliflower head, plant and whole plant at harvest. Means with the same letter for each variable are not significantly different.

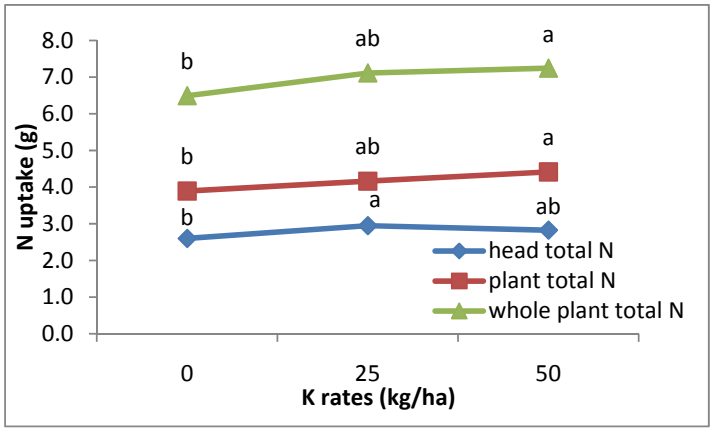

Figure 3.12 Effect of K rates on N uptake in cauliflower head, plant and whole plant at harvest. Means with the same letter for each variable are not significantly different.

Table 3.18 N and K effects on N concentration and total uptake in cauliflower head on the basis of dry weight at 60 DAT and at harvest

		60 DAT		At harvest	
Source	Rate (kg/ha)	Head N (%DW)	N uptake (g/head)	Head N (%DW)	N uptake (g/head)
N	0	2.98 d	0.42 d	2.14 d	1.5 c
	55	3.66 c	0.65 c	2.45 c	2.5 b
	110	4.28 b	0.82 b	2.82 b	2.7 b
	165	4.79 a	0.87 ab	3.28 a	3.4 a
	220	4.99 a	0.93 a	3.34 a	3.9 a
K	0	4.11 a	0.61 c	2.89 a	2.6 b
	25	4.16 a	0.89 a	2.75 a	3.0 a
	50	4.15 a	0.73 b	2.79 a	2.8 ab

Means with the same letter are not significantly different at a =0.05 using LSmeans

Table 3.19 N and K effects on N concentration and total uptake in cauliflower plants on the basis of dry weight at harvest

Source	Rate (kg/ha)	Head N (%DW)	Plant N (%DW)	N uptake (g/head)	N uptake (g/plant)	N uptake (g/whole plant)
N	0	2.14 d	1.9 c	1.5 c	2.4 d	3.9 e
	55	2.45 c	2.2 bc	2.5 b	3.8 c	6.2 d
	110	2.82 b	2.4 b	2.7 b	4.3 bc	7.0 c
	165	3.28 a	2.9 a	3.4 a	4.8 b	8.2 b
	220	3.34 a	3.1 a	3.9 a	5.5 a	9.4 a
K	0	2.89 a	2.7 a	2.6 b	3.9 b	6.5 b
	25	2.75 a	2.4 b	3.0 a	4.2 ab	7.1 ab
	50	2.79 a	2.5 ab	2.8 ab	4.4 a	7.2 a

Means with the same letter are not significantly different at a =0.05 using LSmeans

Cauliflower total N uptake did not significantly differ among varying K rates when combined with N rates (0, 55, 110 and 165 kg/ha) (Figure 3.13). However, when high N rate of 220 kg/ha was applied, K_{25} significantly increased N uptake in cauliflower whole plant compared with $N_{220}K_0$. In addition, whole plant total N in $N_{220}K_{50}$ was 16% higher than that in $N_{220}K_0$. Results suggest that the effect of K fertilization was more significant when combined with high N rate.

Total N uptake in cauliflower ranged from 3.6 to 10.3 g/whole plant (Table 3.20). The maximum N uptake of 10.3 g/plant was achieved in $N_{220}K_{25}$, while that was not significantly different from 9.6 g/plant in $N_{220}K_{50}$. The minimum total N uptake of 3.6 g/plant was obtained in both N_0K_0 and N_0K_{25}. With no N fertilizer applied, the treatments of N_0 and K rates (0, 25 and 50 kg/ha) resulted in significantly lower N uptake in cauliflower head, plant and whole plant than any other NK treatment combinations (Table 3.20). $N_{220}K_{25}$ had the highest N accumulation respectively in cauliflower head and plant leaf-stem-root. No significant difference was found on that between $N_{220}K_{25}$ and $N_{220}K_{50}$. The range of head N concentration was between 2.1% and 3.5%. N concentration did not vary among the treatment combinations of N_{220} or N_{165} and K rates (Table 3.20).

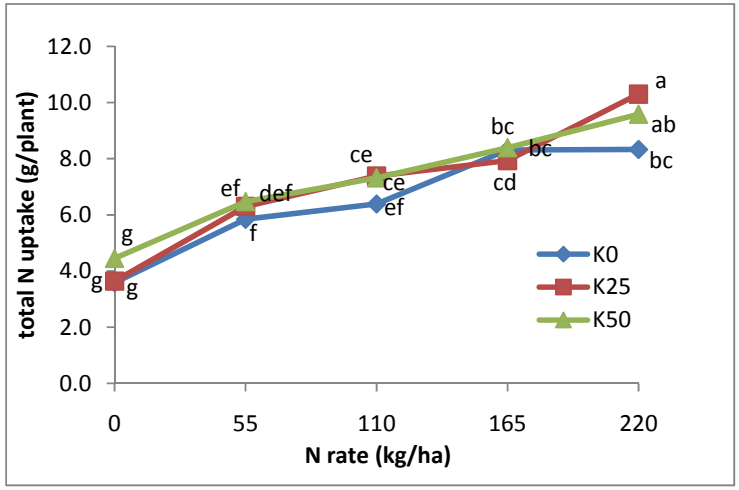

Figure 3.13 Interactive effect of NK treatments on total N uptake in cauliflower whole plant

Table 3.20 Interaction effect of N and K treatment combination on N accumulation in cauliflower head, plant leaf-stem-root and total N uptake in whole plant at harvest

NK treatments (kg/ha) / Variables	K rate =0					K rate =25					K rate =50				
	N0	N55	N110	N165	N220	N0	N55	N110	N165	N220	N0	N55	N110	N165	N
total N uptake (g/plant)	3.6g	5.8f	6.4ef	8.3bc	8.3bc	3.6g	6.3ef	7.4ce	7.9cd	10.3a	4.5g	6.5def	7.3ce	8.4bc	9
plant N (g)	2.1	3.6	3.9	5.0	4.8	2.3	3.7	4.3	4.5	6.0a	2.8	4.0	4.8	4.9	5
head N (g)	1.5	2.3	2.5	3.3	3.5	1.3	2.6	3.1	3.4	4.3a	1.6	2.5	2.5	3.5	4
head N content (%)	2.13	2.60	2.86	3.48	3.36	2.16	2.24	2.85	3.23	3.25	2.15	2.52	2.76	3.13a	3

Means followed by different letters within the same row (variable) are significantly difference at 0.05 using LSmeans.

3.2.7. Relations between Cauliflower Plant, Head, Tissue N and N/K Rates

3.2.7.1 Whole Plant Biomass versus N/K Rates

N and K significantly influenced cauliflower plant growth as mentioned above, whereas plant growth did not significantly respond to NK treatment combination. Regression analysis was conducted in the present study in order to evaluate the relationship between applied N and K rates, cauliflower plant growth and yield. N rates showed a significant positive linear impact on cauliflower whole plant biomass (P <0.0001) that accounted for 47% of variations (Figure 3.14). Interestingly, a quadratic model was also suited to account for the effect of applied N on cauliflower whole plant biomass (Figure 3.14). The effect was highly significant, accounting for 48% of variation (Figure 3.14).

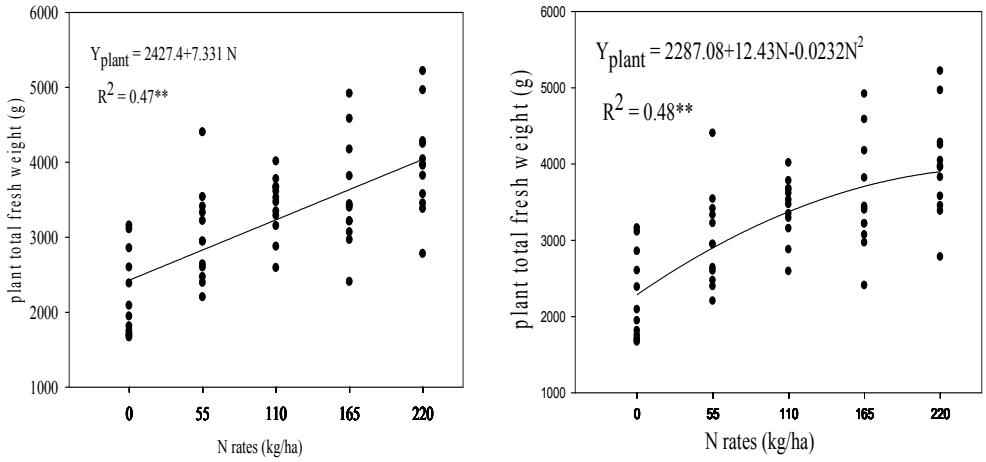

Figure 3.14 Relationship between applied N and cauliflower whole plant biomass

In addition to N rates, K fertilization had a quadratic effect on cauliflower whole plant biomass. This effect was significant (P<0.05), despite that the model explained only 11% of variation (Figure 3.15). The highest cauliflower whole plant biomass was achieved at K_{25}. A linear model was tested and disregarded here due to rather low percentage of total variation explained by the regression model.

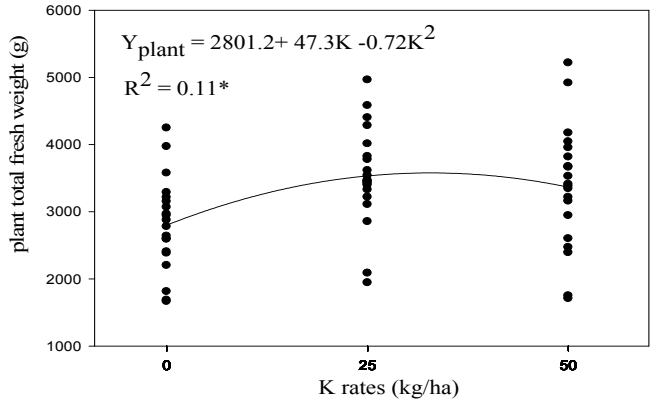

Figure 3.15 Relationship between applied K and cauliflower whole plant biomass

3.2.7.2 Head yield versus N/K rates

At the early heading and head maturity stages, the relation between N, K and cauliflower head yield was studied. Figure 3.16 indicated that cauliflower yield appeared to have a significant (P <0.05) linear relation with N fertilization at early heading stage (60DAT), however, it only accounted for 5.25% of variation. The quadratic model failed to analyze the relationship between the two variables at a significant probability of 0.05. N effect on cauliflower yield became more visual at harvest (Figure 3.17). Cauliflower head yield was linearly related to N rates applied with a significantly high coefficient R^2 =53.4% (P <0.0001).

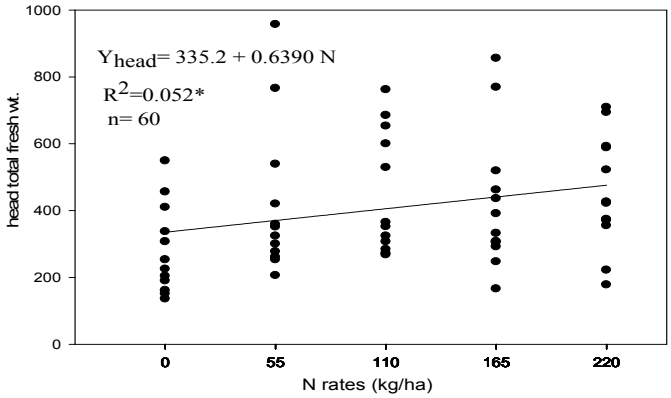

Figure 3.16 Relationship between applied N and cauliflower curd yield at 60 DAT

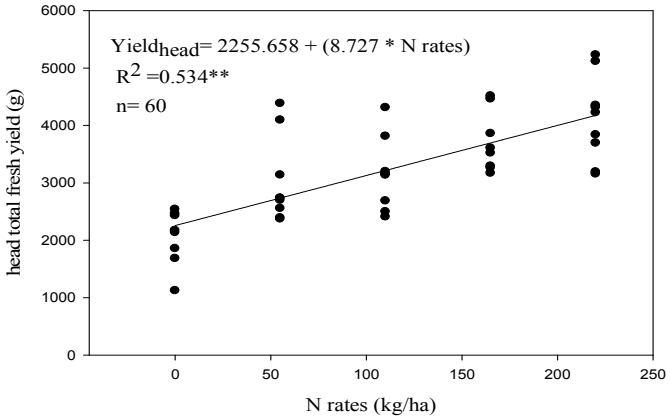

Figure 3.17 Relationship between applied N and cauliflower curd yield at harvest

K rates did not affect head yield as strongly as N rates at either the early heading stage (60 DAT) or head maturity. Unlike N treatments, the limited number of K rates treatments resulted in a relatively high variability when determining the relation between K application rate and head yield. In fact, cauliflower head yield demonstrated a quadratic response to K application during cauliflower head development. At 60 DAT, applied K had a significant quadratic effect on fresh yield of immature cauliflower heads, although 24% of variation was explained (Figure 3.18). Similarly to the early head stage at 60 DAT, there was a marginally significant quadratic relation between K rates and cauliflower head yield at harvest, accounting for 13.5% of variation (figure not present).

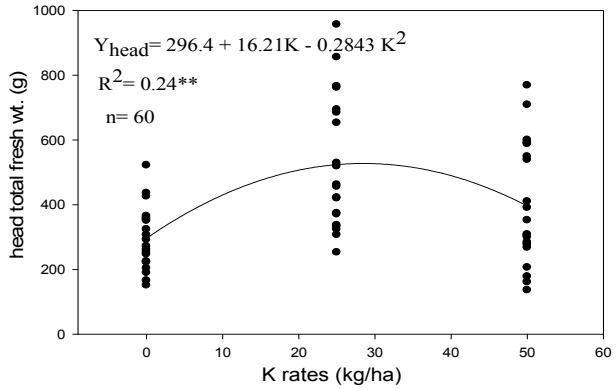

Figure 3.18 Relationship between applied K and cauliflower head yield at 60 DAT

3.2.7.3 Relations between Whole Plant, Head Yield, Tissue N and Total N Uptake

Cauliflower head N concentration did not correlate with fresh yield of cauliflower whole plant and head, plant leaf-stem-root N, head N and total N uptake in whole plant (Table 3.21). All the parameters except for head N concentration have demonstrated greatly significant ($p<0.001$) correlations between each other.

Table 3.21 Correlation values for tissue N, whole plant and head fresh yield at harvest

	head N	plant N	total N uptake	head yield	Whole plant yield
head N (%DW)	NS	NS	NS	NS	NS
plant N (%DW)	0.841**	0.865**	0.864**	0.696**	0.654**
head total N		0.954**	0.986**	0.96**	0.94**
plant total N			0.991**	0.9**	0.895**
total N uptake				0.938**	0.926**

*, ** denote significant correlation at p=0.05 and p=0.001, respectively

Cauliflower whole plant fresh biomass and head yield were significantly correlated with total N uptake in cauliflower. Furthermore, regression analysis between the variables indicated a significant (P >0.0001) linear relationship between total N uptake and both yields of cauliflower whole plant and head. As total N uptake in cauliflower increased, cauliflower whole plant and head yields increased. Linear models for whole plant and head accounted for 86.9% and 85.7% of total variations, respectively (Figure 3.19). On the other hand, applied N rates had a significant linear effect on increasing total N uptake in cauliflower, which explained nearly 91% of total variation in the model (Figure 3.20). Therefore, in order to achieve a higher level of production of cauliflower plant and head yields, higher levels of N fertilizer can be applied which results in a higher amount of total N uptake by cauliflower.

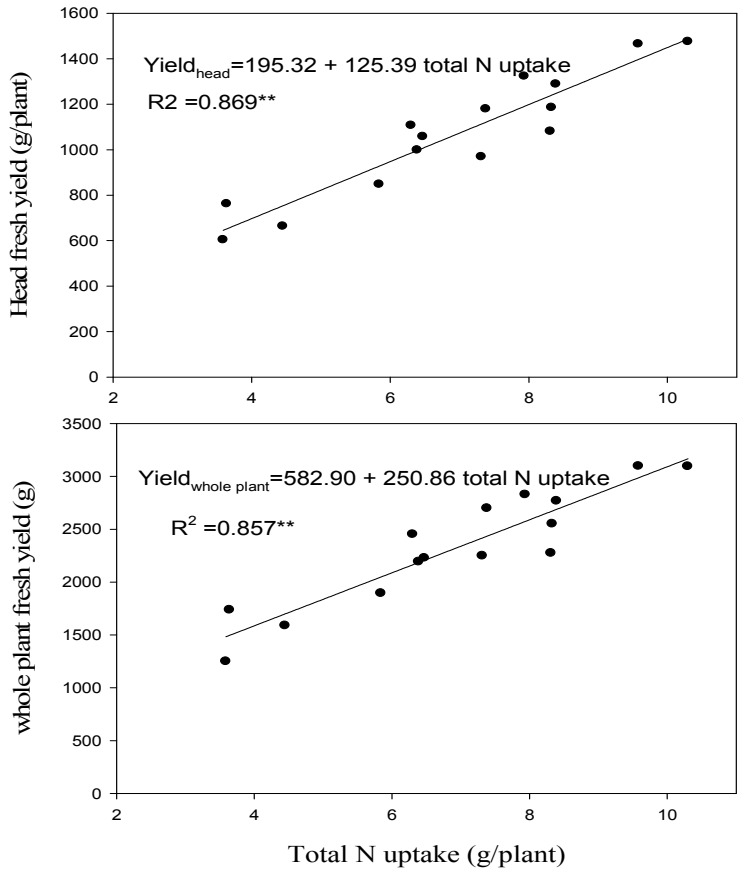

Figure 3.19 Relationships between total N uptake and yields of cauliflower whole plant and head at harvest (** indicates significant difference at P<0.001)

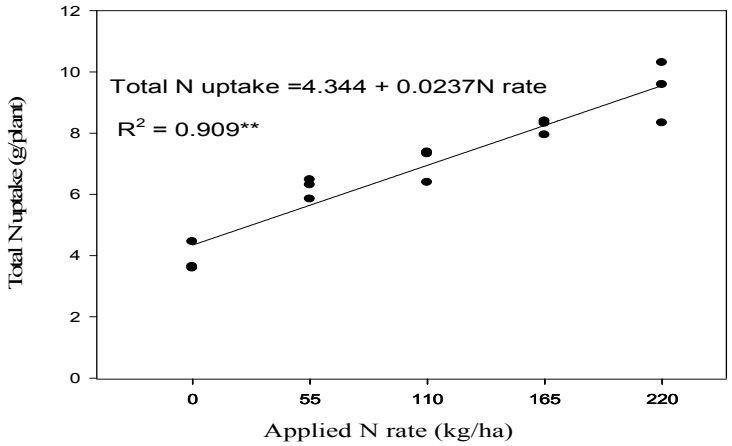

Figure 3.20 Relationship between N rates and total N uptake in cauliflower at harvest (** indicates significant difference at P<0.001)

3.2.8 Leaf Chlorophyll

The three-way interaction time*N*K and two-way interactions such as N*K, time*N and time*K did not significantly influence leaf chlorophyll content in cauliflower plant (Table 2.22). Leaf chlorophyll was significantly affected by N and time. This suggests that leaf chlorophyll content varied with time starting from head initiation when it was first measured. N had the most significant effect on cauliflower leaf chlorophyll content according to its F-value. Moreover, N main effect was consistent overtime which is indicated by the non-significant interaction effect between N and time.

Table 3.22 ANOVA table for fixed effects of applied N, K and time on leaf chlorophyll content in cauliflower during head development

Source	DF	F value	P >F
time	3	6.79	0.0002
N	4	54.08	<.0001
time*N	12	1.18	0.3011
K	2	0.51	0.5985
time*K	6	1.24	0.2895
N*K	8	0.61	0.7719
time*N*K	24	0.52	0.968

Cauliflower leaf chlorophyll measurement was first taken on the fully expanded leaves on September 2, 2009 when head initiation occurred and ended on September 22 during the early heading stage. Means of leaf chlorophyll at four different dates during head development were shown in Table 3.23. Leaf chlorophyll in cauliflower plant was relatively low at the beginning of head initiation (September 2) and significantly increased after one week on September 9 2009 (Figure 3.21). Results indicated that cauliflower leaf chlorophyll remained stable after about one week from head initiation. In addition, applied N significantly affected leaf chlorophyll regardless of time. Leaf chlorophyll content increased linearly with N

fertilizer rates. N_{220} treatment had the highest leaf chlorophyll content overtime although it did not significantly differ from N_{165}. Compared with N applications N_{50} and N_{110}, leaf chlorophyll content increased in cauliflower plants with high N fertilizer applications (Table 3.23).

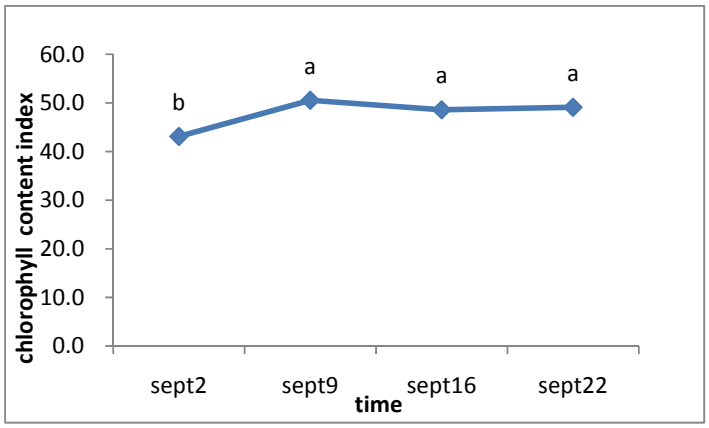

Figure 3.21 Leaf chlorophyll versus time during cauliflower head development
(Means with the same letter are not significantly different at a =0.05 using LSmeans)

Table 3.23 Effect of applied N and time on cauliflower leaf chlorophyll content

Source	Rate (kg/ha)	Sept. 2	Sept.9	Sept.16	Sept.22	Average of date
N	0	32.1	32.7	32.6	32.7	32.6d
	55	41.9	46.3	45.3	40.1	43.4c
	110	44.3	51.9	50.6	51.9	49.7b
	165	48.7	58.0	55.4	57.8	55.0a
	220	48.5	63.8	59.1	63.0	58.6a
Time		43.11b	50.5a	48.6a	49.1a	
Contrast	d.f.					
0 vs N rates	1	70.63**	33.56**	41.61**	40.61**	
55 vs 110, 165 & 220	1	9.74**	8.60**	9.34**	27.62**	
110 vs 165 & 220	1	5.64*	4.51*	3.80 ns	5.84*	
165 vs 220	1	0.01 ns	1.40 ns	0.88 ns	1.62 ns	

*&** denote significant correlation at p=0.05 and p=0.001, respectively.

Applied N rates demonstrated a significant (P <0.0001) linear effect on leaf chlorophyll at all the dates during the head development stage (Figure 3.22). Linear

regression varied slightly between the dates September 9, 16 and 22, which may refer to stable leaf chlorophyll content over the three dates as discussed before. The linear model for September 22 accounted for the highest percentage (57.4%) of total variation.

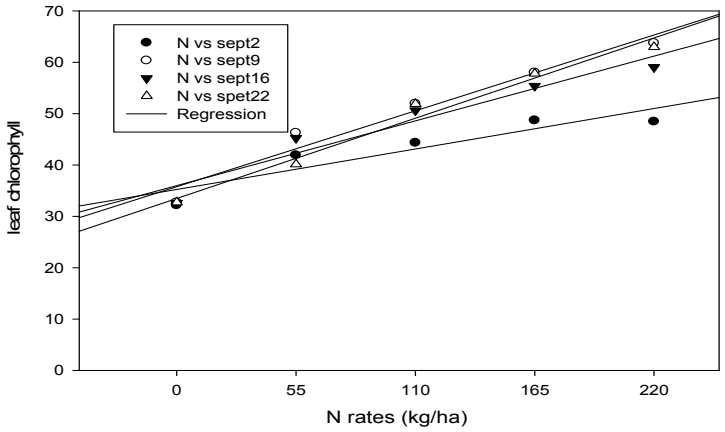

Figure 3.22 Relationship between N rates and leaf chlorophyll in cauliflower

sept2: chlorophyll= 35.23 + 0.07165 N R^2= 46.8%**
sept9: chlorophyll= 35.8 + 0.134 N R^2= 43.4%**
sept16: chlorophyll= 36.0 + 0.115 N R^2= 48.5%**
sept22: chlorophyll= 33.5 + 0.142 N R^2= 57.4%**

3.2.9 Gas Exchange Measurement

The main effects of applied N and K as well as their interactive effect on net photosynthesis, intercellular CO_2 concentration and water use efficiency were not significant in cauliflower plants (Table 3.24). Transpiration rate and stomatal conductance were significantly affected by N and K fertilizations. The highest transpiration rate was achieved in $N_{220}K_{25}$. K application rate of 25 kg/ha greatly increased transpiration rate of cauliflower plants under all N treatments (Figure 3.23). The treatments of $N_{220}K_{25}$ and N_{110} K_{25} had significantly higher transpiration rate than any other NK treatment combinations. Transpiration rate was correlated with applied

N to a certain extent; it increased with N rates up to 110 kg/ha under all K rates, while tended to decrease with N rates above 110 kg/ha under K_{50}. However, under K_0 and K_{25} a decrease on transpiration rate occurred exclusively between N_{110} and N_{165}.

In addition, N and K fertilization have shown a significant interaction effect on stomatal conductance (Gs) in the cauliflower plant (Table 3.24). Stomata conductance was positively related to K applications under N treatments of 0-110 kg/ha. However, when high N rates (165 and 220 kg/ha) were applied, stomata conductance decreased with K rates (Figure 3.24). Plants under $N_{110}K_{50}$ treatment had the highest value of stomatal conductance among NK treatment combinations. Overall, a consistent increase of stomatal conductance was obtained when increasing N rates from 0 to 110 kg/ha under all K treatments. Results in Table 3.25 indicate that Gs were exclusively significantly *(P*<0.5) correlated with E rather than other photosynthetic parameters.

Table 3.24 Effects of N and K on cauliflower plant photosynthesis parameters

Source	d.f	A (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	Gs (mol m^{-2} s^{-1})	Ci vpm	A/E (μmol $mmol^{-1}$)
N	4	0.15 ns	9.59**	8.25**	0.15 ns	0.15 ns
K	2	0.31 ns	3.31*	4.12*	0.27 ns	0.35 ns
N*K	8	0.56 ns	2.2 ns	3.87**	0.54 ns	0.53 ns

value of F, ns: non-significant; * and ** significant at 5 and 1% probability level, respectively. A: photosynthetic rate, E: transpiration rate, Gs: stomatal conductance, Ci: intercellular CO_2 concentration, A/E: water use efficiency.

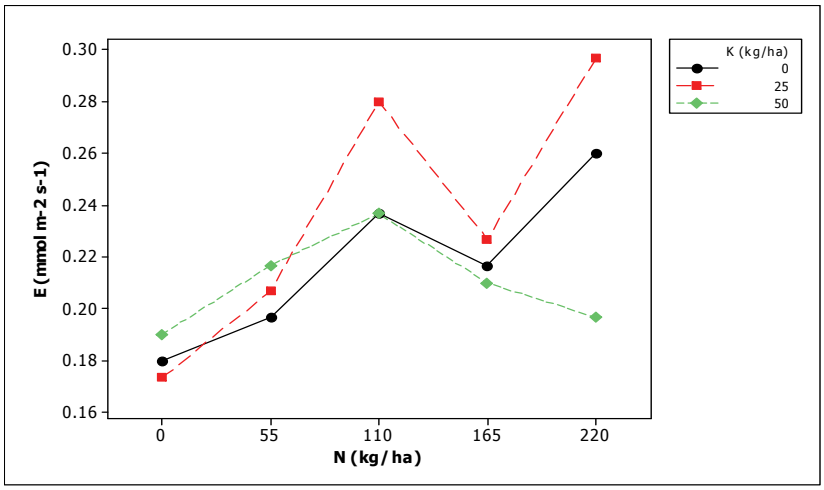

Figure 3.23 Interactive effect of N and K rates on transpiration rate (E)

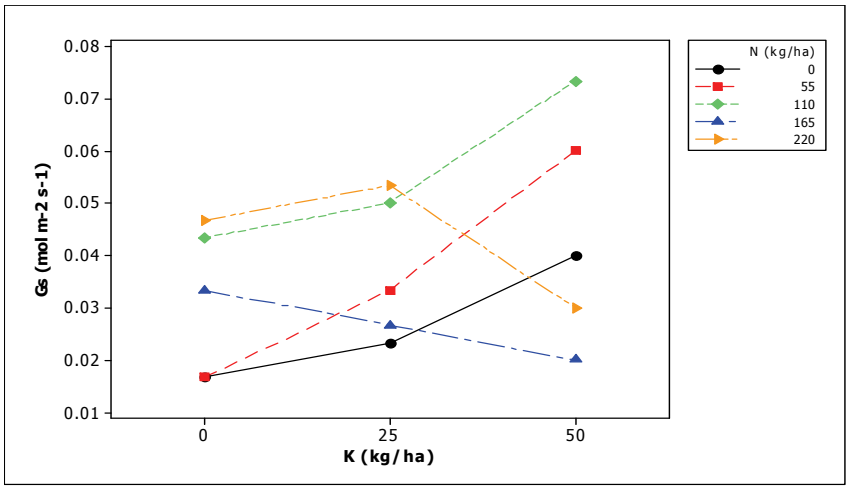

Figure 3.24 Interactive effect of N and K rates on stomatal conductance (Gs)

Table 3.25 Pearson correlation coefficient between photosynthetic parameters

	A	E	Gs	Ci	A/E	A/Ci
PAR	0.414**	NS	NS	-0.504**	0.423**	0.620**
A		NS	NS	-0.927**	0.98**	0.848**
E			0.661**	NS	NS	NS
Gs				0.345*	NS	NS
Ci					-0.949**	-0.767**
A/E						0.84**

Photosynthetic active light (PAR), photosynthesis (A), transpiration (E), stomatal conductance (Gs), leaf internal and external CO2 concentration ratio (Ci/Ca), instantaneous water use efficiency (A/E), instantaneous carboxylation efficiency (A/Ci). ** significant at P<0.05, * significant at P<0.1, NS: not significant at P=0.05

3.2.10 Soil, Plant and N/K Rates Relations

3.2.10.1 Effects of N and K Rates on Soil Parameters and N Concentration

Soil moisture, pH and EC but not soil available N concentrations (NO_3^- and NH_4^+) varied between two sampling times (Table 3.26). Applied N, applied N*time and K fertilization significantly affected soil EC but not soil moisture and pH. The three-way interaction for time*N*K and two-way interactions including time* K and N*K were not significant for soil moisture, pH and EC. In addition, applied N and N*time respectively showed marginally significant effect on soil NO_3-N. Besides those two, soil NO_3-N and NH_4-N were not significantly affected by the main factors of applied N, K and sampling time as well as the 2-way interactions and 3-way time*N*K interaction.

Cauliflower soil sampling was conducted twice, first on August 25 before the second split N application and secondly at harvest. Soil moisture did not change among NK treatments prior to the second split N application as well as at harvest. However, soil at harvest contained overall higher moisture content than before second split N application (Table 3.27 and Table 3.28). Soil pH overtime was stable around 6. Applied N and K did not affect soil EC at harvest, while significantly influenced soil EC on August 25 before the second split N application during mid-season (Figure 3.25).

Soil EC measured with a soil: water ratio of 1:1 was significantly low in the control and N_{55} treatments, whereas it linearly increased with increasing N fertilizer application. Similarly, higher K application of 50 kg/ha significantly increased soil EC on August 25 (Figure 3.25). Moreover, similar patterns of N and K effects were observed on soil EC measured under 1:2 and 1:3 soil: water ratios as shown in Figure

3.26. The interaction of N and K fertilizations on soil parameters and available N concentrations at two different dates were present in Table 3.27 and Table 3.28.

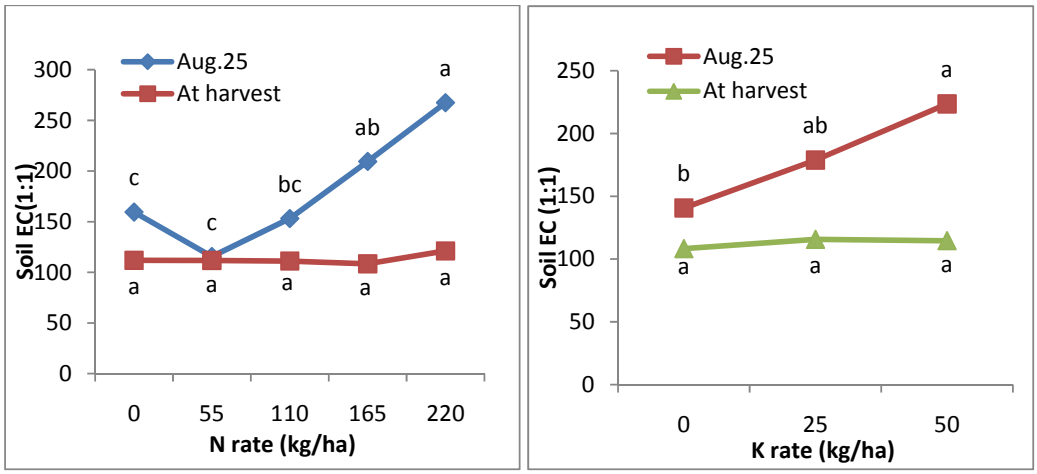

Figure 3.25 Effects of N and K on soil EC on August 25 (before 2nd split N application) and at harvest (Means with the same letter at each date are not significant at 0.05)

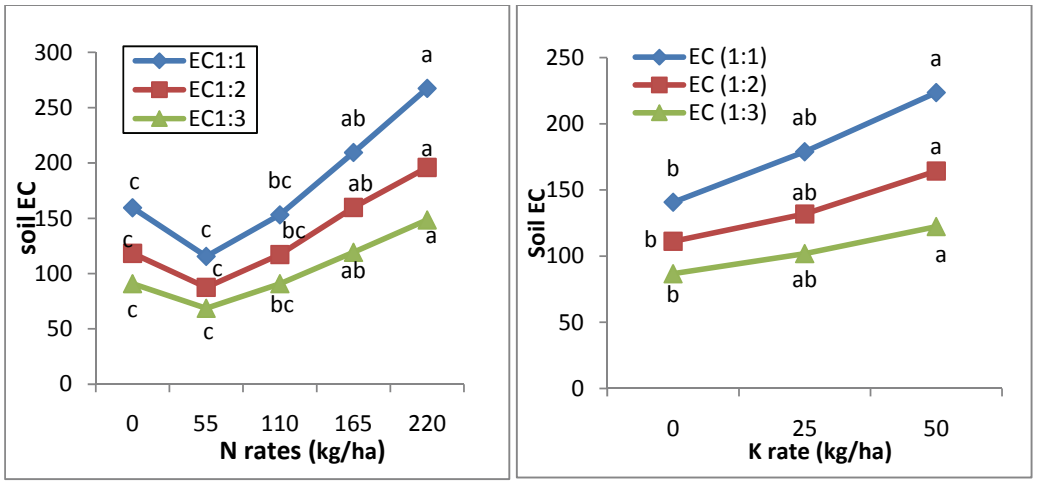

Figure 3.26 Effects of N rates on soil EC under different soil: water ratios on August 25th before 2nd split N application (Means with the same letter at each ratio are not significant at 0.05)

3.2.10.2 Relations between Soil N, Yield and N/K Rates

During mid-season, soil NO_3-N, NH_4-N and total available N appeared negatively correlated with applied K on August 25 (Table 3.29). Similar results were

found at harvest except that soil NH_4-N showed positive correlation with applied K (Table 3.30). Soil N concentration and applied N relationship differed for the two dates. Prior to the second split N application on August 25, soil NO_3-N and total available N were significantly correlated with applied N (Table 3.29). However, the residual available N in the soil at harvest did not show significant correlations with applied N rates (Table 3.30). For the relationships between soil N and cauliflower yield, there were no significant correlations found between soil available N and cauliflower whole plant and head yields at the two dates. At harvest, soil N did not have strong correlations with the following parameters: plant leaf-stem-root N concentration, head N, plant N and total N uptake in cauliflower whole plant (Table 3.30). However, head N concentration showed significant (P<0.0001) correlations with soil NO_3-N and total available N and increased with soil N at harvest (Figure 3.27).

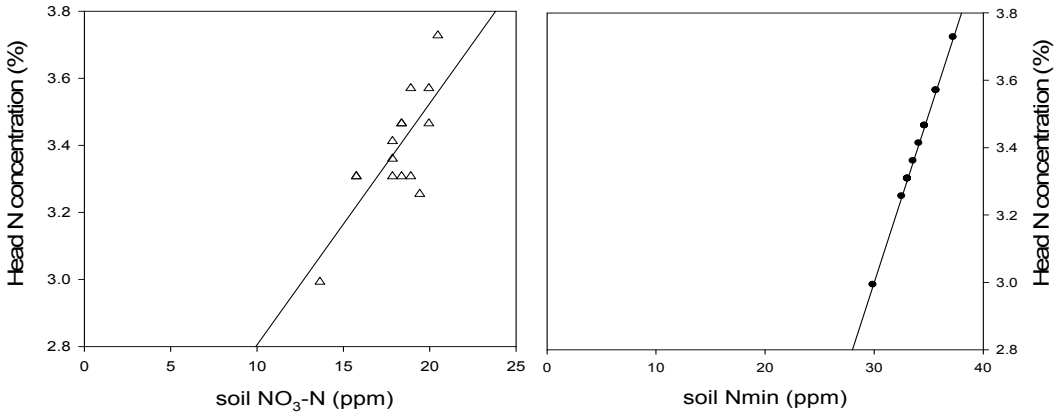

Figure 3.27 Relationships between head N concentration and soil NO_3-N and mineral N at harvest

Table 3.26 ANOVA table for effects of applied N and K on soil parameters and N concentrations between two soil sampling dates (before split 2nd N application and postharvest)

Source	DF	moisture	pH	EC(1:1)	EC(1:2)	EC(1:3)	soil NO_3-N	soil NH_4-N
time	1	<.0001	0.0008	<.0001	<.0001	<.0001	ns	ns
N	4	ns	ns	0.0061	0.0006	0.0018	0.0519	ns
time*N	4	ns	ns	0.0147	0.0038	0.0054	ns	ns
K	2	ns	ns	0.039	0.0142	0.039	ns	ns
time*K	2	ns	ns	ns	ns	ns	ns	ns
N*K	8	ns	ns	ns	ns	ns	ns	ns
time*N*K	8	ns	ns	ns	ns	ns	ns	ns

Data indicate P value, ns: not significant at P =0.05 EC (ratio of soil: water); Nmin: soil mineral/available N

Table 3.27 N and K treatment combinations on soil parameter and N concentration before 2nd split N application

NK treatment (kg/ha)	K rate =0					K rate =25					K rate =50				
	N0	N55	N110	N165	N220	N0	N55	N110	N165	N220	N0	N55	N110	N165	N220
moisture	13%	11%	12%	11%	12%	12%	13%	14%	12%	12%	12%	12%	12%	12%	12%
pH	6.0	6.0	6.0	5.9	5.8	6.0	6.0	6.0	5.9	6.0	6.0	6.1	6.0	6.1	5.8
EC (1:1)	115	106	122	151	209	171	112	186	232	193	192	129	151	246	401
soil NO_3^-	14.7	17.9	18.9	17.9	24.2	16.8	14.7	17.9	20.0	18.9	17.9	15.8	16.8	16.8	24.2
soil NH_4^+	13.7	18.9	11.6	15.8	18.9	13.7	14.7	12.6	14.7	12.6	13.7	11.6	12.6	13.7	21.0

Note: moisture in %; EC in µs/cm; soil NO_3^- & NH_4^+ in ppm

Table 3.28 N and K treatment combinations on soil parameter and N concentrations at harvest

NK treatment	K rate =0					K rate =25					K rate =50				
(kg/ha)	N0	N55	N110	N165	N220	N0	N55	N110	N165	N220	N0	N55	N110	N165	N220
moisture	14%	14%	15%	14%	15%	15%	14%	14%	14%	15%	14%	13%	13%	13%	15%
pH	6.1	6.0	6.0	6.1	6.0	6.3	6.2	6.2	6.1	6.0	6.2	6.2	6.1	6.1	6
EC(1:1)	103	110	112	106	111	113	114	115	112	124	119	111	106	108	129
soil NO_3-N	20.5	18.4	17.9	17.9	20.0	19.4	15.8	18.4	20.0	17.9	18.9	18.4	13.7	15.8	18.9
soil NH_4-N	16.8	14.7	15.2	15.8	14.7	13.1	17.3	16.3	15.8	16.3	14.2	16.3	16.3	17.3	16.8

Note: moisture in %; EC in µs/cm; soil NO_3^- & NH_4^+ in ppm

Table 3.29 Correlation values for soil N concentration, tissue N and cauliflower biomass on August 25th before 2nd split N application

	K rate	N rate	WP N (%DW)	WP total N	WP biomass
soil NO_3-N	-0.0628	0.725**	0.508	0.366	0.386
soil NH_4-N	-0.187	0.378	0.329	0.101	-0.04
Nmin	-0.137	0.603*	0.458	0.255	0.189

WP: whole plant; *&** denote significant correlations at a =0.05 and 0.01, respectively

Table 3.30 Correlation values for soil N concentrations, tissue N and cauliflower yield at harvest

	K rate	N rate	head N (%DW)	plant N (%DW)	head N	plant N	WP total N	Head yield	WP biomass
soil NO_3-N	-0.413	-0.0841	0.772**	-0.0717	-0.134	-0.337	-0.249	-0.163	-0.227
soil NH_4-N	0.259	0.321	0.245	0.217	0.459	0.43	0.449	0.477	0.448
Nmin	-0.26	0.135	1**	0.0752	0.179	-0.0584	0.0484	0.16	0.0721

WP: whole plant; *&** denote significant correlations at a =0.05 and 0.01, respectively

3.3 Discussion

3.3.1. NK Treatment Efficiency on Cauliflower Head Yield

Nitrogen is essential for plant growth and required for achieving a high crop yield, as the importance of N on crop performance is well-known (Havlin et al., 1999; Li et al., 2010). Increasing N application rates (0 to 220 kg/ha) increased cauliflower curd yield. This finding agrees with the observations of several studies on cauliflower that curd yield increased with N rates (Nilsson, 1980; Hart, 1992; Kaniszewski and Rumpel, 1998). In the study of Kaniszewski and Rumpel (1998), an increase in cauliflower yield was obtained with N application rate up to 500 kg/ha. However, those field trials were conducted in other regions or countries, not in Atlantic Canada.

In Nova Scotia, the recommendation rate of N fertilizer for cole crops is 220 kg/ha, regardless of soil available N and soil type (Soils & Crops Branch, 1994). In the filed trial, N_{220} resulted in the highest cauliflower yield that was significantly higher than N_{165}. However, as the curd yield increased linearly with N rates (0-220 kg/ha), this suggests that N_{220} may not be the best N rate for maximizing cauliflower yield and the optimum N application rate could be higher. Therefore, N application rates higher than 220 kg/ha can be further studied on cauliflower production in Atlantic regions of Nova Scotia.

Based on the literature, it is reported that the optimum range of N applications for cauliflower is between 100 and 300 kg/ha (Cutcliffe and Munro, 1976; Nilsson, 1980; Welch et al., 1987; Everaarts and De moel, 1995). Additionally, Cutcliffe and Munro (1976) found that no significant differences in cauliflower yield were found between 224 and 336 kg N/ha at all of nine experiment locations in PEI. However, how N rate exceeding 220 kg/ha would affect cauliflower production in Nova Scotia remains unknown. On the basis of the study conducted in Atlantic PEI, further

research can look at the effects of N rates ranging 0 - 336 kg/ha or higher on cauliflower yield in Nova Scotia. Beside that, experiments should be conducted in different regions in Nova Scotia, in order to provide the optimum N rate with a higher precision to the commercial growers for maximizing cauliflower production.

Head size is one of the major components for determining the yield and quality of cauliflower. In this field trial, head size slightly varied among N treatments (55-220 kg/ha). When increasing N rate from 55 to 220 kg/ha, there was an average of 1.8 cm increase in head size. N_{110} did not resulted in significant difference in head size from N_{55} and N_{165}. However, when compared with N_{55}, high N application of 165-220 kg/ha did produce significantly larger head size. N_{220} had the biggest head size, while it was not significantly different from N_{165}. This would suggest that N fertilization (>110 kg/ha) did not highly influence cauliflower head size. A similar result was found by Nilsson (1980). Results showed that an increase of N rates (150 - 300 kg/ha) had no effect on the size quality of cauliflower (Nilsson, 1980). Everaarts and De moel (1995) also revealed that cauliflower size quality was affected little or not at all by the N availability.

In addition to N application, K fertilization also affected cauliflower curd yield and size. Compared to the control, cauliflower yield and szie was signifcantly increased with K applications. Guo et al. (2007) also found that K fertilization significantly increased caulilflower yield. In contrast, K applications have less of a signficant impact on caulifower yield than N. This agrees with the findings of Cutcliffe and Munro (1976) that applied K had less effect on yields than applied N. However, the smaller number of K treatments may cause limited variation of cauliflower yield response to K rates compared with the N rates.

Results indicated that there was a signficant quadratic relationship between cauiflower curd yield and K rates. In literature, few studies were undertaken to investigate the relationship between K application and cauliflower. However, a similar observation was found on other crops. Coltman and Riede (1992) found that the yield was quadratically related to increasing external K concentration in tomato plants.

According to the soil test conducted in the experiment field, soil K was rated to a medium plus level. Research has found that it is often difficult to investigate plant K requirements due to the high level of the nutrient in the soil. The effect of K fertilizaton on increasing cauliflower yield is highly related with the dose of fertlizer K and soil K status (Guo et al., 2007). Therefore, in order to evaluate K effects on cauliflower and optimize K fertlization rate, it is suggested that more rates of K fertilzation would be applied in different field sites in Nova Scotia.

Results indicated that the interaction between N and K fertilizations overtime did not show a significant effect on cauliflower yield or size. $N_{220}K_{25}$ resulted in an 18.8% increase on curd yield and 30% on head dry matter when compared with $N_{220}K_0$ treatment. Yang et al. (1994) also reported that increasing K rates can increase the yield and production of cauliflower under the same N application. In addition, K applied together with N favoured increased broccoli yield, a plant in the same species as cauliflower (Ying et al., 1997). Therefore K fertilizer is recommended to be applied together with N in order to achieve a higher production of cauliflower.

3.3.2 NK Treatment Efficiency on N Uptake

Increasing N application consistently increased N uptake in head, plant leaf-stem-root and whole plant of cauliflower in the field trial. Compared to the control, N

fertilizer input significantly enhanced N uptake in the organs of cauliflower plants. Everaart (2000) also indicated that N uptake in cauliflower was significantly increased by N fertilization.

Total N uptake by cauliflower varied from 6.2 to 9.4 g/whole plant on average with N applications of 55-220 kg/ha. Li et al. (2009) reported cauliflower plants were able to take up total N between 6.2 and 9.0 g/plant depending on variety. Results in this experiment suggested cauliflower took up N slowly prior to head initiation and about 72.3-78.7% of total N uptake took place around 30 d before harvest. This observation is similar to the findings of other studies (Welch et al., 1987; Doerge et al., 1991).

In previous research, it is reported that about 30% of total N was contained in the harvested portion of cauliflower (Doerge et al., 1991; Stivers et al., 1993). However, a relative higher percent of head N was observed in this study. N accumulation in harvested heads accounted for 34.5 to 42.4% of whole plant total N uptake among all the NK treatments. N application applied with proper K fertilization (25 kg/ha) may contribute to the allocation of N into edible parts of cauliflower when compared with N fertilization alone in the previous studies. This would produce a higher quality of cauliflower head, since protein content can be converted by multiplication of total N x 6.25, which is commonly applied for foods and plants (Fujihara et al., 2001). In addition, increased N uptake in head can significantly enhanced cauliflower head fresh weight (Figure 3.19, Table 3.21).

K application significantly increased head total P as well as N in cauliflower head and plant. The favourable effect of K on nutrients uptake such as N and P has been proven by the studies on several crops (Sharma and Singh, 1992; Li et al., 1997;

Armstrong, 1998; Guo et al., 2007). Guo et al. (2007) also reported that K fertilization enhanced the uptake of N, P and Ca by cauliflower plant.

3.3.3 Photosynthesis Related to NK Treatments

Nitrogen plays an important role on photosynthetic capacity and efficiency as more than 50% of leaf N is in components associated with photosynthesis (Evans, 1989b). This study showed no significant effect of N and K on photosynthesis rate of cauliflower under field conditions. Excluding N and K factors, there can be other uncontrolled variables that can affect the results. Results in Table 3.25 also indicate that photosynthesis rate (A) was significantly (P<0.05) correlated with photosynthetic active light (PAR) and water use efficiency (A/E). It is very likely that climate (rainfall) and weather conditions can influence the photosynthesis. Photosynthetic measurements were taken during Late September, when frost occurred, temperature was quite low and PAR may be low. Besides, it took a few hours to complete the measurements in all plots.

Transpiration rate (E) of cauliflower plant increased with N rates (0-110 kg/ha) and did not vary with further increasing N rates. Dordas and Sioulas (2008) indicated that N fertilization can influence both the amount of water extracted by a crop and crop growth, and consequently can affect WUE. When compared to the control, K_{25} enhanced transpiration rate of cauliflower under all N applications. This can be due to changes in the morphology of cauliflower plant such as higher plant growth with K_{25} application. Brag (1972) also indicated that K affected the transpiration rate of plants and revealed that stomatal frequency and stomatal aperture were correlated with the potassium concentration in the leaves. However, an increase and a decrease in E

depended on the age of plants, younger plants showing a decrease, older an increase (Brag, 1972).

Increasing N application (0-110 kg/ha) increased Gs. A similar effect of N fertilization on increased Gs was found on other crops such as wheat and safflower (Shangguan, 1997; Dordas and Sioulas, 2008). There was a significant correlation obtained between E and Gs in the study. Therefore it suggests N fertilization can influence stomatal transpiration of cauliflower through stomatal conductance, as controlling stomata aperture is the most important mechanism to regulate water loss.

Cauliflower plants in the control N_0 had the lowest photosynthesis rate, which is supported by Dordas and Sioulas (2008) that N deficiency reduced the radiation interception and radiation use efficiency, resulting in reduced photosynthesis capacity. High N fertilization can increase photosynthesis rate on several crops (Evans, 1989a; Cechin and Fumis, 2004). Plants in N_{220} also had a higher photosynthesis rate than N_{165} and N_{110}. However, the highest photosynthesis rate was obtained in N_{55}. This suggests plants with high N fertilization take up more N while it may not be utilized for photosynthesis during the early heading stage of cauliflower. In addition, photosynthesis rate (A) demonstrated a significant negative correlation with intercellular CO_2 concentration (Ci). In the study of Dordas and Sioulas (2008), this relationship between A and Ci was also found in safflower (*Carthamus tinctorius* L.).

3.3.4 Limitations of Soil Testing

In Nova Scotia, N rate recommendations for crops are not being made on the basis of soil testing. Soil testing is normally conducted for determination of nutrients including P, K, Ca, Mg, Na, S, Fe, Mn, Cu, Zn and B. On the basis of soil testing, soil

nutrients such as P and K are rated from low to high level. Nutrient requirements for crop are subsequently determined based on the soil nutrient rating.

However, in order for optimize N fertilization for vegetable crops like cauliflower, soil mineral N should be taken into account. In the research field pre-planting soil NO_3-N and NH_4-N at a sampling depth of 15-20 cm is around 18 and 15 ppm, which are converted to approximately 54 and 45 kg/ha. This would add up to nearly 100 kg/ha of mineral N in the soil before planting. With an N application of 220 kg/ha, the range of optimum N application for cauliflower can be estimated around 320 kg N per hectare or higher. However, attention should be paid to nitrate accumulation in edible cauliflower head subjected to high N application in a further study on cauliflower.

Based on the results of soil analysis for mineral N (NO_3^- & NH_4^+), in the control with no fertilizer applied, soil NO_3-N and NH_4-N at harvest were 4-6 ppm higher than in the mid-season. This could be an error due to several uncertain factors. First, soil mineral N was analyzed for two random blocks in the midseason, while at harvest soil samples from four blocks were all analyzed; Secondly, soil nutrient fertility may differ with spots in the field; Thirdly, there can be error while doing soil analysis. On the other hand, during the growing season the acidic rainfall and soil mineralization may contribute a small proportion to the soil mineral N at harvest.

Chapter IV

Effects of Nitrogen Supply on Nitrate Reductase Activity and Growth of Cauliflower (*Brassica oleracea* var. *botrytis*)

4.1 Introduction

N fertilizer plays an important role in crop yield and quality. Today more and more nitrogen fertilizers are applied in agricultural fields in order for high crop production (Ludwig et al., 2010). Nitrate is the main N form taken up from the soil by most plants. When high or excessive amounts of N fertilizers are applied to the field, concerns of food safety and environmental health are raised, which is being given attention by consumers (Gastal and Lemaire, 2002).

Nitrate not taken up by plants and left in the soil would potentially contributed to ground and surface water pollution due to nitrate leaching and soil erosion (Gastal and Lemaire, 2002; Wang et al., 2007). Nitrate accumulation in plants is another major concern for human health, as high NO_3^- concentration in edible plant parts has been implicated in the occurrence of methaemoglobinemia and as a possible cause of gastric cancer (Cardenas-Navarro et al., 1999). With respect to environmental concerns and food quality, nitrate uptake and assimilation to useful N-containing compounds such amino acid and protein are of great importance. Therefore it is important to understand the relation between N supply and nitrate assimilation in plants, especially vegetables.

Nitrate taken up by plants is reduced to nitrite as an intermediate, subsequently reduced to ammonia that is further synthesized to amino acids, thus protein and other

nitrogenous compounds for plant development. Nitrate reductase (NR) is the key enzyme essential for nitrate reduction to nitrite, thus limiting the further steps of N assimilation and metabolism. Many studies have agreed that nitrate and other factors such as light or sugars control NR gene expression (Galangau et al. 1988; Becker et al. 1992; Vincentz et al. 1993). Additionally, nitrate reductase in leaves and roots is very dependent on nitrate supply (Man et al., 1999). The main purpose of this study was to investigate the effects of nitrate supply on nitrate accumulation, leaf nitrate reductase activity (NRA), plant growth and photosynthetic response of cauliflower plants grown under greenhouse conditions. The response of head and leaf sap mineral composition to various nitrate supply rate was also determined. The relationship between leaf NRA and nitrate-N content was discussed.

4.2 Materials and Methods

4.2.1 Experimental Design and Treatment

The greenhouse study was undertaken to focus on nitrate reductase activity of cauliflower plants with different N supplies. Experimental treatments involved five rates of N supply throughout the growth period in the greenhouse. Based on the results of my field trial (cv. 'Minuteman' tested in Truro) and Dr. Hong Li's study (Li et al., 2009a, 2009b) on N uptake of three cauliflower cultivars ('Minuteman', 'Sevilla' and 'Whistle' tested in Annapolis Valley), a daily N supply of 50 mg for cauliflower was estimated to be the efficient rate of N supply for cauliflower growth.

To test this assumption, a series of daily N supply treatments were set up, i.e. 0, 33%, 60% and 133% of 50 mg N per day. The daily N supplies were then determined to be 0, 16.5, 33 and 66.5 mg N in the form of KNO_3 (Table 4.1). Total N supply was calculated for each treatment according to a 60-day growing period for

cauliflower plants (Table 4.1). Cauliflower plants were fertilized with modified Hoagland solution to ensure adequate nutrients supply. Modified N free Hoagland solution was used as the control N treatment. For other N treatments, different N supplies were achieved by adding appropriate amounts of KNO_3 to the control solution, in order to study the conversion of nitrate to nitrite. All of the treatments were arranged in a completely randomized design in the greenhouse and each treatment was replicated 9 times.

Table 4.1 Greenhouse N treatments

N supply	0	33%	66%	100%	133%
Daily N supply (mg)	0	16.5	33	50	66.5
Total N supply for 60 days (g)	0	0.99	1.98	3	3.99
Treatments	N0	N1	N2	N3	N4

4.2.2 Nitrogen Application Methods

Plants can take up nutrients when roots develop. In order to simulate the trend of plant nutrient uptake, N supply was proportionally distributed to different growth stages through the growing period in the greenhouse. In literature, little information on N uptake and accumulation of cauliflower during plant development is available. Based on the results from my field trial and the previous study by Li et al. (2009), 20% and 80% of total N supply for 60 days were determined to apply in the first 4 weeks and the later 4 weeks, respectively in this greenhouse study. Percentages of N supply for each individual week are described in Table 4.2 and Table 4.3.

Table 4.2 N supply distribution during the growing period

N supply	0	33%	66%	100%	133%
Daily N supply (mg)	0	16.5	33	50	66.5
Total N supply for 60 days (g)	0	0.99	1.98	3	3.99
subtotal N from week 1 to 4	20% total N for 60 days				
subtotal N from week 4 to 8	80% total N for 60 days				

growth stages (DAT)		% subtotal N from week 1 to 4
week 1	0-7 day	10%
week 2	7-14 day	20%
week 3	14-21 day	25%
week 4	21-28 day	45%
		% subtotal N from week 4 to 8
week 5	28-35 day	15%
week 6	35-42 day	20%
week 7	42-49 day	30%
week 8	49-56 day	35%

DAT: day after treatment application.

Table 4.3 Percent of total N from week 1 to 8

Week 1 to 4 = 20% total N	Week 4 to 8 = 80% total N
week 1= 10%*20%* total N = 2% total N	week 5= 15%*80%* total N = 12% total N
week 2= 20%*20%* total N = 4% total N	week 6= 20%*80%* total N = 16% total N
week 3= 25%*20%* total N = 5% total N	week 7= 30%*80%* total N = 24% total N
week 4= 45%*20%* total N = 9% total N	week 8= 35%*80%* total N = 28% total N

4.2.3 Plant Materials and Treatment Application

The greenhouse experiment was started on April 20, 2010 in the Plant Science greenhouse at the Nova Scotia Agricultural College. Three seeds of cauliflower cultivar 'Minuteman' were sown 1 cm deep into each 2-L pot filled with a peat based soilless potting medium (pro-mix) and maintained in the greenhouse with day/night temperature of 20/15^{o}C. Seedlings were watered as needed. At approximately 10 days after sowing, the seedlings in each pot were thinned to 1 seedling of relatively good vigour and similar plant height, in order to maintain uniform size of seedlings in each pot. After thinning, modified Hoagland solution with different N treatments were randomly applied to each pot based on the experimental treatments and application method as described in Table 4.1 &4.2.

The composition of modified N free Hoagland solution (Hoagland and Arnon, 1950) included 5 mM KH_2PO_4 , 2 mM $MGsO_4$, 2.5 mM $CaSO_4$ and micronutrients 46 µM H_3BO_3, 0.3 µM $CuSO_4.5H_2O$, 0.8 µM $ZnSO_4.7H_2O$, 9 µM $MnCl_2.4H_2O$, 0.1 µM $H_2MoO_4.H_2O$, 89 µM Fe-EDTA (pH 5.5-5.8). Each seedling was supplied daily with 30 ml of modified N free Hoagland solution, to which different amounts of N using KNO_3 were added under each treatment. Control plants received zero N only but the same amount of other nutrients. The greenhouse experiment was initially planned to last for 60 days after treatment applications (DAT). However, after the 60 DAT the experiment was extended until 80 DAT in order to look at head formation among N treatments. For the additional 20 days (approximately 3 weeks), cauliflower plants were further supplied with N treatments following the N percentages used for week 6-8 (Table 4.3).

4.2.4 Plant Sampling and Measurements

For determination of cauliflower plant growth under each N treatment, plant height and total leaf number were recorded at an interval of about 2-3 weeks from the beginning of treatment applications. At day 20 after treatments, plants were at the 7-8 leaf stage and several leaves were sampled from one random plant for analysis of enzymatic activity (nitrate reductase activity NRA) and root scanning. Before uprooting the plant, the youngest mature leaf from the centre of the whorl of each plant was sampled for NRA (Spectrum Analytic Inc., 2002; Serve-Ag Analytical services, 2004), which was the 5^{th} leaf from the plant base. Fresh leaves were rinsed with distilled water and blotted with paper towel. Two subsamples of 0.2 g were excised from the leaf and immediately analyzed for NRA under different time induction in the dark.

After NR analysis, the randomly selected whole plant was uprooted. Cauliflower roots were washed several times with distilled water until the promix was removed from the roots. The whole plant including the remainder of the leaf sampled earlier was weighed for fresh weight. Plant height, leaf number and shoot tip width were recorded in order to investigate cauliflower development under different N treatments. The roots were then separated from the plant and scanned under a root scanner for the following measurements: root volume, root length, surface area and number of tips. Total plant dry matter was later determined by drying the whole plant at 70ºC in an oven for 48 hours.

The procedure used to process cauliflower samples also applied to the samples taken at the other sampling times. At day 40, 60 and 80 after treatment application, three cauliflower plants were taken from each treatment. Plants were processed in the same way as day 20 for plant measurements, root scanning and nitrate reductase analysis for both leaves and heads at 80 DAT. At 80 DAT, the fully expanded leaves and a portion of head were taken for determination of sap NO_3^- and K concentration using Cardy nitrate & K meters (Spectrum Technologies Inc.). Samples were cut into pieces, mixed and pressed to obtain the sap using a handheld plant sap press.

4.2.5 Assay of Nitrate Reductase Activity

The NR activity was analysed using the standard curve prepared from Dr. Hong Li's test of cauliflower NRA using the methods shown in Aslam et al. (2001). Fresh leaves were washed and blotted, and cut into 5 mm × 5 mm segments of approximately 25 mm square. Leaf samples of 0.2 g were weighed, placed in test tubes and then 10 ml of assay buffer medium composed of 30 mM KNO_3, 0.1 M potassium phosphate buffer (pH 7.5) and 5% (v/v) 2-propanol was added (Aslam et

al., 2001). All tubes were sealed and placed in the dark at room temperature for different periods: 0, 1, 3, 6, 24, 48 and 72 hours. For the control at time 0, 1 ml of reaction mixture was pipetted from each tube and transferred to a new tube and made up to 10 ml with distilled water. NR activity was stopped immediately by placing the tube in the boiling water bath for 5 minutes. After cooling, the nitrite released from the medium was determined by the method of Aslam et al. (2001). Five millilitre of 1% (wt/v) sulfanilamide solution in 25% (v/v) HCl and 5 ml of 0.02% (w/v) N-(1-napthy)ethelenediamine dihydrochloride were added to the reaction medium. After colour development for 20 min, nitrite was determined at A540 using a spectrophotometer (Taghavi and Bablar, 2007). For each induction time, the same procedure was conducted to determine formed nitrite concentration. NRA was determined by using the average of formed nitrite content within the first 3-hour induction and expressed as μmol NO_2^- g^{-1}fw h^{-1}.

4.2.6 Statistical Analysis

Data obtained from this experiment were submitted for assumptions test including normality and constant variance in Minitab v.14. After assumptions were validated, analysis of variance (ANOVA) was used to test the significance among treatment means using Proc GLM (general linear model) in SAS v8. Least Squares Means method was conducted for means comparison if a significant difference was found among treatment means. Correlation analysis was conducted to analyze the variables of nitrate reductase activity (NRA), tissue sap nitrate-N and K concentrations, using Minitab. A 0.05 probability level of significance was tested for all the data analysis in this study.

4.3 Results

4.3.1 Effect of Nitrate Supply on Plant Growth

4.3.1.1 Plant Biomass and Dry Matter

N supply significantly affected plant growth in terms of plant fresh weight and dry matter accumulation. N treatment, DAT and their interaction had significant effects on both plant fresh and dry weights (Table 4.5). N2 treatment resulted in the highest plant fresh biomass and dry matter accumulation overtime throughout the greenhouse experiment. Plant fresh and dry weight was significantly enhanced by N supply when compared to the control treatment N0 (Table 4.5). Increasing N supply level from N1 to N2 significantly increased plant biomass at all time periods. N3 and N4 resulted in significantly higher plant fresh weight than N1 exclusively at 80 DAT. There was no difference on plant biomass among N2, N3 and N4 at 40 and 80 DAT (Figure 4.1). However, N3 and N4 significantly inhibited plant growth for both plant fresh and dry weight at 60 DAT. Overall, N2 tended to have higher plant fresh and dry weights than N3 and N4 at the later growth stage, even though no significant difference was observed (Table 4.5). Plant dry weight did not vary significantly among N supply treatments at each sampling time except for N2 which was significantly higher compared to N4 and N1 at 60 and 80 DAT (Figure 4.2).

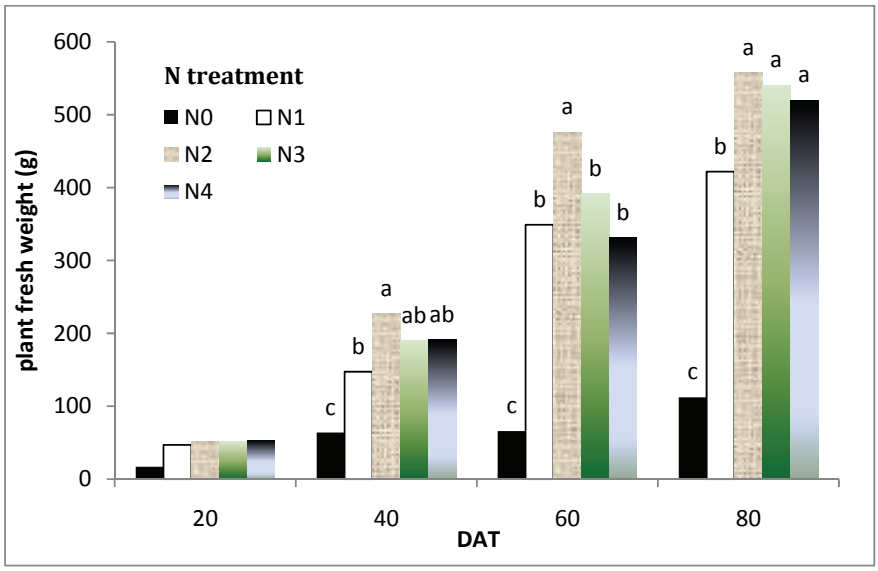

Figure 4.1 Effect of N supply on plant total fresh weight at 20, 40, 60 and 80 DAT. Observations with the same letter within each DAT are not significantly different at $P< 0.05$. N0: 0 mg N/day, N1: 16.5 mg N/day, N2: 33 mg N/day, N3: 50 mg N/day, N4: 66.5 mg N/day.

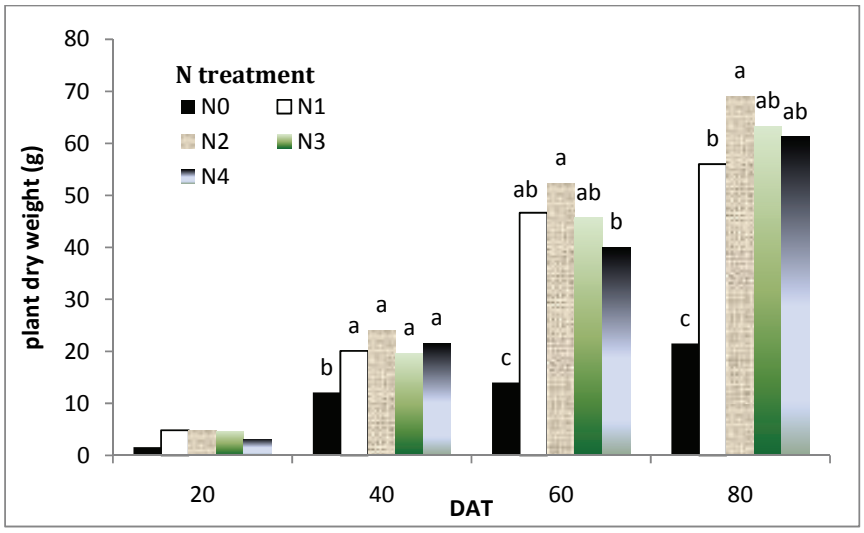

Figure 4.2 Effect of N supply on plant total dry weight at 20, 40, 60 and 80 DAT. Observations with the same letter within each DAT are not significantly different at $P< 0.05$. N0: 0 mg N/day, N1: 16.5 mg N/day, N2: 33 mg N/day, N3: 50 mg N/day, N4: 66.5 mg N/day.

4.3.1.2 Leaf Number, Plant Height and Stem Diameter

N supply rate significantly influenced plant height, total leaf number and stem diameter (Figure 4.3-4.5). As plants grew, plant height and leaf number increased over time with significant differences among the four time periods (Figure 4.3 and 4.4). N supply effect on plant height and leaf number became more significant in the later growth stages compared to the early vegetative stages. No difference was found between N treatments (N0-N4) on the two plant growth variables at 14 DAT. However, plant growth in N0 plants was significantly less at 28 DAT. The main differences in plant height and leaf number over time were between N0 and the other N treatments.

Plant height and leaf number differed among N supply treatments from N1-N4 at 40 DAT (Table 4.5). The two variables significantly increased with higher N supply of N2 when compared to N1. However, the growth variables of plant height, leaf number and stem diameter did not highly respond to N supply treatments at 60 DAT. In contrast to N2, N4 treatment resulted in significantly lower leaf number and stem diameter at 60 DAT (Figures 4.4, 4.5). In general, plants treated with no additional N supply resulted in the poorest growth. Among N supply treatments (N1-N4), the maximum plant growth occurred at N2, while the minimum plant growth occurred at N4.

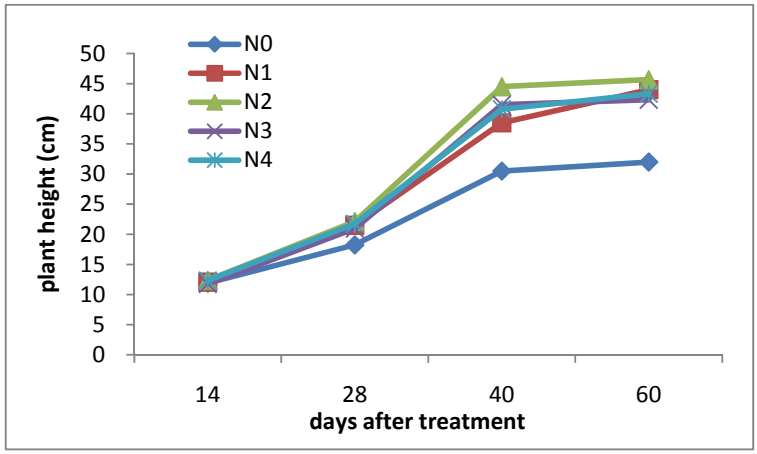

Figure 4.3 Effect of N supply treatments on plant height of cauliflower overtime (N0: 0 mg N/day, N1: 16.5 mg N/day, N2: 33 mg N/day, N3: 50 mg N/day, N4: 66.5 mg N/day)

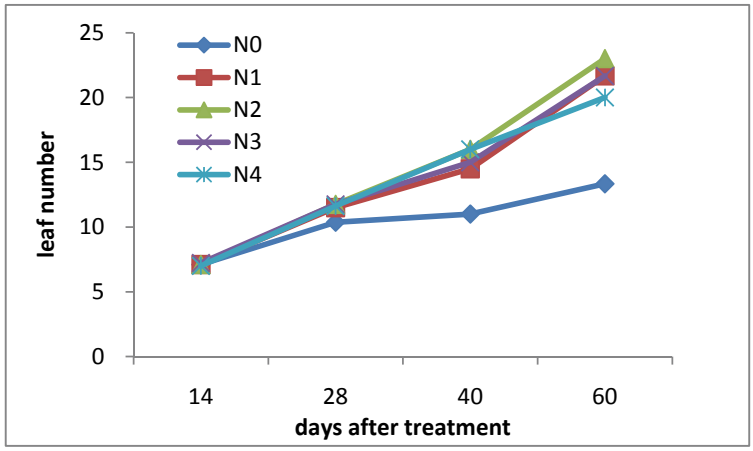

Figure 4.4 Effect of N supply treatments on total leaf number of cauliflower overtime (N0: 0 mg N/day, N1: 16.5 mg N/day, N2: 33 mg N/day, N3: 50 mg N/day, N4: 66.5 mg N/day)

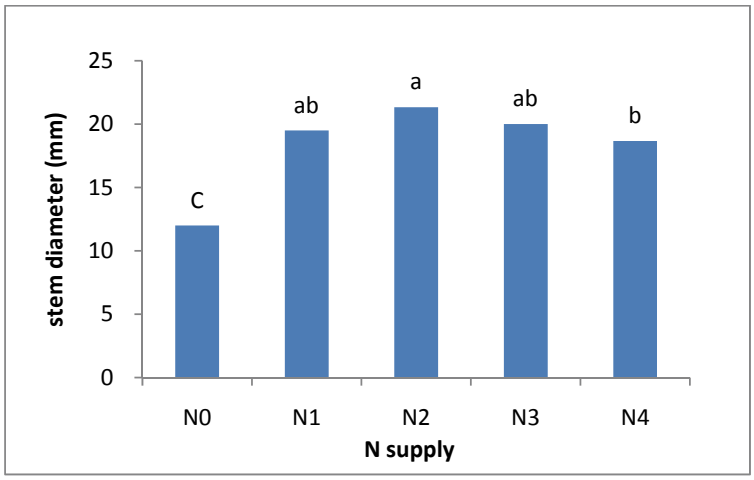

Figure 4.5 Effect of N supply on stem diameter at 60 DAT.
Observations with the same letter for each date are not significantly different at $p < 0.05$. N0: 0 mg N/day, N1: 16.5 mg N/day, N2: 33 mg N/day, N3: 50 mg N/day, N4: 66.5 mg N/day.

4.3.2 Effect of Nitrate Supply on Leaf Chlorophyll and Photosynthesis

4.3.2.1 Leaf Chlorophyll

Leaf chlorophyll was significantly increased with N supply (N1-N4) when compared with the control N0. During the early vegetative stage at 28 DAT, leaf chlorophyll decreased above N1 while there was no significant difference between N1, N2 and N3. Leaf chlorophyll content in N4 was significantly lower than the rest of the N treatments. This may suggest less N uptake in cauliflower plants at 28 DAT when excessive N was supplied. At 63 DAT, leaf chlorophyll content greatly increased with N supply rate up to N2, and furthermore stayed stable in N treatments N2, N3 and N4 (Figure 4.6).

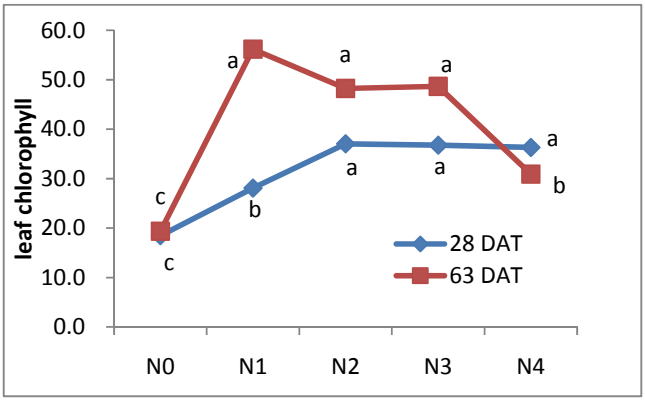

Figure 4.6 Effect of N supply on leaf chlorophyll at 28 and 62 DAT.
Observations with the same letter for each date are not significantly different at p< 0.05 using LSmeans.

4.3.2.2 Photosynthetic Measurements

Results indicate that the N treatment effect was significant on most of photosynthetic variables: photosynthetic rate (A), intercellular CO_2 concentration (Ci), water use efficiency (WUE) and instantaneous carboxylation efficiency (A/Ci) (Table 4.4). Transpiration rate (E) was the only variable affected by time. Moreover, the interactions of N treatment and time on photosynthetic rate, intercellular CO_2 concentration and instantaneous carboxylation efficiency were significant.

Table 4.4 ANOVA table for N supply treatments and DAT on photosynthetic measurements

Source	DF	A	E	Gs	Ci	WUE	A/Ci
N supply	4	15.23**	NS	NS	4.91**	9.52**	11.86**
DAT	1	NS	9.26**	NS	NS	NS	NS
N supply*DAT	4	3.36*	NS	NS	2.7*	NS	3.5*

A: photosynthetic rate, E: transpiration rate, Gs: stomatal conductance, Ci: intercellular CO_2 concentration, WUE: water use efficiency, A/Ci: instantaneous carboxylation efficiency. **, * and NS denote significant at 0.01, 0.05 and non-significant, respectively.

Results for the N supply effect on photosynthesis measurements at 35 and 60 DAT are present in Table 4.6. N treatments increased photosynthetic variables except Ci compared to the control. At 35 DAT, photosynthesis rate (A) linearly increased with N supply from N0 to N2. With a further increase in N supply, no significant difference was found in A between N2, N3 and N4. However, at the later growth stage of 60 DAT, photosynthesis rate declined as N rates exceeding N2 were supplied (Figure 4.7a). The highest photosynthetic activity was observed in the N2 treatment over time. Furthermore, the same pattern for the N supply treatment x DAT interaction effect on A was also found in instantaneous carboxylation efficiency (A/Ci). Transpiration rate did not vary among N treatments over time; while it was observed that E of plants supplied with high N rates was relatively low at the later growth stage of 60 DAT (Figure 4.7b).

As N supply increased, intercellular CO_2 concentration (Ci) decreased at both 35 and 60 DAT (Figure 4.7c). At 30 DAT, Ci under low N supply such as N0 and N1 was much higher than the rest of N treatments, whereas there was no significant difference among N treatments including N0 at 60 DAT. Overall, the highest Ci was obtained in the control treatment. Water use efficiency positively related with N supply rate up to N2. However, when further increasing N supply, WUE responded differently with growth stages. At early growth stage (35 DAT), WUE did not significantly differ between N2, N3 and N4 treatments. At 60 DAT, WUE dramatically decreased above N2, and N4 resulted in significantly lower WUE than N2 (Figure 4.7d). Water use efficiency among N treatments ranged from 1.9 to 5.3 μmol $mmol^{-1}$ at 60 DAT (Table 4.6). N2 treatments had the highest water use efficiency over time.

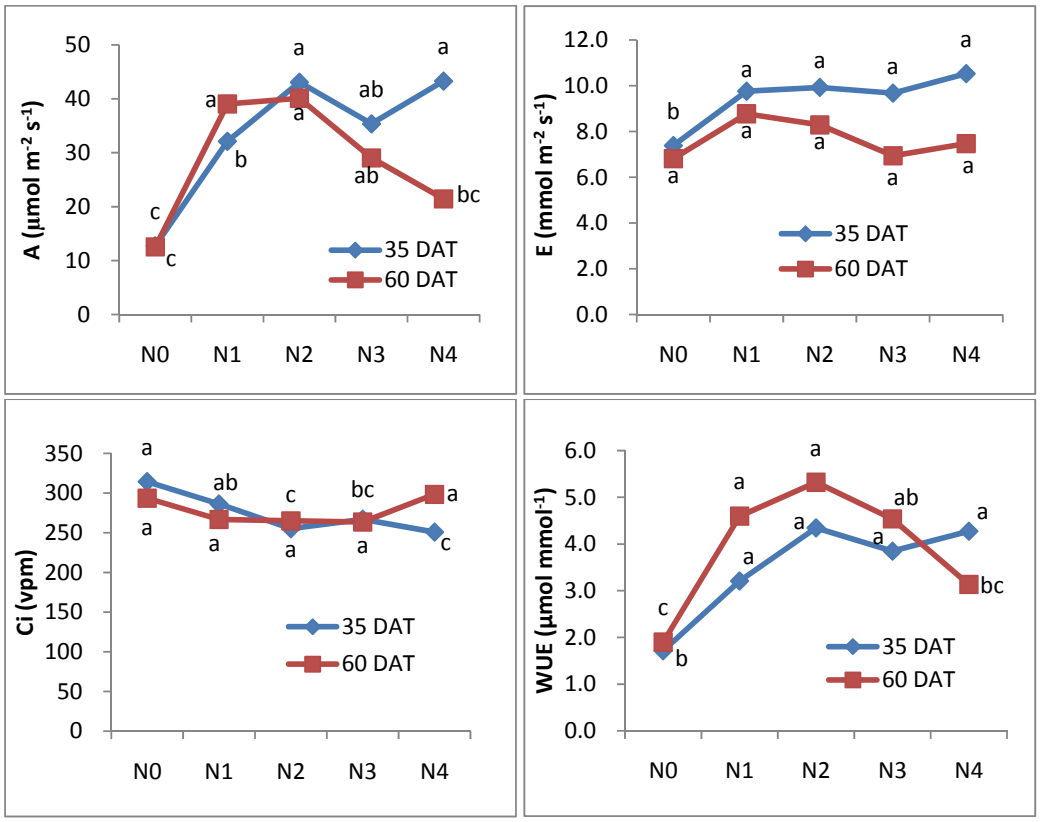

Figure 4.7 Effect of N supply on photosynthetic rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci) and water use efficiency (WUE) at 35 and 60 DAT. Observations with the same letter for each date are not significantly different at $P< 0.05$ using LSmeans.

Table 4.5 N effect on plant measurement variables at 20, 40, 60 and 80 DAT (days after treatment application)

	Whole plant FW				Whole plant DW				Stem diameter		Root FW			Head size		Head FW	
DAT	20	40	60	80	20	40	60	80	40	60	40	60	80	60	80	60	80
N0	17	64	66	112	1.6	12	14	22	9	12	7.1	6.0	13.8	N/A	N/A	N/A	N/A
N1	47	147	349	422	4.82	20	47	56	12	20	12.6	32.3	26.8	2.0	11.0	5.7	117
N2	51	227	476	558	4.75	24	52	69	11	21	20.5	27.0	33.8	5.6	10.7	32.0	119
N3	51	190	392	541	4.68	20	46	63	12	20	15.2	20.3	26.4	2.9	8.7	10.0	75
N4	53	191	331	520	3.14	22	40	61	12	19	19.1	15.3	23.7	3.1	7.2	11.7	33
ANOVA[a]																	
N supply		87.43**				60.92**			20.24**			29.03**		5.33**		7.23**	
DAT		79.36**				98.65**			200.48**			25.31**		115.05**		68.83**	
N supply * DAT		3.94*				3.49*			5.73**			NS		3.58*		4.98*	

[a] value of F, ns: non-significant; * and ** significant at 5 and 1% probability level, respectively.

Table 4.6 Effects of N supply on photosynthesis parameters

	35 DAT					60 DAT				
Variable[b]	N0	N1	N2	N3	N4	N0	N1	N2	N3	N4
A	12.8c	32.1b	43.1a	35.4ab	43.3a	12.5c	39.0a	40.1a	29.0ab	21.4bc
E	7.4b	9.8a	9.9a	9.7a	10.5a	6.8a	8.8a	8.3a	6.9a	7.5a
Gs	1.7a	4.0a	1.7a	4.3a	0.7a	0.8a	3.0a	4.5a	1.5a	3.3a
Ci	314.4a	286.3ab	255.3c	267.0bc	250.9c	293.6a	266.8a	265.0a	263.6a	298.4a
A/E	1.7b	3.2a	4.3a	3.8a	4.3a	1.9c	4.6a	5.3a	4.5ab	3.1bc
A/Ci	0.04c	0.12b	0.17a	0.14ab	0.18a	0.04c	0.15a	0.15a	0.11ab	0.07bc

Means of the same variable followed by different letters are significantly different at $p < 0.05$ using LSmeans

[b] A: photosynthetic rate (µmol m^{-2} s^{-1}), E: transpiration rate (µmol m^{-2} s^{-1}), Gs: stomatal conductance (mol m^{-2} s^{-1}), Ci: intercellular CO_2 concentration (vpm), A/E: water use efficiency ((µmol $mmol^{-1}$), A/Ci: instantaneous carboxylation efficiency (mol m^{-2} s^{-1}).

4.3.3 Effect of Nitrate Supply on Root Development

N supply influenced root development in terms of root fresh biomass production. Root FW was significantly affected by N treatment and time individually but the interaction between the two factors was not significant (Table 4.4). Root developed relatively slowly in the period of 40 DAT to 60 DAT with fresh biomass increasing by 24.3%, whereas root biomass increased by 34.4% from 60 to 80 DAT (data not presented). Within the period from 40 to 80 DAT, root fresh weights doubled in most of the N supply treatments expect N4 (Figure 4.8). Root biomass increased with increasing N supply and declined when N supply rate exceeding N2. There was no significant difference in root fresh weight between N1, N2 and N3 over time (Figure 4.8). At 40 DAT, root biomass did not greatly vary among N treatment N1-N4. However, the excessive N rate of N4 inhibited root development at the later growth stage (60 and 80 DAT) and caused a significant reduction in root biomass when compared to N1 and N2.

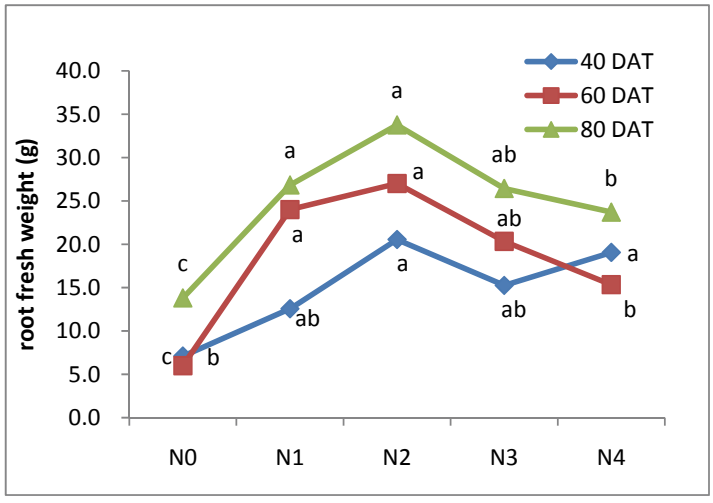

Figure 4.8 Effect of N supply on root biomass at 40, 60 and 80 DAT
Observations with the same letter within the same date are not significant different at $P < 0.05$.

Root scanning was conducted to determine N treatment effects on root measurement variables: total length, projected area, surface area, average root diameter and root volume as shown in Table 4.7. Those are the main factors affecting root function for absorbing water and nutrients from the soil. As roots developed, it became more difficult to scan the root and determine root variables. Therefore, it was only possibly to conduct root scanning at the early growth stage. For the later growth stages, root scanning pictures under N treatments were presented and compared with root systems at 60 and 80 DAT, respectively. As mentioned earlier, root biomass was not significantly affected by N treatments at 40 DAT. N supply also did not significantly affect root variables at 40 DAT (Table 4.7). However, higher N treatments N3 and N4 tended to increase values on all the root measurements, especially in root surface area and total volume.

Table 4.7 N treatment effects on root scanning measurements at 20 and 40 DAT

	20 DAT					40 DAT				
Variable[§]	N0	N1	N2	N3	N4	N0	N1	N2	N3	N4
RootLen	200	667	819	1136	772	2609a	2561a	2198a	2633a	2769a
ProjArea	14	46	67	79	75	395a	336a	409a	516a	510a
SurfArea	45	145	209	248	236	1240a	1055a	1284a	1619a	1601a
AvgDiam	0.8	2.5	4.3	4.5	5.8	1.6a	1.4a	1.8a	2.0a	2.0a
RootVol	0.7	0.7	0.8	0.7	1.0	57.7a	45.8a	65.8a	81.4a	77.8 a
Tips	836	3876	4249	6083	3165	23669a	21291a	24894a	29720a	30437a

[§]RootLen: root total length; projArea: root projected area; SurfArea: root surface area; AvgDiam: average root diameter; RootVol: total root volume; Tips: total number of root tips. Means with the same letter are not significantly different at 0.05

4.3.4 Effect of Nitrate Supply on Head Development and Tissue Sap Composition

4.3.4.1 Head Fresh Weight and Size

Head initiation in cauliflower plants grown in the greenhouse occurred approximately 6 weeks after the start of the N supply treatments. Plants in the control N0 treatment did not produce heads throughout the greenhouse experiment. Among N

supply treatments, except the control, maximum head width ranged from 1.75 to 7 cm in size at 50 DAT, which were obtained respectively in N4 and N3. Moreover, the fraction of plants with head developed was 1/2, 1/3, 1/3 and 1/6 in treatments N1, N2, N3 and N4, respectively, at 50 DAT. Generally, N supply treatments significantly affected head fresh biomass and size of cauliflower plants grown under greenhouse conditions. The interaction between N supply treatment and DAT was significant on head fresh weight and size (Table 4.5). It was observed that the response of head size to N treatments was similar to head fresh weight response over time (Figure 4.9). At 60 DAT, both head fresh weight and size increased with an increase N rate of N1-N2 while declined above N2, where the largest head was obtained in terms of fresh weight and maximum head width. Differently from 60 DAT, no significant difference of head FW and size was found between N1 and N2 at 80 DAT (Figure 4.9). Head production was greatly reduced by high N supply over N2. Low N supply treatments N1 and N2 resulted in significantly high head FW and large head size at the later growth stage.

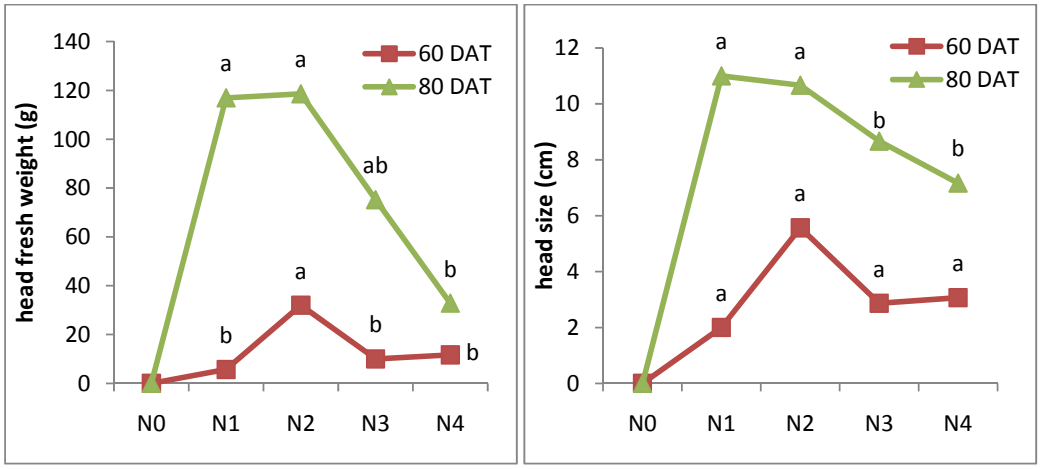

Figure 4.9 Effect of N supply on head fresh weight and size at 60 and 80 DAT
Observations with the same letter within the same date are not significant different at $P<0.05$ using LSmeans.

4.3.4.2 Head and Leaf Sap Composition

Plants in the control treatment N0 failed to form heads, and instead shoot tips formed a straightened bush of green inflorescence and leaves. For the control, the shoot tips were taken in order to determine the sap composition including NO_3-N, K and TDS that were compared to head sap in N treatments at 80 DAT. N treatments significantly affected sap NO_3-N content in both leaf and head. Leaf and head NO_3-N concentration correlated positively with N treatments and increased with increasing N supply from N0 to N4 (Figure 4.10). Head sap NO_3-N concentration overall was relatively high compared with leaf. This may be due to more N transported from the source leaf to the sink head during heading stage, which resulted in higher N accumulation in the head. The N4 treatment had the highest sap NO_3-N in both leaf and head (Figure 4.10). Furthermore, N supply did not influenced sap K concentration in the head but did in the leaf. Head sap K concentration did not vary among N supply treatments (Figure 4.10). High N supply N3 and N4 significantly increased K concentration in leaf sap compared to N1 and N2 supply treatments.

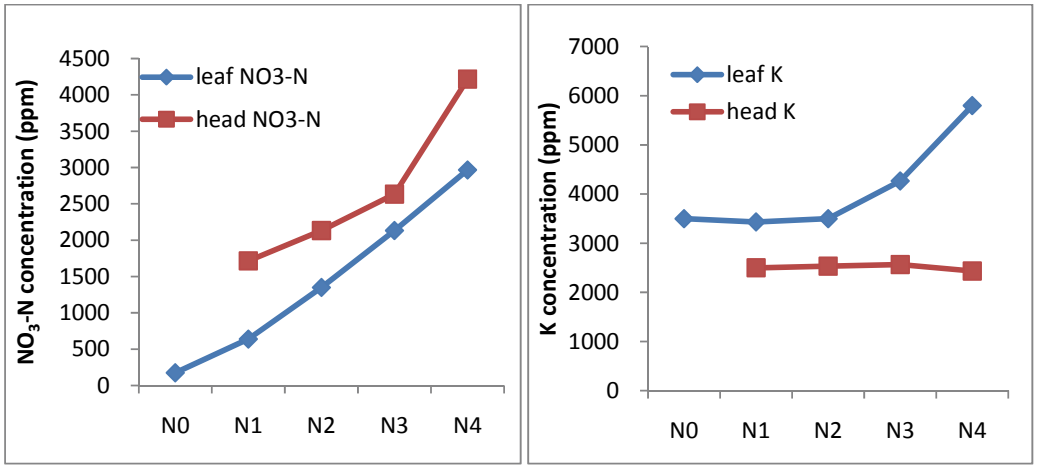

Figure 4.10 N treatments on head/leaf sap NO_3-N and K concentrations at 80 DAT Observations with the same letter within the same date are not significant different at $P<0.05$ using LSmeans.

The results suggest that both leaf and head total dissolved solid (TDS) increased with N supply treatments excluding N0, although there was no significant difference on leaf TDS among treatments (Table 4.8). However, head TDS was significantly increased by N supply. N4 had the highest head TDS that was significantly different from N0 and N1 treatment. Additionally, no differences were found among N supply treatments N2-N4 as well as treatments N0-N2. Generally, the N4 treatment resulted in the highest values of leaf and head sap variables, except for head K concentration (Table 4.8).

Table 4.8 Effects of N treatments on leaf and head sap compositions: NO_3-N, K and TDS at 80 DAT

	leaf			head		
Treatment (g)	NO_3-N (ppm)	K (ppm)	TDS (%)	NO_3-N (ppm)	K (ppm)	TDS (%)
N0	175d	3500bc	8.8a	1043c	2300a	6.9a
N1	639cd	3433c	6.8a	1715bc	2500a	8.1bc
N2	1350bc	3500c	7.7a	2133bc	2533a	8.9ac
N3	2133ab	4267b	8.2a	2633b	2567a	9.8ab
N4	2967a	5800a	8.6a	4217a	2433a	10.2a

Means with the same letter are not significant different at $p<0.05$ using LSmeans.

4.3.5 Effect of Nitrate Supply on NRA

4.3.5.1 NRA versus Induction Time

In order to study NR activity overtime through incubation in the dark, NRA was compared among N supply treatments in terms of formed nitrite concentration that was spectrophotometrically determined. The higher concentration of nitrite formed within the same period of dark induction suggests the higher NRA, as the nitrate concentration in the buffer solution is consistent for each treatment. At the early growth stages of 20 DAT, nitrite concentration did not change among all N

treatments including the control N0, whereas at the later growth stage, significant difference was observed between the control and other N treatments N1-N4, where N0 resulted in the lowest NRA based on the variation of nitrite concentration (Figure 4.11). Additionally, NRA also responded differently among N treatments N1-N4. Generally, NRA under N treatments (N1-N4) increased overtime and stopped around 24 hours after dark induction throughout the greenhouse experiment as spectrophotometrically measured leaf nitrite concentration remained stable (Figure 4.11).

For the control, NRA was inhibited and maintained close to zero over time except at 20 DAT. This could be due to no N supply for plants in the control treatment, whereas N originated from the promix resulted in N uptake and thus NRA of plants in N0 at the vegetative growth stage (20 DAT). The highest NRA occurred in N1 at both 20 and 40 DAT, while increasing N supply from N1 to N4 did not make a significant difference on nitrite content over time during the period of 72 hours of dark induction (Figure 4.11). However, at the later growth stage N2 resulted in the highest nitrite concentration and NRA over 72-hour induction. At 60 DAT, NRA was significantly increased by N2 treatment. Among N treatments, there was no difference on NRA in the first 3 hours of induction, whereas after 3 hours NRA in N2 greatly increased and remained significantly higher than other N treatments (Figure 4.11). In contrast, at 80 DAT no significant difference was observed on NRA among N supply treatments through 72-hour induction (Figure 4.11).

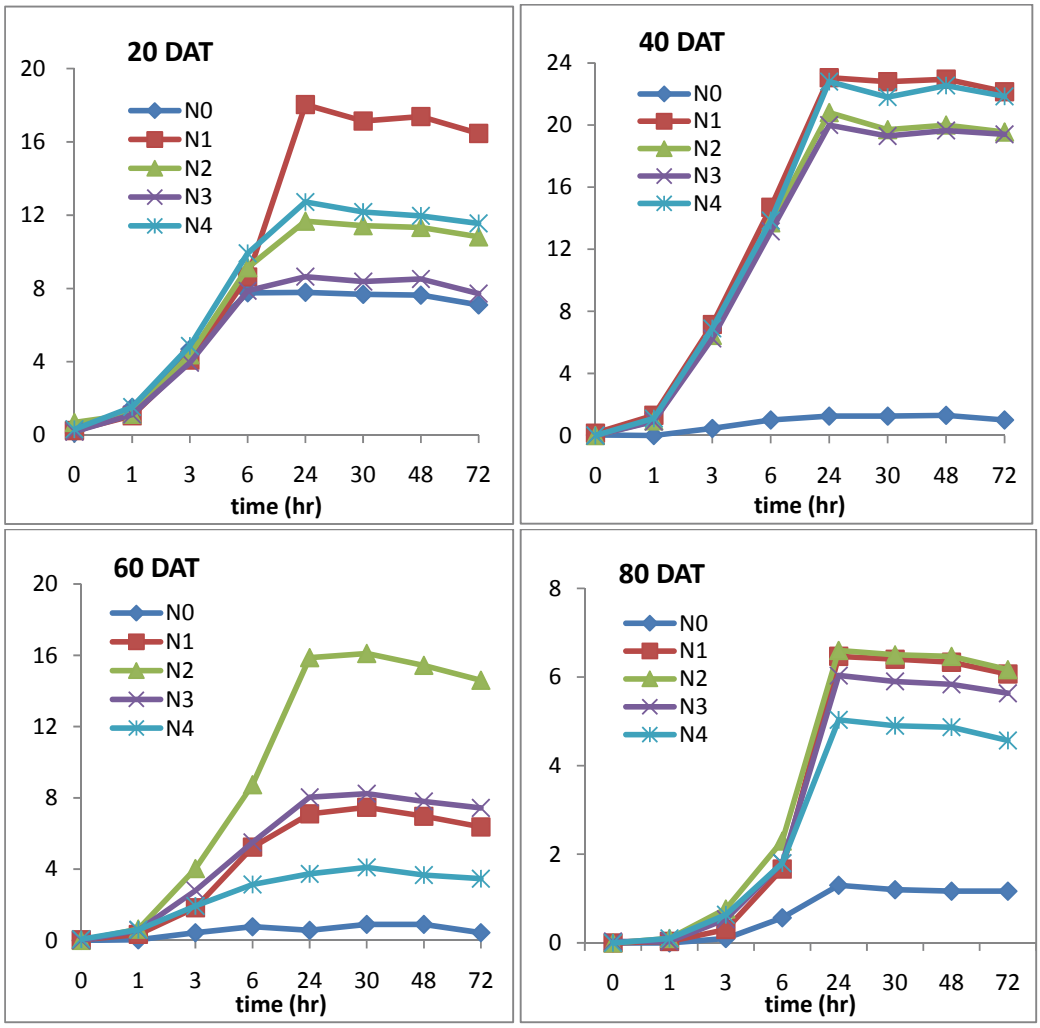

Figure 4.11 Leaf formed nitrite concentration (μM NO_2^-) versus induction time in the dark under N treatments at 20, 40, 60 and 80 DAT

4.3.5.2 NRA versus N Treatments

NRA of cauliflower leaves was significantly affected by N treatments and growth stages. The NRA of the youngest fully expanded leaves decreased as plant grew (Figure 4.12). At the early growth stage of 40 DAT, maximum NRA rate was 2.48 and 7.68 times of that at 60 and 80 DAT, respectively (date not presented). The effect of N treatments on NRA rate responded differently with plant growth stages. N supply overtime significantly promoted NRA when compared with the control N0

treatment (Figure 4.12). NRA did not significantly vary among treatments N1-N4 at both 40 and 80 DAT. At 60 DAT, increasing N rate linearly increased NRA, however, NRA decreased with a N supply rate exceeding N2. Overall, NRA increased as N supply rate increased up to N2 and tended to decrease above N2. Results suggest that N2 would be the most appropriate N rate for maximizing NRA that consequently results in less nitrate accumulation and higher production of N-containing compounds such as amino acids and protein.

In addition, N treatments did not influence NRA in the cauliflower head at 80 DAT. No significant differences were found among N supply treatments, however, head NRA appeared to slightly increase with N treatments except for N0 in which no head was formed (Figure 4.13). N2 had the highest leaf NRA while also resulting in the lowest head NRA. Moreover, NRA in leaves was significantly higher than in head among treatments N1-N4 (Figure 4.13).

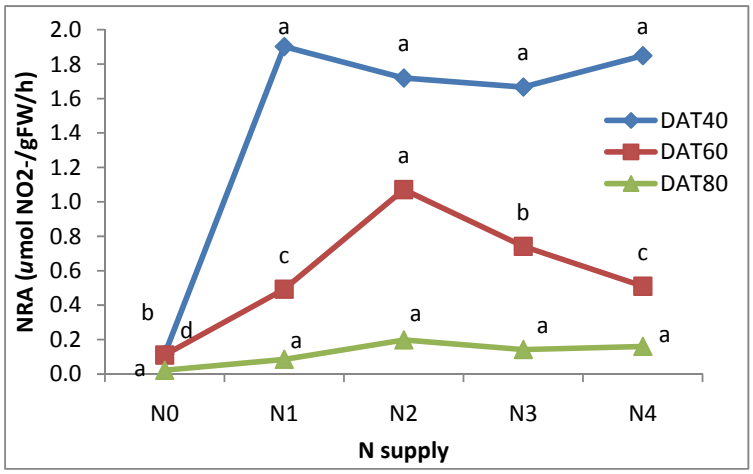

Figure 4.12 NRA in the youngest fully expanded leaf of cauliflower plants under different N supply rates at 40 DAT, 60 DAT and 80 DAT.
Means with the same letter are not significantly different at 0.05 using LSmeans.

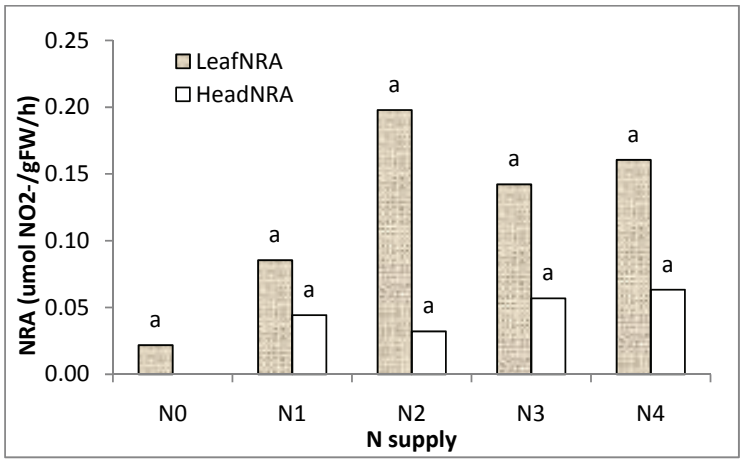

Figure 4.13 NRA in cauliflower leaves and head under different N supply rates at 80 DAT. Means with the same letter for the same variable are not significantly different at 0.05 using LSmeans.

4.3.5.3 Relationship between N Supply, NRA and Tissue Sap N/K

N supply treatments showed significant correlations with sap NO_3-N content of leaves and head as well as leaf sap K content (Table 4.9). A significant positive correlation was also found between leaf sap NO_3-N and head sap NO_3-N. This may suggest increasing N supply can increase NO_3-N content in both leaf and head. In contrast, NRA was not correlated with N supply or tissue sap NO_3-N and K content. Additionally, leaf K was significantly correlated with sap NO_3-N in leaf and head of cauliflower plants (Table 4.9).

Table 4.9 Correlation between N supply, leaf and head NRA, tissue sap NO3-N and K concentrations

	Leaf NRA	Head NRA	Leaf NO_3-N	Head NO_3-N	Head K	Leaf K
N supply	NS	NS	0.936**	0.855**	NS	0.786**
Leaf NRA		NS	NS	NS	NS	NS
Head NRA			NS	NS	NS	NS
Leaf NO_3-N				0.864**	NS	0.815**
Head NO_3-N					NS	0.766**

*, ** denotes significant correlations at a=0.05 and 0.01, and NS denotes non-significant at P=0.05.

4.4 Discussion

4.4.1 Plant Growth and Nitrate Accumulation Associated With N Supply

Cauliflower plants were supplied with nitrate-N as KNO_3. Plant TFW and TDW increased with N rates up to N2 and decreased above N2. Increasing N supply from N2 to N4 reduced plant TFW and TDW by 7.3% and 13.1%. Similar results were obtained in the study of Chen et al. (2004) that investigated five levels of N supply (N1-N5) from 0-0.6 g N/kg soil with an increment of 0.15 g. Results indicated that whole plant FW of three vegetables increased with increasing N supply up to the N3 level and then decreased.

In the present study, however, there is no significant difference on plant TFW and TDW among N2, N3 and N4 treatments. This would suggest that N supply (N2-N4) did not affect cauliflower plant growth in terms of whole plant fresh biomass and dry matter; however, it significantly influenced cauliflower head development. Compared with N2 treatment, cauliflower head FW significantly decreased by 37% and 72% in N3 and N4. This suggests that cauliflower head development can be inhibited when an excessive N rate of 66 mg N/day is supplied that refers to N4 treatment.

Cauliflower leaf/head sap NO_3-N concentration increased linearly with nitrate supply rates. It is well established that nitrate uptake and accumulation are highly related to exogenous nitrate concentration (Devienne-Barret et al., 2000; Darnell and Stutte, 2001). Clarkson (1986) claimed that nitrate supply in excess of current demand can contribute to nitrate accumulation in the plant. Plants supplied with N4 rate had abnormally high sap NO_3-N concentration of 4217 ppm. Head sap nearly doubled when increasing N supply from N2-N4. It is likely that the dramatic decrease of head yield in N4 treatment can be associated with high nitrate accumulation in cauliflower.

Nitrate accumulation in cauliflower head is not only harmful to human health by consumption but also have adverse impacts on plant growth.

Plants in N4 treatment appear to exhibit toxicity symptoms that can be evidenced by a minimal size of root system and an abundance of foliage with a small head. In addition, necrotic lesions occurred in both head and leaves, and was severe along the margin of leaves surround the head. Recent studies have supported that due to nitrate accumulation, nitrite can be converted into nitric oxide (NO) in plants, and nitrate reductase (NR) can rapidly convert NO into peroxynitrite ($ONOO^-$) that is highly toxic to plant (Chen et al., 2004).

In general, head sap NO_3-N was higher than in leaves of cauliflower. Kaniszewski and Rumpel (1998) pointed out that nitrate accumulation occurs to a greater extent in the leaves than in the curds, while nitrate-N content in leaves appeared to lower than in curds when low N rates were applied. On the contrary, the reverse result was obtained in cauliflower plant with high N supply in the current study. This may be because that nitrate-N content of cauliflower was measured in the early head stage and increasing N accumulation in head can occur as head develops.

4.4.2 Leaf Chlorophyll and Photosynthesis

Compared with the control, leaf chlorophyll content was significantly increased by N treatments. At the vegetative stage of plant growth (28 DAT), leaf chlorophyll content increased with N supply (N0-N2) and did not vary with further increasing N rates (N2-N4). However, N4 resulted in significantly low leaf chlorophyll content from other N treatments (N1-N3) at the later growth stage (63 DAT). Leaf chlorophyll is related to leaf N content as the majority of leaf N is contained in chlorophyll molecules (Netto et al., 2005). Results did not indicate a

linear relationship between leaf chlorophyll content and leaf sap NO_3-N of cauliflower plants, although nitrate-N is as much as 60% of total N in plant (Cárdenas-Navarro et al., 1999). Leaf sap NO_3-N consistently increased with N supply, whereas increased chlorophyll content with N supply rate occurred at N0-N2.

Leaf chlorophyll was found to be highly related to photosynthesis. Higher leaf chlorophyll content resulted in a higher rate of photosynthesis in cauliflower. N supply showed a similar effect on photosynthesis like leaf chlorophyll content. This can be seen from the pattern of photosynthesis change with N treatments at 35 and 60 DAT when compared with the response of leaf chlorophyll at 28 and 63 DAT. Hence, photosynthesis is associated with leaf chlorophyll content in cauliflower. Studies have proven that lower photosynthesis is often attributed to the reduction in chlorophyll content and Rubisco activity (Evans and Terashima, 1987; Fredeen et al., 1991).

4.4.3 Relationship between NRA and N Supply

Nitrate reductase activity (NRA) in cauliflower was significantly affected by N supply and time. Results clearly indicated NRA in cauliflower leaves declined as plants grew overtime. Based on the substantial reduction from 40 to 20 DAT, leaf NRA achieved the highest at 40 DAT near curd initiation, and NRA was the lowest at 80 DAT. This observation is in agreements with other studies on cauliflower. Yang et al. (1994) stated that NR activity in cauliflower leaves was higher during the curd formation while lower with further flower bud differentiation and at head maturity.

The response of nitrate reductase activity (NRA) to N treatments differed with growth stages. Cauliflower plants in the control treatment had the lowest NRA that was close to zero because of lacking N supply. At 40 and 80 DAT, NRA did not vary with N supply. However, there were significant differences found on NRA between N

treatments at 60 DAT. Increasing N supply (N0-N2) significantly increased NRA in cauliflower, while NRA decreased above N2, where the highest NRA rate occurred. The results agreed with most of the observations by Chen et al. (2004). Nitrate supply showed a significant impact on leaf NRA of 9-week old cabbage plants that are in the same family as cauliflower. Leaf NRA significantly increased with nitrate supply to the medium level after which no large difference was observed (Chen et al., 2004).

Cauliflower head NRA was not significantly influenced by N supply. An increased N supply from N2-N4 tended to increase NRA in the cauliflower head. Numerous studies have been conducted on several crops and established that to some extent, nitrate reductase activity can increase with nitrate supply or nitrate concentration in hydroponics studies (Yang et al., 1994; Lo´pez-Cantarero et al., 1997; Darnell and Stutte, 2001; Chen et al., 2004; Taghavi and Babalar, 2007).

Chapter V

Conclusions

1. Requirements of N and K Fertilizers for Cauliflower in Nova Scoita

Cauliflower is well adapted to the long and cool growing season in Canada's Maritime Provinces. In Nova Scotia, N rate required for cauliflower is estimated to be 220 kg/ha. Results in the field trial indicted that cauliflower curd yield increased with N application in an increment of 55 kg/ha from 0 to 220 kg/ha. The application of 220 kg/ha had the highest head fresh biomass and dry matter that are significantly higher than in the treatment of 165 kg N/ha. However, head size and head nutrients N, P & K concentration did not differ between N_{220} and N_{165}. As cauliflower is marketed based on size but not weight, N input rate of 165 kg/ha is recommended to apply to cauliflower crops in Nova Scotia. Local commercial growers can benefit from reduced input cost due to reduced dose of fertilizer N that is also environmentally healthy.

In addition, due to a consistent increase on cauliflower yield with N rates applied, the N application rate to maximize cauliflower head yield would need to be further studied. Therefore, for future field research on the relationship between higher N fertilization and cauliflower production and quality, N application exceeding 220 kg N/ha should be tested on cauliflower or other cole crops in Nova Scotia.

In this field study, K fertilization significant affected cauliflower curd yield and head size. A K application of 25 kg/ha caused a significant increase on head biomass and dry weight as well head size. Based on the soil testing and plant K requirements in Nova Scotia, soil existing K_2O was 333 kg/ha and to reach the

recommendation rate of K fertilization for cauliflower, a K application rate of 50 kg/ha is suggested.

However, higher K application (50 kg/ha) reduced cauliflower yield from the aspects of fresh biomass, dry matter and head size. Moreover, it decreased head total N and thus protein content. Generally, K application enhanced N uptake in head, plant leaf-stem-root and whole plant of cauliflower. However, it did influence head total K accumulation but not K concentration (%DW). Results indicate that the applied K rate of 25 kg/ha is sufficient for cauliflower growth and maximizing the yield in soils with a medium rating for K nutrients. On the basis of soil K availability, estimated K fertilization required for cauliflower or cole crops is around 300 kg/ha. K application exceeding 300 kg/ha can be disadvantageous to cauliflower growth and yield.

Overall, N fertilization combined with K application resulted in an ideal cauliflower curd. Cauliflower curd biomass and dry weight increased by nearly 19% and 30% from $N_{220}K_0$ to $N_{220}K_{25}$. Besides, cauliflower curd yield did not significantly vary among $N_{220}K_{25}$, $N_{220}K_{50}$ and $N_{165}K_{25}$. Therefore, it is possible to reduce N fertilization rate by applying an approximate amount of K fertilizer together with N fertilizer. In practice, K fertilizer is recommended to apply to the commercial cauliflower production in Nova Scotia. For achieving sustainable agriculture in Nova Scotia, other factors beside fertilization need to be considered. From environmental and agronomic aspects, factors that can contribute to the variation on crop performance and production include climate, rainfall, weed and disease control, soil type, etc. As it is well known that fertilizer N is more subjected to leaching and loss under such conditions as sandy soil or heavy rainfall, N fertilizer source and timing of fertilization application play an important role.

2. Cauliflower Response to Varying N Supply under Greenhouse Conditions

In terms of plant fresh biomass, dry weight, stem diameter and leaf number, cauliflower plant growth increased the most with N supplied at a rate of N2. In the vegetative growth stage cauliflower responded similarly to N supply treatments (N1-N4), while in the later growth stage nitrate appeared to have been supplied in excess to cauliflower plants that were symptomized by inhibited development of cauliflower head and root as well as significantly reduced leaf chlorophyll content in N4 treatment. The control treatment (N0) overtime resulted in the poorest plant growth variables as well as inhibited root growth.

High N supply (N3-N4) favoured the establishment and development of roots in the vegetative growth stage. Increasing N supply increased total root volume, root surface area and average root diameter. However, root measurements did not vary among N supply treatments (N1-N4) when plants were 40-day old after treatment application.

Leaf photosynthesis and chlorophyll content increased with N supply (N0-N2), but no significant difference was found among N2, N3 and N4 treatments at the early growth stage. However, both variables tended to decrease with N supply rate above N2 at the later growth stage. Increasing N supply linearly increased leaf/head sap NO_3-N concentrations and the concentrations doubled from the N2 to N4 levels. Head NO_3-N was significantly higher than in leaves ($P < 0.05$). Head sap K concentration was constant (2400 μg/g) among treatments N1-N4, but leaf sap K concentration increased by 22-66% only in plants supplied with N rate above N2.

The control plants did not form a curd. Low N supply (N1-N2) produced better cauliflower curd based on fresh biomass and head size. There was a 37% and 72% decrease of head fresh weight in N3 and N4 treatments when compared with N2.

In addition, head size of cauliflower with N3 and N4 levels was 36% smaller than plants in N1 and N2 treatments.

The in-pot cauliflower nitrate reductase activity varied between 0.1-2.0 μmol NO_2^-/gFW/h. The highest NRA was measured at vegetative stage and NRA decreased with the age of leaves to reach the lowest level at head maturity. In contrast to the control, NRA was significantly higher in the plants receiving N treatments ($P < 0.01$). N2 overall had the highest leaf NRA. Head NRA was higher in N3 and N4 than in the other treatments. It was suggested that high NRA was associated with high photosynthesis that could lead to high cauliflower yield and quality.

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