The Role of Variation in Nitrogen and Water Uptake on the Performance of Lettuce Genotypes under Organic Field Conditions

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Plant breeding Group

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A thesis Submitted to the Department of Plant breeding in partial fulfillment of the requirements for Master of Science in plant science

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Abstract

The experiment was designed to examine the relationship between genetic variation in nitrogen and water uptake and crop performance under organic field conditions. One hundred fifty butter head lettuce genotypes were evaluated for their performance and nitrogen uptake from different soil depths. Biomass yield, head nitrogen content and amount of nitrogen left at different soil depth at time of harvest were measured. Crop performance was evaluated by scoring uniformity, size and overall performance of genotypes. The results showed significant genotypic difference in dry matter yield and nitrogen use efficiency. There were strong positive relationships between NUE, head N content and dry matter yield indicating the important role of N uptake efficiency for genotypic variation in dry matter yield. Uniformity and overall performance of genotypes had positive associations with dry matter yield and NUE indicating the possibility of selecting genotypes which combine high quality and yield with high NUE. The total amount of nitrogen taken up per plant had positive correlation with head N content and dry matter yield. There was significant genotype by soil layer interaction in amount of nitrogen taken up that indicated variation in rooting pattern of genotypes. Percentage soil moisture content showed significant positive association with N uptake. The results indicated that nitrogen uptake measurement from different soil layers can give an indication about the rooting pattern and uptake efficiency characteristics of genotypes under field condition.

The weather data, mean soil moisture content, the genotypes yield and performance indicated that the growing condition was optimal for the majority of genotypes but a few genotypes showed mild stress symptoms. Genotype by environment interaction evidently influenced crop growth, yield, rooting pattern, crop nutrient requirement and root to shoot ratio. Thus, through repeated experiments across growing seasons and years the functional association of plant N uptake per soil layer with rooting pattern and uptake efficiency can be established.

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Abbreviations

DM	Dry matter yield
ISE	Ion- selective electrode
MV	Milli volt
M	Molarity
N	Nitrogen
NUE	Nitrogen use efficiency
PPM	Part per millions
QTL	Quantitative trait locus/loci
RSA	Root system architecture

Glossary

Critical nitrogen concentration: the minimum nitrogen concentration which allows maximum growth rate.

Field performance: represents the phenotypic response of lettuce genotypes under field condition; which include yield, uniformity, size, head nitrogen content and over all field performance score.

Head nitrogen content: the amount of total nitrogen (both organic and inorganic) in the head of lettuce genotypes calculated as mg nitrogen per mean dry weight (g) of a genotype.

Nitrogen Use Efficiency (NUE): represents the combined effect of both uptake efficiency, the ability of plants to take up N from the soil and utilization or acquisition efficiency, the ability of plants to utilize N taken up to produce dry matter or grain yield.

Nitrogen productivity: dry matter yield per unit nitrogen present in the plant.

Nitrogen depleted per soil layer (Nitrogen taken up per plant per layer): the two terms were used interchangeably as the amount of soil N depleted was considered to be equivalent to the amount of N taken up by the plant. It was calculated as the difference between the amounts of nitrogen (mg) found in bare soil and nitrogen left in root zone at time of harvest. Refer to the material and method section for details of the calculation.

Total Nitrogen Depleted (Total Nitrogen taken up by plant): the sum of the amount of nitrogen depleted (take up by a plant) from four different soil depths.

Rooting zone: soil volume that was considered to be exploited by a single lettuce plant

Root System Architecture: the structure and distribution of roots with in a rooting media.

Uptake potential: indicates the capacity of root system to take up water and nutrient.

Uptake efficiency: indicates the ability of root system to take up higher amount of nutrient and water while having relatively low root mass / with less carbon investment.

1. Introduction

1.1 Back ground

Lactuca sativa L. is produced commercially in many countries worldwide. It is especially important as a commercial crop in Asia, North and Central America and Europe. China, U.S., Spain, Italy, India and Japan are among the world's largest producers (Morf, 2008).

Consumer's preference to organically produced vegetables like lettuce continues to grow. Even though the price of organically produced food items is much higher than conventionally produced food, consumers are increasingly interested in organically produced foods due to health concerns. In order to satisfy consumers' needs big firms are becoming interested in sourcing and production of organic products. However, supply of organic lettuce in the market is limited and inconsistent in terms of volume and quality. This can be because of limited availability of cultivars well adapted to organic farming condition, challenge in the propagation of planting materials under organic condition, higher possibility to get diseased planting materials under organic seed production, less availability of organic seeds because of the difficulty of propagation, and high cost involved in organic seed production.

Environmental sustainability concerns are also increasingly crucial. For example nitrogen fertilizer application is useful to increase yield. However, its use is inefficient with on average about 33% of the total N applied will actually be utilized by plants (Raun and Johnson, 1999). The remaining N will be lost through surface runoff, leaching of nitrate (NO_3-) in groundwater, volatilization to the atmosphere and by microbial de-nitrification. All of these losses cause environmental concerns (Vitousek et al., 1997).

Efforts have been made to produce lettuce under organic conditions. However, most of the commercially available lettuce cultivars are less adapted to organic farming conditions which are characterized by low and variable nutrient and water availability. Modern lettuce cultivars have high water and nutrient requirement. However, lettuce in general has shallow roots that hamper the extraction of water and nutrient from lower layer of the soil. Under organic farming conditions the approach is to enhance the adaptation of crops to environmental conditions than adjusting the environment to the crop needs. Lettuce requires high nutrient and water. Thus under organic filed conditions lettuce varieties that are efficient in nutrient and water extraction capacity can perform well.

Breeding for improved root system architecture is vital. There is genetic difference among cultivated and wild varieties of lettuce that can be used to generate varieties that are better adapted to organic conditions. Therefore, the objective of this experiment was to examine the difference in nitrogen extraction and use efficiency of various genotypes of lettuce.

1.2 Problem statement

Plants that are capable of exploring large soil volume both horizontally and vertically to the deeper soil horizons can be best suited to organic and low input conditions, where availability of water and nutrient to the plants is limited. Root architecture has a major impact on plant survival and productivity especially under low input and organic production conditions. The inherent root architecture of a plant determines its ability to explore deeper soil horizons. Rooting depth and root architecture differ significantly between crop species (Manschadi et al., 2008). There is genetic variation in root traits, which needs to be exploited. However, the root systems are challenging to study (Myers et al., 2007). This is because it is hard to uproot field grown plants while all fine roots are intact. In addition, pot trials and hydroponic trials can hardly represent the condition under field, because rooting characteristics are highly influenced by many factors under field conditions. The development and proliferation of the roots in soil are affected by intrinsic and extrinsic parameters such as the supply of photosynthesis from the shoot, the nutrient status of the plant, soil type and compaction (Bloom et al., 2002), water potential at the root surface, availability and distribution of nutrients (Forde and Lorenzo, 2001).Breeders are aware of the importance of selection for root traits as consumer's health concerns, environmental sustainability and resource unavailability traits are increasingly important problems that urge for low input agriculture. There is a need to design selection criteria for root traits that ensure easy and reliable screening of large set of genotypes without uprooting plants. As uprooting plants is inefficient and unreliable way of screening. To design reliable selection criteria several aspects need to be studied.

In general genotype by environment interaction of various root traits with environmental conditions like soil water and nutrient availability need to be studied, then based on all results designing of a model that captures the effect of all factors as much as possible will be profitable. There is a PhD project that is under way to design such a model. The current experiment is part of that PhD project. This experiment was designed to examine the relationship of soil nitrogen depletion at different soil depth with genetic variation in crop performance under field condition. The experiment was designed in such a way that lettuce genotypes were assigned to experimental plots which were fertilized with relatively low and equal amount of organic fertilizer in order to examine genetic variation in nutrient extraction efficiency while the supply was limited.

1.3 Objective of the research

To examine the variation in nutrient and water uptake capacity among lettuce genotypes based on crop performance and the amount of nitrogen depletion from different soil depths.

1.4 Research questions

The general research question is: What is the relationship between the performance of lettuce genotypes and their uptake and utilization of nitrogen under field conditions?

This general research question can be refined into the following sub questions:

A. What is the relationship between biomass production and the amount of nitrogen taken up from the soil?
B. What is the relationship between above ground nitrogen accumulation in the crop and nitrogen depletion from the soil?
C. How does plant nitrogen use efficiency (NUE) correlate with field performance?
D. How does the soil moisture content influence the nitrate uptake?
E. Is there genetic variation in nitrate uptake per soil horizon?

2. Literature review

Efficient root system is vital for resource capture and high biomass production under low input and organic farming. Efforts have been made to study the root system of plants and to find a reliable way of screening genotypes for root traits. The current study was intended to examine the possibility to get information about variation in root system among genotypes using phenotypic data and quantifying amount of soil nitrate taken up by each genotype from different soil layers. Hence in order to show the position of the current trial in the big picture of studying root system of plants. This review tries to summarize available literatures on the role of nitrogen, water and genetic variation in root system on phenotypic responses of plants.

This review begins with a general description of lettuce, highlights the significance of breeding for root traits in the context of lettuce cultivation, then factors that govern root to shoot capture and resource utilization will be discussed. Next to that NUE and the impact of root system architecture on soil nitrogen and water uptake will be presented, then genetics of root development and environmental factors that influence root functioning will be discussed respectively. Subsequently, various efforts and underling challenges towards the development of selection criteria for root traits in breeding programs will be discussed and lastly modeling approach, its significance as a means of studying root system indirectly and efforts made on the field will be explained in order to indicate the possibility to use the data obtained from current trial in modeling approach.

2.1 The lettuce plant and its root system

Lettuce (*Lactuca sativa*) belongs to the Asteraceae family (formerly Compositae), subfamily *Cichorioideae* and tribe *Lactuceae.* It is a diploid (2n = 18) and self-pollinating annual which produces a dense rosette of leaves in the early season, followed by flower stalk initiation. There are seven groups of lettuce based on their morphology (Lebeda et al., 2007), namely butter head lettuce, crisphead lettuce, cos lettuce, cutting lettuce, stalk (asparagus) lettuce, latin lettuce and oilseed lettuce. The current study focuses on the raw eaten butter head lettuce type, which is a heading type with soft and tender leaves. It is most popular in England, France, the Netherlands and other western and central European countries (Ryder, 1986).

Cultivated lettuce (*Lactuca sativa*) which is raised from transplants has a shallow root system with a short taproot and prolific lateral branches in the upper layers of the soil; whereas wild lettuce (*Lactuca serriola)* has a deeper root system with more laterals emerging at the tip of the tap root (Jackson, 1995). Lettuce roots develop rapidly. Lateral root branches begin to appear on the first 2.5 to 3.8cm of taproot only 6 days after the seed is planted (Weaver, 1927). However, due to its shallow root system, the cultivated lettuce is mainly adapted to high water and nutrient availability conditions. Hence, to cultivate lettuce under low input and organic condition, selection of lettuce genotypes that have efficient root system architecture and crossing for instances using wild lettuces (*Lactuca serriola)* as a donor for desirable root traits can be useful.

2.2. Root to shoot ratio

The balance between dry matter allocation to root and shoot determines the adaptation of plants to environmental stress, their resource use efficiency and ultimately their desired yield. Roots play a key role in acquiring nutrients and water but root development involves a major cost to plants in terms of carbon allocation (Jackson, 2004). Uptake capacity and efficiency of root system has a significant role on the dry matter yield of plants. Less carbon investment to the root associated with sufficiently high water and nutrient uptake to grant a high yield is a desired trait. However, selection for such trait is not straight forward because of two challenges: one comes from the difficulty to measure root traits and the second challenge comes from the inevitability of genotype by environment interaction in shoot to root dry matter allocation. Hence in order to select genotypes that are efficient in resource utilization; it is important to have insight on an interactive effect of genetic and environmental factors on shoot to root dry matter allocation. This section tries to explain the interactive effect of genetic and environmental factors in determining root to shoot.

In general plants adjust their root to shoot ratio based on their resource need and environmental conditions. Genetic variation in plasticity of root to shoot ratio is associated with difference in uptake and utilization of resources. Plants can also adjust their morphology and chlorophyll concentration of leaves to gain limiting resources

(Aikio and Mari Markkola, 2002). The nutrient status of the plant and supply of photosynthetic product from the shoot determine the extent of root growth (Walch -Liu et al., 2006). On other hand, photosynthesis rate depends on leaf area and nitrogen content per unit leaf area (Gastal and Lemaire, 2002). As nitrogen is major component of chlorophyll uptake of nitrogen is key for photosynthesis. However, root development for nitrogen mining involves carbon investment. High carbon costs for root construction affect photosynthetic carbon allocation for shoot production. Root architectural traits that increase the exploitation of resources with a minimal root biomass allocation are desirable (Johnson et al. 2000).

Environmental factors such as water and nutrient availability have great influence on root to shoot ratio. Generally, when water and nutrient availability increases, plants allocate relatively less to their roots because less effort is required to acquire this resource (Agren and Franklin, 2003). There are evidences that shows a tendency of increase in root to shoot ratio when water and nutrient availability is limiting (Gonzalez dugo et al., 2010). Light intensity also has an influence on nitrogen demand and the shoot to root ratio. Plants invest more resource to leaf area increment to intercept more radiation under low light intensity and to take up higher amount of nitrate for osmotic adjustment. There are evidences that showed a tendency of higher nitrate accumulation under low light intensity (Burns et al., 2011b). The supply of nitrate nitrogen in the rooting medium is also recognized as a factor controlling the distribution of dry matter between shoot and root (Drew et al., 1973).

2.3 Nitrogen use efficiency and crop performance

Nitrogen is a critical nutrient that determines crop growth and productivity. Thus, nitrogen use efficiency is very crucial especially under low input condition. Nitrogen use efficiency (NUE) includes both uptake efficiency, the ability of plant to remove N from the soil and utilization or acquisition efficiency, the ability of plants to transfer N to the shoot (Benincasa et al., 2011). According to Pathak et al. (2008) NUE can be quantified in different ways: as agronomic efficiency which is total economic outputs relative to the available soil nitrogen; as apparent nitrogen recovery which is related to the efficiency of N uptake, and as physiological NUE that quantify N utilization to produce grain or total plant dry matter.

Genetic variation plays a key role both N uptake and use of absorbed N as different genotypes can differ in morphological and functional characteristics of shoot and root (Thorup-Kristensen and Sørensen, 1999). However, a clear understanding of the major mechanisms and inheritance of NUE is lacking (Dawson et al., 2008).

Environmental factors such as water availability, light intensity, temperature, availability of other nutrients, the availability and form of nitrogen influence NUE (Benincasa et al., 2011). Environmental factors influence either crop growth and development or nitrogen availability to the plant (Benincasa et al., 2011, Gonzalez dugo et al., 2010). As NUE is a function of multiple interacting genetic and environmental factors disagreements often arise in partitioning variation to genetic or environmental causes and even on the definition of NUE itself (Dawson et al., 2008).

2.4 Root system architecture and nitrogen use efficiency

Nitrate is the most preferable form of nitrogen and is taken up by active transport through the root. Garnett et al. (2009) stated that the role of root traits in NUE is not well understood. The knowledge gap is associated with the limited availability of information on root biology under field condition (Garnett et al., 2009). However, in QTL studies on rice and maize positive coincidences between QTLs for N uptake and QTLs for root architecture traits was found (Coque et al., 2008). Dupuy et al. (2010) reported the existance of considerable evidence on the role of root architecture in water and nutrient acquisition efficiency. The root system architecture is an essential part of plant nitrogen uptake capacity as it determines root exchange area and exploitation potential (Fitter et al., 2002). The rooting depth penetration rate and depth distribution of root density are found to be the most important parameters that determine crop N uptake from deeper soil layers (Pedersen et al., 2010). Similarly, Robinson and Rorison (1983) reported the important role of root length per unit soil volume for the uptake of nitrate from soil. Breeding for a root system trait that ensure efficient N uptake can improve NUE. However, it is important to note the growing condition as high or low N availability can have major influence on what factors explain observed differences in NUE (Coque and Gallais, 2006).

2.5 Genetics of root system architecture

Root system architecture (RSA) plays an important role for the plant to access limiting soil resources efficiently (Lynch, 1995). Genetic variation in RSA among lettuce genotypes and other plant species is reported by many authors (Thorup-Kristensen, 2006,Garnett et al., 2009, Jackson, 1995). In most cases a suite of quantitative trait loci (QTLs) that interact with the environment govern the genetic variation for RSA (de Dorlodot et al., 2007). In order to utilize this variation, the difficulties associated with evaluating and selecting root traits are obvious. (Wissuwa et al., 2009) suggested that using tightly linked markers to indirectly select for a trait of interest as ideally suitable way to transfer important root traits to modern varieties.

Breeding for root traits is now becoming promising as information on genetic control of RSA is advancing rapidly (De Dorlodot et al., 2007). There are QTLs that individually explain up to 30% of phenotypic variation for root traits in rice for the response of RSA to environmental factors (De Dorlodot et al., 2007). However, (Wissuwa et al., 2009) reported that very few of identified QTLs have been used in practical breeding programs due to the lack of relevant QTLs identified in target environment. The challenge in identification and confirmation of benefits of QTLs in target environments is associated with large spatial variability in nutrient availability or toxicity traits on field trials that limit accuracy of phenotyping in mapping (Wissuwa et al., 2009)**.** In addition, other constraints related to the nature of the underlying genes, low heritability caused by small effects of individual loci, presence of genotype by environment interactions, gene by gene interactions (epistasis) and multiple effects of one gene (pleiotropy) limits success (de Dorlodot et al., 2007).

2.6 Environmental factors that influence root functioning

A range of environmental factors influence root growth and development. These environmental factors can be grouped as soil physical property (soil texture, structure), soil chemical property (PH, nutrient). Biological factors (soil microbial population, root disease and pests), soil temperature and water availability, the above ground weather conditions such as light intensity also influence roots functioning indirectly as a result of their influence on photosynthesis and shoot growth. Root system architecture is a highly plastic trait. Genetically identical plants can highly differ in root system architecture, depending on their macro- and microenvironment (Osmont et al., 2007). The response of plants to soil environmental changes influence water and nutrient uptake by the root systems and ultimately productivity of plants (McMichael and Quisenberry, 1993).

Soil temperature influences water and nutrient uptake, metabolic processes, roots and shoot growth (Dong et al., 2001). Dalton and Gardner (1978) showed that the viscosity of water, permeability of membranes, and the amount of active uptake of ions are influenced by temperature. When temperature is higher, there will be more water uptake and root growth. However, excessively high soil temperature can decrease the amount of root tissue and eventually result in death (Terry J. Moore, 1981).

Soil water plays a major role for nutrients uptake by plant roots as nutrients are available in solution. The influence of soil water content is reported by many authors. Root depth and root density increase in a dry soil and root elongation rates may be significantly decreased (Mcmichael and Quisenberry, 1993). Excess soil water can causes oxygen deficiency that retard growth and uptake. Soil water deficits reduce shoot growth resulting in increased root to shoot ratios in water-stressed plants (Andrews et al., 2001). Severe water stress can result in a reduction or cessation of root growth, that inhibits water and mineral absorption (Gonzalez dugo et al., 2010).

Soil penetration resistance is another factor that influence root growth. Roots are able to force their way into the soil because of the turgor pressure of roots. When turgor pressure exceeds the resistance of soil, root extension is possible (McMichael and Quisenberry, 1993). As water content increases, soil penetration resistance decreases. There are some evidences that indicate root morphology change as a result of soil resistance for root penetration (McMichael and Quisenberry, 1993). Responses of plant root system to soil compaction includes reduction of number and length of roots, restriction of downward penetration of the main root axes, decrease in leaf thickness, increase in dry matter shoot to root ratio and decrease in crop grain yield (Grzesiak, 2009).

The development of roots is also sensitive to changes in internal and external concentrations of nutrients. Nutrient specific signal transduction pathways interpret external and internal concentrations of nutrients to modify root development (López-

Bucio et al., 2003). Some regulatory genes that play pivotal roles in nutrient-induced changes in root development are also identified in Arabidopsis (López-Bucio et al., 2003).

2.7 Challenges towards breeding for root traits

In the past root system has not been given much emphasis and breeding efforts were mainly focusing on traits of above ground parts. Plant root system is relatively less explored probably due to the difficulty of observing and sampling the disruption of root systems in soil (Fageria et al., 2011).

Currently, there is an increasing interest towards breeding for efficient root system in order to develop high yielding genotypes that are adapted to stress full conditions and to minimize nutrient loss to the environment due to inefficient use. However, there are many factors that limit success in the field. Time consuming and highly laborious job of measuring root characteristics and lack of reliable and efficient screening techniques hinders breeding efforts (ALl, 1999).

Many root studies have relied on soil cores and on minirhizotron observations. however, the data collected using these methods may not represent the crop as a whole (Fageria et al., 2011). Root traits that are measured sometime may not be relevant to plants under field condition (de Dorlodot et al., 2007). In addition, root traits are prone to environmental plasticity (de Dorlodot et al., 2007). Although this plasticity is well documented, the underlying molecular mechanisms are poorly understood (Osmont et al., 2007). Polygenic control of the root traits makes further complications in the improvement program (ALl, 1999).

2.8 Modelling approach towards the study of plant roots

Simulation modeling has value in helping the researchers to define the relevant processes and interactions in assessing the impact of single variables on system performance through sensitivity analysis, and in suggesting issues and hypotheses for experimentation (Wullschleger et al., 1994). The difficulties in analyzing the architecture of actual root systems make simulation modeling an attractive approach. Models can be used to simulate the behavioral response of roots under different scenarios of environmental and internal factors. This enables the representation of a genotype through a set of response parameters that are valid under a range of conditions (de Dorlodot et al., 2007). Root system architecture models can provide new insights in studying root development, comparing genotypes, and quantifying the effects of the environment on the root system. Models might reveal an interesting relation between dynamic and static features of root system architecture that would help to reduce the phenotyping effort (de Dorlodot et al., 2007).

The current study which is part of a four years PhD project that is intended to investigate the relationship between the amount of soil nitrogen taken up and the phenotypic characteristic of a large set of lettuce genotypes. It is known that a wide range of factors are involved in determination of phenotypic response of plants. As measuring the effect of all factors at the same time is impractical, additional trials namely, pot trial to examine root response to various watering regimes, field root trial and QTL analysis are undergoing. Finally, the information that will be obtained from the overall project will be used to develop a simulation model for characterizing root system architecture.

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3. Materials and methods

3.1 Experimental set up

The experiment was conducted in the field at Droevendaal, Wageningen. One hundred fifty genotypes were tested. The experiment was conducted starting from June 22 to July 21, 2010. Land and seedling preparation was done in advance to make sure that transplanting could be done on time. Before transplanting seedlings into the field, soil samples were taken from a depth of 0-20 cm and 20-40 cm. The samples were taken after every 10 m distance in the field. Twenty five samples were taken from each of the above mentioned soil depth ranges. The soil samples were analysed in order to check the homogeneity of the field in terms of moisture and nitrogen content at the time of planting.

At the time of planting 100 kg NO_3/ha was applied. The fertilizer "Eco-Fertiel" (seaweed pellets) which has N-P-K in proportion 9-3-3% respectively was used. The experiment was laid out using RCBD with two blocks. Individual plot size was (120 cm × 120 cm). The planting density was twenty five plants per plot. Spacing between plants and rows was (25 cm × 25 cm). Data on soil moisture content and nitrogen depletion was collected three times, before planting, at the mid growth season and at the time of harvesting. Data analysis was done using 14th edition of Genstat statistical software.

3.1 Soil moisture content analysis

Soil moisture content was analysed using gravimetric moisture measurement method. Soil samples was collected using plastic bags from each plot at the depth of 0-10, 10-20, 20-30 and 30-40 cm. when the samples reached laboratory the weight of each sample was measured. Samples were transferred into aluminium plates; large soil granules (aggregates) in the samples were crushed to facilitate drying. After that the samples were kept in the oven for 48 h at 40 °C. The weight of dried soil samples was measured. Percentage soil moisture content was calculated as the difference between fresh weight and dry weight divided by fresh weight of soil samples times hundred.

3.2 Soil nitrate content analysis

The dried soil sample was made ready for nitrate analysis by grinding and sieving using a 2 mm sieve to facilitate dissolution. A sample of 30 gram was taken from the sieved soil for analysis. Nitrate content was measured using selective ion electrodes. The nitrate ion-selective electrode (NO_3^-–ISE) provides a rapid and reliable method for quantitative analysis of soil nitrate (Dahnke, 1971). Ion-selective electrode (ISE) is a transducer (or sensor) that converts the activity of a specific ion dissolved in a solution into an electrical potential, which can be measured by a voltmeter or pH meter. The voltage is theoretically dependent on the logarithm of the ionic activity, according to the Nernst equation. The NO_3^- ISE electrochemically generates a voltage across its sensitive membrane that varies with ionic strength (molarity) of the solution according to the Nernst equation(Morf, 1981).

$$E = E_o + S \log (A)$$

Where E is the electrochemical cell potential (mV), E0 is the standard potential (mV) in a 1M solution, ideally a constant, S is the electrode slope (mV per decade of concentration), and A is the nitrate activity (effective concentration moles L^{-1}) in the solution.

Through calibration with known standards, the logarithm of solution molarity was related to electrode output voltage to determine a linear calibration curve for determining nitrate concentration (mg L^{-1} or ppm) of subsequent soil samples (Sibley et al., 2010)

The measurement of nitrate concentration of the soil samples was done by mixing 30 g of a soil sample with 100 ml of deionized or distilled water. After a one minute extraction (dissolution) time, the extract in the mixture was poured from the soil particles and clarified by filtration. Then the molarity of the clarified extract was measured with the NO_3^- ISE. The resulting electrode voltage output was converted to concentration NO_3 (M/liter) using a calibration curve, and subsequently to nitrate content (mg kg^{-1} soil). The calibration curve was set using KNO_3^- stock solution which was diluted to 0.001 M, 0.0005 M and 0.0001 M NO_3^-.

The concentration of NO_3^- (M/ liter) was converted to mg NO_3^- in 30 g of soil by using the formula (62 g X (NO_3^- M/ liter)/10); where 62 stands for molecular weight of nitrate; the NO_3^- concentration M/ liter value divided by ten because 30 g of soil was diluted in 100 ml of water. Subsequently, the amount of NO_3^- g/30 g soil was converted to a standard unit milligram NO_3^- per kg of soil (PPM). The calculated PPM values at this step represent the amount of NO_3 left in each of the four soil layers at time of harvest.

3.3 Calculation of plant nitrate uptake from different soil layers

The amount of nitrogen taken up (depleted) was calculated as the difference between the amount of nitrate found in bare soil and amount of nitrate left in soil samples from root zone. Bare soil nitrate content was calculated for each layer as a mean value of three bare soil samples taken per block from each soil layer. After the difference in amount of nitrate in mg/ kg of soil between bare soil and soil from root zone was calculated. The nitrate taken up per plant from each soil depths was calculated as nitrate depletion in mg from the soil mass in rooting volume. The mass of soil within the rooting volume was calculated by multiplying soil density value by the rooting volume. Rooting volume at each layer was assumed to be a cylinder having 10.5 cm radius and 10 cm depth; this volume was multiplied by 4 in order to represent the surrounding of a plant in which roots can be spread. Then nitrate uptake of individual genotype from the four soil layers was added up to get the total amount of nitrate taken up per plant. The total amount of nitrate taken up per plant was converted to the amount of nitrogen in order to make direct comparison with head total nitrogen content.

4. Results and discussion

4.1 Relationship of dry matter production and nitrogen uptake from soil

Analysis of variance showed significant difference among lettuce genotypes in amount of dry matter production in gram per plant (p= 0.004). Dry matter yield of the genotypes was in range of 37 to 67 g per plant. The genotypes can be grouped as high yielding (57-67 g DM / plant), medium yielding (47-57g DM / plant) and low yielding (37-47g DM /plant). Figure 1 shows the yield of some selected genotypes from high, medium and low yielding groups. The mean yield of each genotype is presented in Appendix 1: Genotypes yield and field performance score. The yield of most genotypes falls in the medium yielding category (47-57 g / plant). Genotypes that are in the high yielding and low yielding category can have a significantly different property in uptake and utilization of nitrogen as compared to medium yielding once. However, the results from other repeated experiments will be important to draw final conclusions.

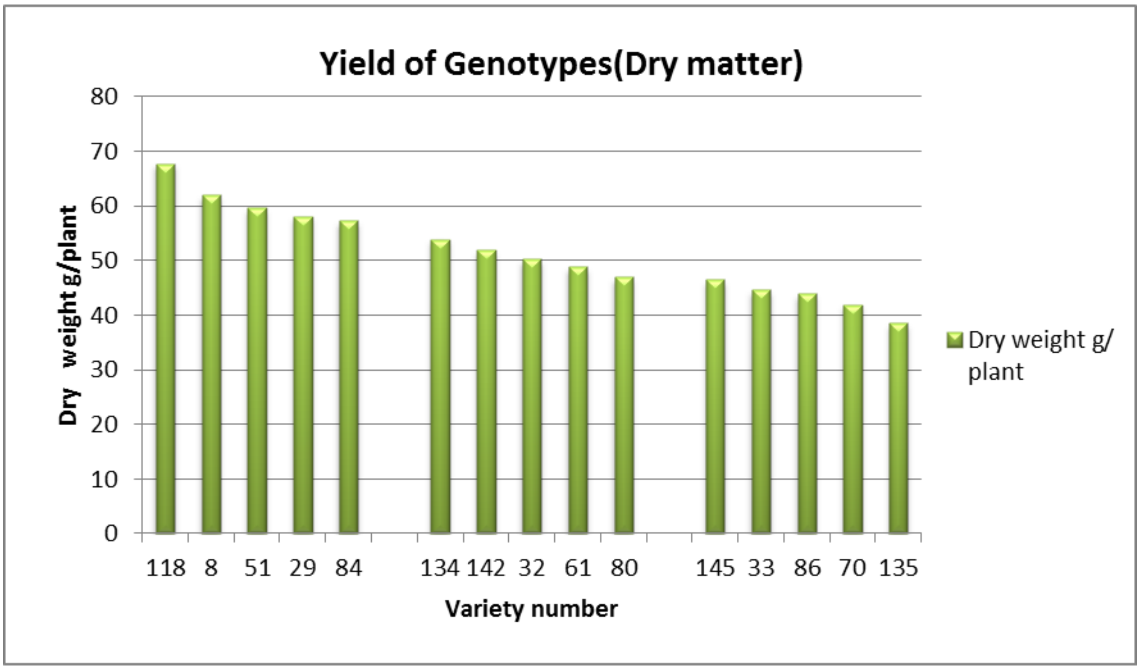

Figure 1: Dry matter yield of some genotypes from high, medium and low yielding group

Correlation analysis showed highly significant ($p<0.001$) positive correlation between dry weight and head nitrogen content with a correlation coefficient of 0.32. Figure 2 shows the relationship between dry weight per plant versus head N content in mg / plant. The scattered data points represent considerable variation in head N content among genotypes that had very similar dry matter yield. The variation in head N content can be due to the difference in nitrate accumulation property of lettuce genotypes. Many authors have reported the existence of considerable variation in nitrate accumulation among lettuce genotypes (Burns et al., 2011b, Burns et al., 2011a, Reinink, 1991, Reinink and Eenink, 1988, Anjana et al., 2009).

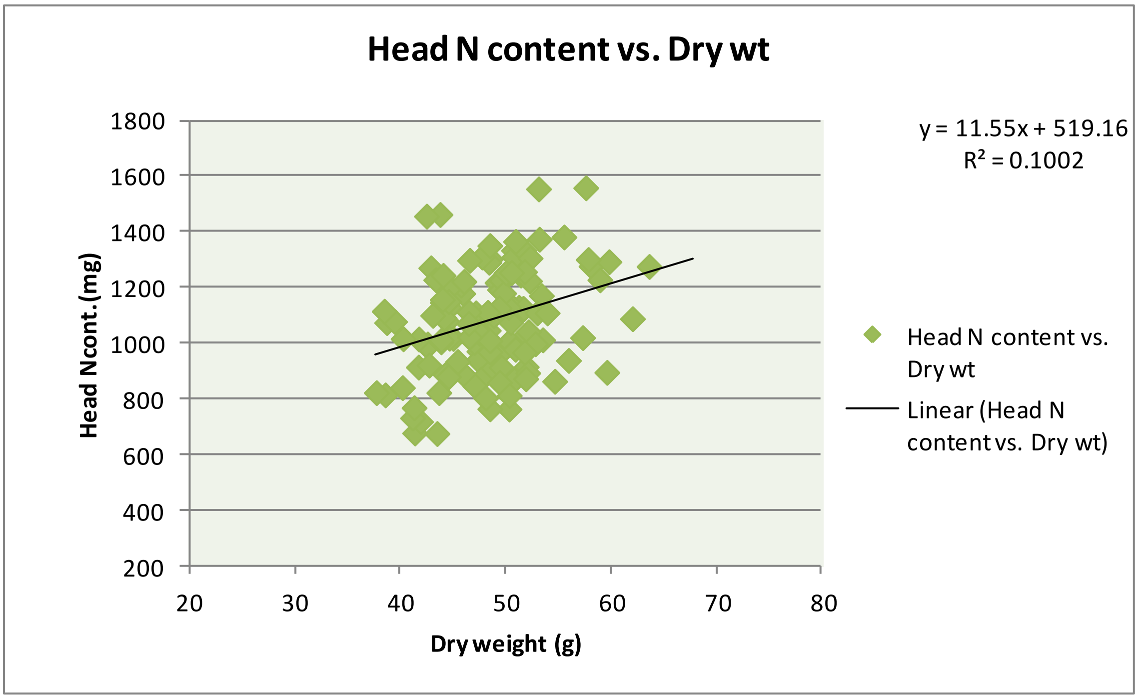

Figure 2: Relationship of N content and dry weight of the heads of different genotypes

The association between dry weight and total nitrogen taken up from soil was positive (Figure 5) with correlation coefficient of 0.088 and p=0.4269. Positive correlation indicates the positive effect of nitrogen uptake on dry matter production.

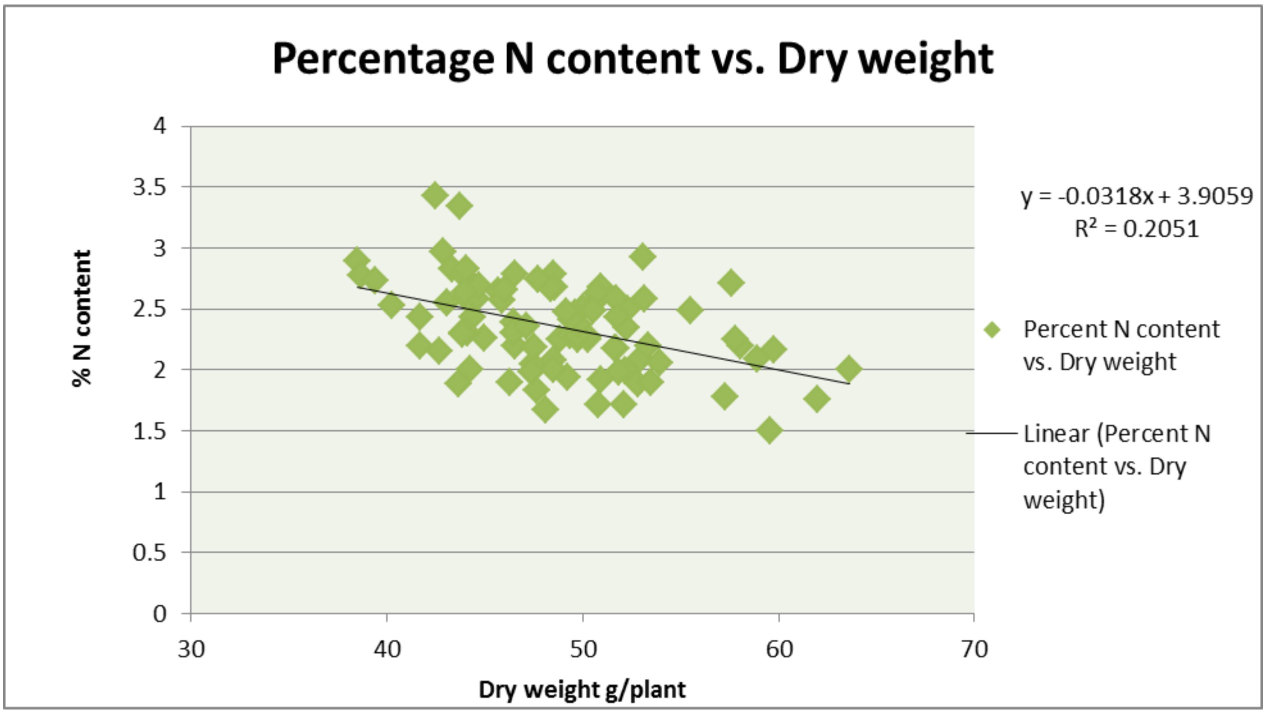

Figure 3: Relationship of percentage nitrogen content and dry weight

There was a negative association between percentage N content and dry matter yield (Figure 3). This trend was expected: when N content per unit dry matter increases nitrogen productivity/utilization efficiency will decrease. Nitrogen productivity was defined as dry matter production per unit N content (Berendse and Aerts, 1987). Vegetable crops like lettuce can accumulate high quantities of nitrate in their vacuole which may result in high head N content in relation to their DM weight. Nitrate accumulation in plants can be associated with either low assimilation efficiency or high demand of nitrate as a tool for osmotic adjustment especially under low light condition. High nitrate accumulation is not a desirable trait as N uptake involves carbon investment; higher N uptake as compared to utilization capacity can waste carbon that could be allocated to shoot dry matter production.

The percentage head nitrogen content of the genotypes was in a range of 1.5 to 3.5 present on dry weight basis. Several researchers have studied the nitrate content variation in lettuce on fresh weight basis. European commission regulations set 2500 mg/kg NO_3 per kilogram fresh weight during summer and 4000 mg NO_3/ kg during winter as a maximum acceptable limit for lettuce grown under field conditions (Anjana et al., 2007).

Data on total head N content range of lettuce per dry weight basis is hardly available. A data based on Boston type of lettuce showed 4.5 % to 5.1 % leaf nitrogen content depending on the maturity of stage of leaves (NMSU, 2005). However, data on butter head type of lettuce could not be found. The variation in N content of lettuce genotypes can be associated with variation in physiological age of plants as the genotypes differ in their time of maturity (Anjana et al., 2007), genetic variation in N uptake capacity (Burns et al., 2011a), and genetic variation in nitrate accumulation characteristics (Reinink and Eenink, 1988).

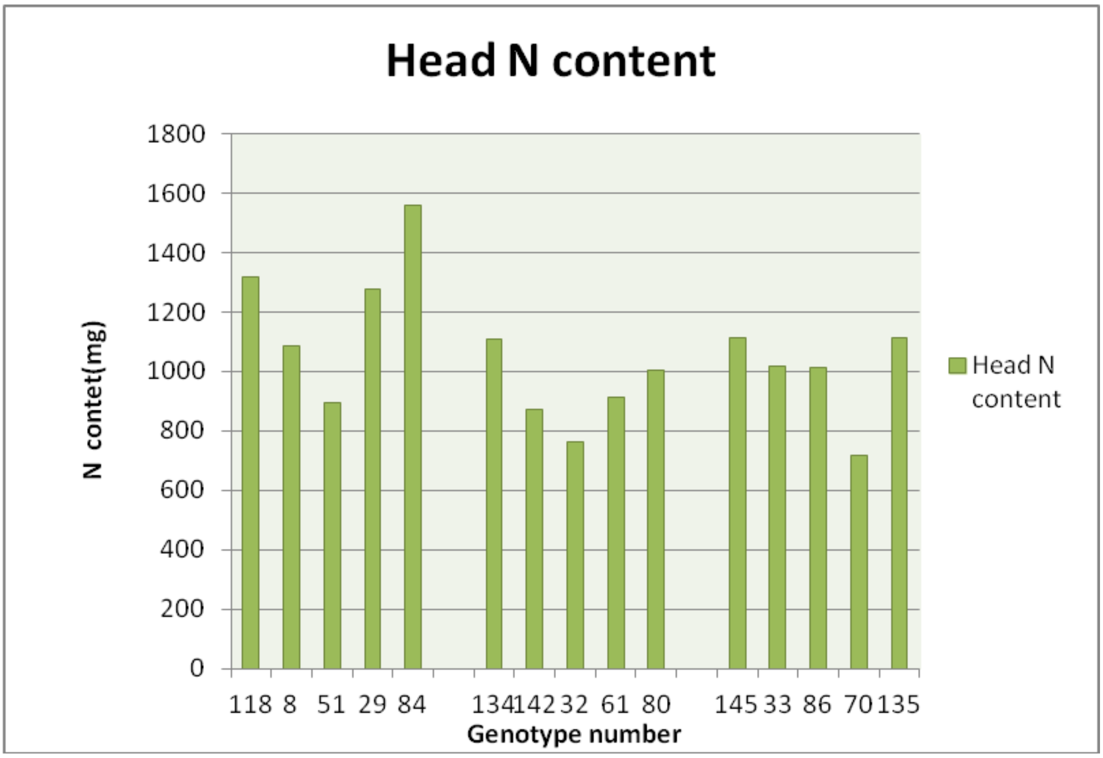

Figure 4: Head N content of selected genotypes from the high, medium and low yielding groups

High yielding genotypes tend to have high head N content as compared to medium and low yielding groups (Figure 4). As N content had positive impact on yield, the relatively higher N content of high yielding genotypes indicated the higher N requirement associated with high yield. The low head N content of genotype 51 in the high yielding group showed a genotypic difference in crop N requirement, as genotype 51 utilized relatively less N while it gave high yield. This can be associated with genetic difference in N utilization efficiency.

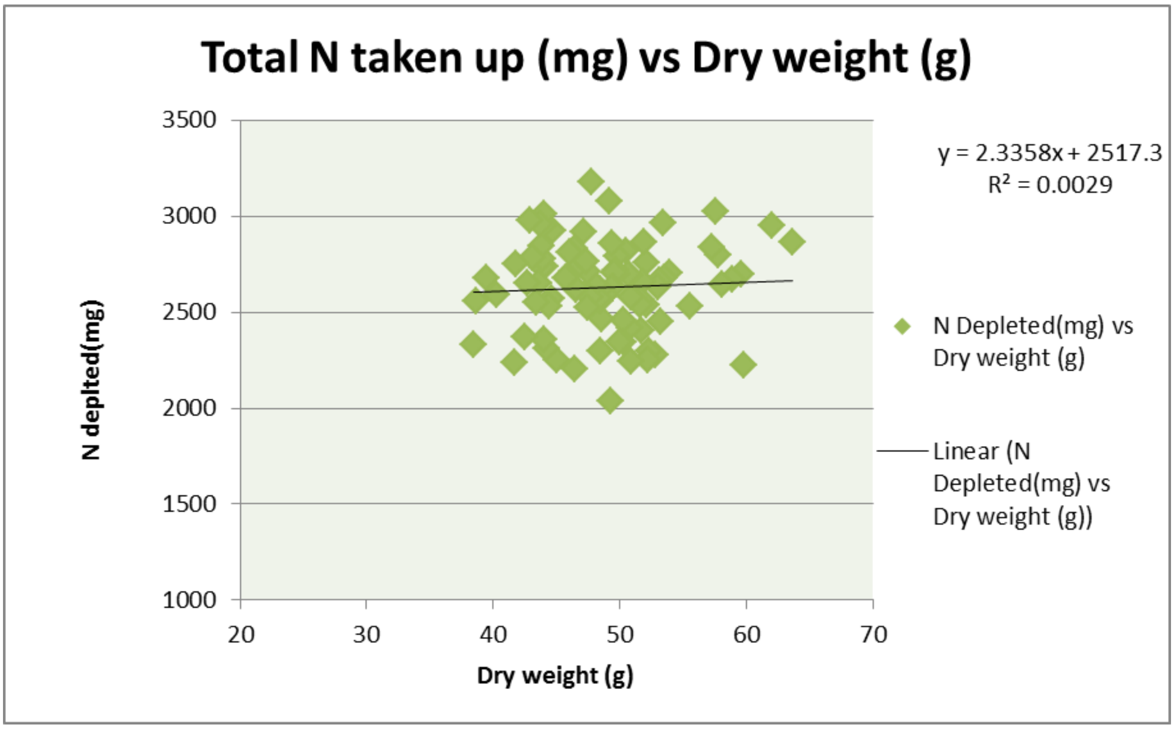

Figure 5: Relationship of total nitrogen (mg/plant) taken up from soil and dry weight (g /plant)

Several researchers have studied the association of nitrogen nutrition with dry matter and fresh yield of lettuce and other species of crops. However, for a comparison it is important to take into account the growing condition and make a distinction between nitrate content, total nitrogen content and organic nitrogen content, as most of the researchers used a nutrient solution culture and made different kinds of N measurements. The result of the current experiment was in agreement with the generally accepted fact as many researchers reported the positive effect of N uptake on dry matter production. According to (Yin et al., 2003) the influence of N uptake on dry matter production is linked with the effect of nitrogen on leaf area index and the amount of N per unit of leaf area (specific leaf N). Specific leaf N in turn determines the rate of photosynthesis. (Mooney et al., 1981) reported a linear relationship between the rate of photosythesis per unit leaf area and the nitrogen concentration in the leaves of what crop. Schenk (1996) proposed that N demand of a crop is dependent on the increase in dry mass. Schenk (1996) indicated that this relationship might not be linear if the critical level of nitrogen in plant dry matter changes during crop development or if retranslocation of nitrogen from older leaves to meristematic tissue occurs.

The difference among genotypes in total amount of N uptake indicated their difference in uptake potential. Genotype 118 and 84 from the high yielding group and genotype 33 from the low yielding group showed higher N uptake potential (Figure 6). The higher N uptake of genotype 33 from the low yielding group showed that high N uptake may not necessarily grant high yield unless it is accompanied with efficient N and carbon utilization.

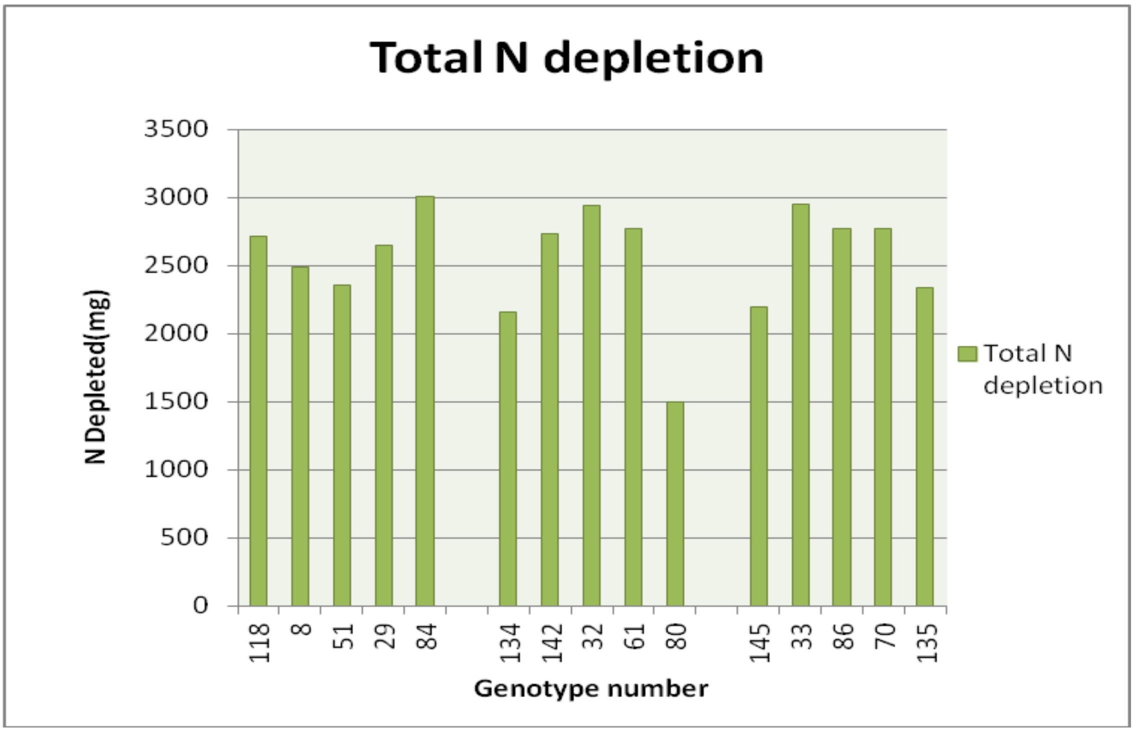

Figure 6: Total N uptake (mg/plant) of selected genotypes from the high, medium and low yielding groups.

4.2 Relationship of head nitrogen content and nitrogen uptake from soil

The association between head N content and total nitrogen taken up from soil was positive (Figure 7) with correlation coefficient of 0.111 and p=0.313.Even though the correction is not significant, the positive association indicates the positive effect of nitrogen uptake on amount of nitrogen translocation to the head of lettuce plants. The result was in agreement with previous results. It is believed that the large proportion of N taken up from the soil will translocate to the shoot. (Rufty et al., 1981) reported that

N translocation to the shoot is predominantly a function of total N absorbed. As it can be seen from Figure 7 there was high variation in amount of nitrogen taken up from soil among genotypes that differ slightly in head N content. This variation indicates difference among lettuce genotypes in uptake efficiency and translocation capacity of nitrogen taken up from root to shoot. The existence of genetic variation in translocation capacity is not well documented but effect of environmental factors on translocation is reported (Erica et al., 1996). There is genetic variation in nitrate uptake capacity of lettuce genotypes (Burns et al., 2011a). Nitrogen loss due to root turn over and soil heterogeneity under field condition may also have slight contribution for the variation.

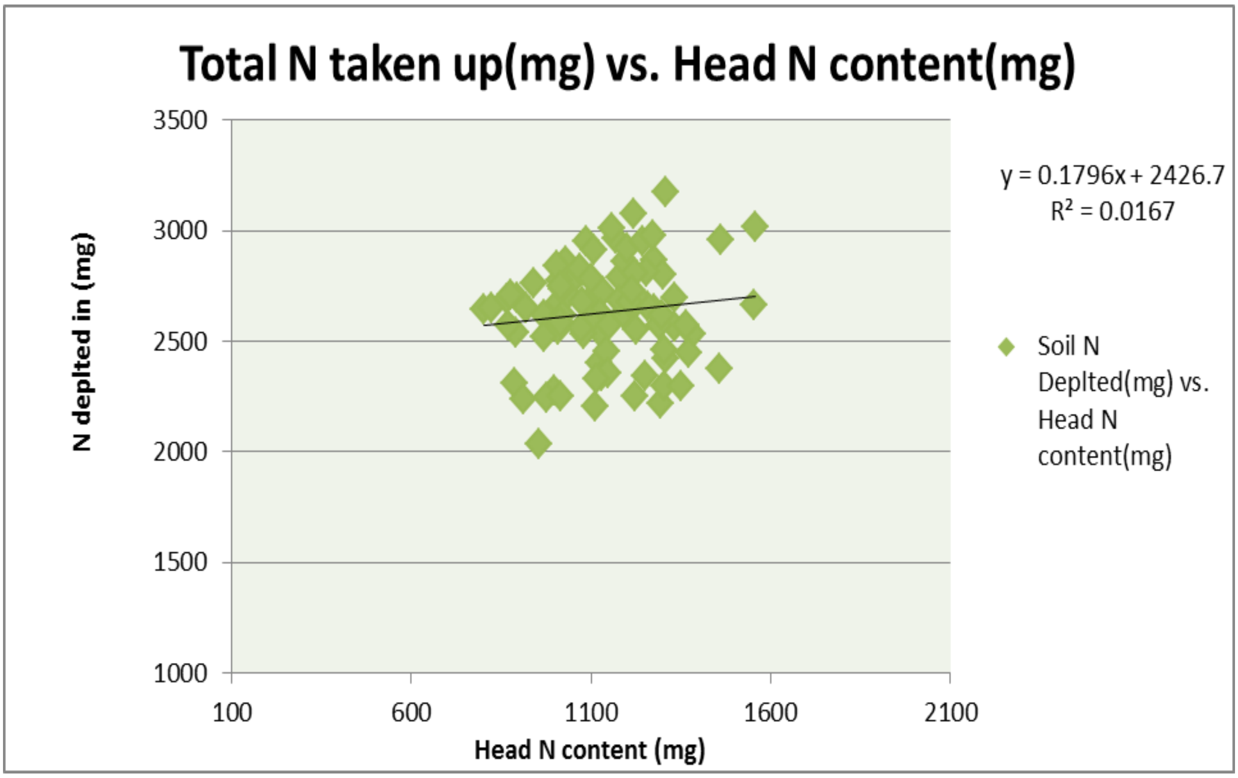

Figure 7: Relationship of total nitrogen taken (mg/plant) up from soil and head N content (mg).

4.3 Relationship of nitrogen use efficiency with field performance

Nitrogen use efficiency has different components namely uptake, translocation, and utilization efficiency. We are mainly interested in genetic differences in uptake efficiency, because genetic potential to take up nutrient efficiently from deep soil layers is an important trait for organic farming. Nitrogen uptake efficiency can be measured as N uptake per unit root weight. However, in this section the translocation efficiency component of NUE was evaluated, because, as it is described in the problem statement, there is no reliable method to measure the weight of roots under field condition. Uptake and translocation efficiency can be related, as nitrogen in the shoot (head) plays a key role in photosynthesis and dry matter production; genotypes that are less efficient in the translocation of N taken up to the shoot will definitely either accumulate the rest amount of N in their root or lose higher amount of N due to high root turnover rate. This implies the lower uptake efficiency of their root system; as excess N uptake than the actual translocation and utilization will definitely minimize energy allocation for dry matter yield.

Correlation analysis of nitrogen use efficiency with field performance parameters namely dry matter yield, uniformity, size and overall performance was done. The summary of the results is presented in Table 1 below.

Table 1: Correlation analysis results of nitrogen use efficiency and field performance parameters

Parameters	Correlation coefficient	P value	Relationship
Dry weight vs. NUE	0.1767	0.1079	Positive (non-significant)
Head N vs. NUE	0.8318	<0.001	Positive (significant)
Total N depleted vs. NUE	-0.4544	<0.001	Negative (significant)
Uniformity vs. NUE	0.135	0.2209	Positive (non-significant)
Head Size vs. NUE	-0.0691	0.5325	Negative (non -significant)
Overall performance vs. NUE	0.0184	0.8682	Positive (non-significant)
Uniformity vs. Dry weight	0.0354	0.6995	Positive (non-significant)
Head Size vs. Dry weight	-0.1669	0.0673	Negative (non -significant)
Overall performance vs. Dry weight	0.0203	0.8247	Positive (non-significant)

Nitrogen use efficiency had a positive correlation with dry matter yield, head N content, uniformity and overall field performance (Table 1). As nitrogen is a key nutrient for plant growth and development the positive effect of nitrogen use efficiency on yield under low input condition was as expected. The availability of nitrogen in the growing media has an influence on the NUE of plants (Benincasa et al., 2011). Under high input condition nitrogen uptake can be mainly governed by plant demand. However, under low input condition root uptake capacity can be crucial (Garnett et al., 2009). Nitrogen uptake by the root system is a complex process. (Lea and Azevedo, 2006) in their review presented the existence of four different transportation system of nitrogen in plants depending on soil N availability and plant demand. However, very little is known about how the various N transporters contribute to net N uptake by crops under field conditions (Garnett et al., 2009).

The negative association of NUE with head size (Table 1) can be associated with large leaf area that demands high nitrogen for chlorophyll formation while radiation use efficiency is low because of overlapping of leaves. The positive association of NUE with the overall performance indicates the possibility to breed for both quality and NUE at a time. The negative association of NUE with total N depleted from soil (Table 1) may be associated with negative impact of excess N uptake on shoot dry matter allocation.

Nitrogen utilization efficiency is also an important parameter in determination of NUE. Especially in case of lettuce; there is wide genetic difference in shoot nitrate accumulation character. Thus, if a genotype tends to accumulate high nitrate, high nitrate uptake from soil may not necessary results in high yield. In addition, high nitrate accumulation is not a desired trait as it can pose health risk (Anjana et al., 2007). Therefore, it is important to take into consideration the proportion of organic and nitrate concentration in shoot while assessing NUE of lettuce genotypes.

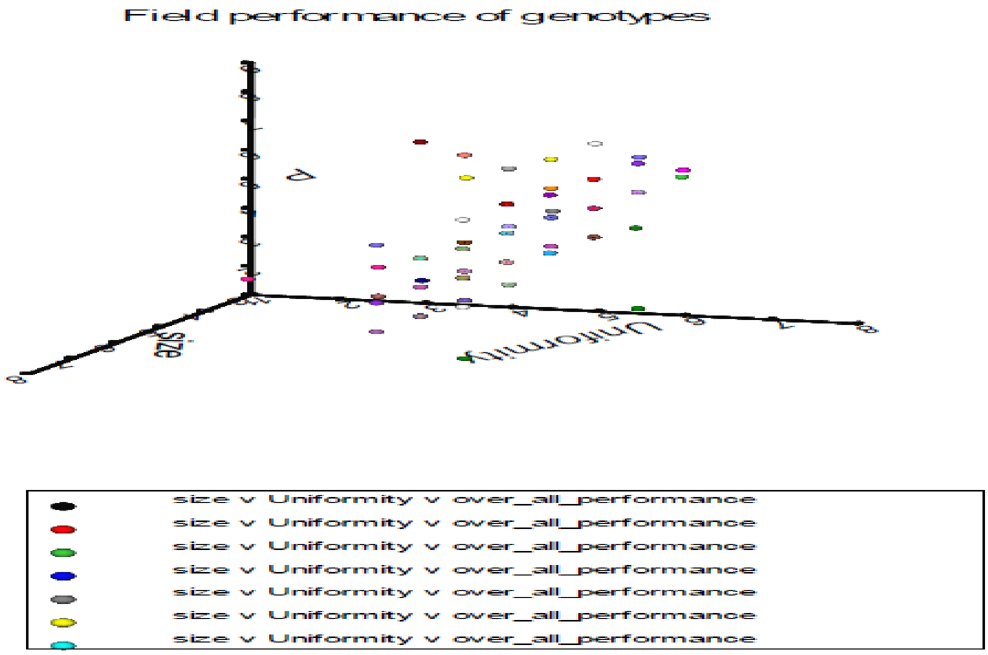

Figure 8: Scatter plot of individual genotypes uniformity, size and overall performance score

Figure 8 shows genotypes performance as a function of size, uniformity and overall performance. The scattered data points represent individual genotypes. Figure 8 shows high genetic variability among genotypes in overall performance and uniformity indicating the availability of wide genetic variation among lettuce genotypes that can be used to develop varieties for organic and low input conditions.

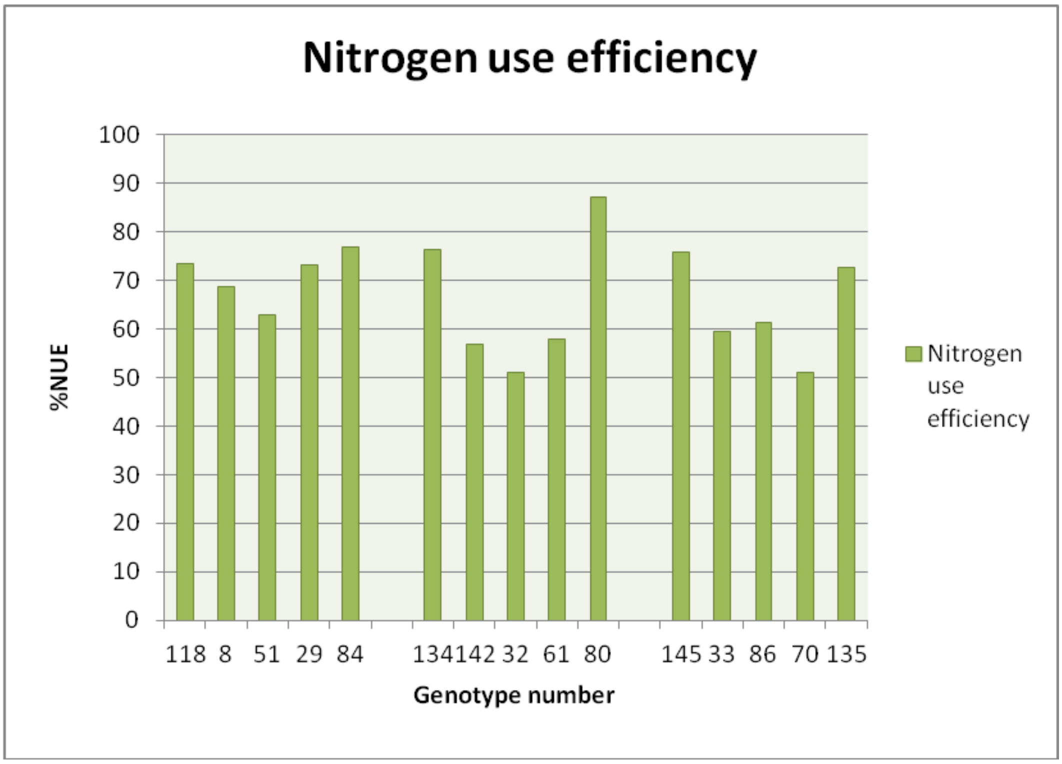

Figure 9: NUE of some selected genotypes from the high, medium and low yielding groups

High yielding genotypes tend to have high nitrogen use efficiency as compared to medium and low yielding groups, though medium and low yielding genotypes such as genotype 80,145 and 135 also showed high NUE

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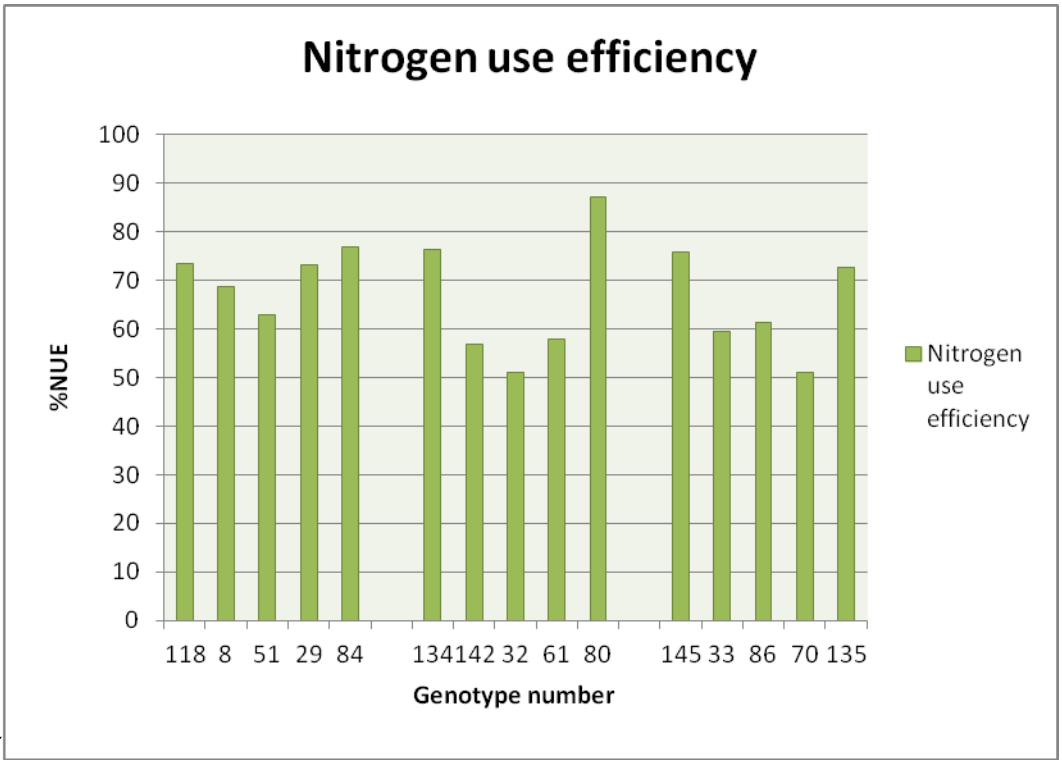

Figure **9**). Genotypes 80, 145 and 135 showed a good potential to efficiently utilize the amount of N taken up. However, their low yield can be due to their low genetic yield potential as compared to high yielding genotypes. These genotypes potentially may serve as donor for NUE traits. If their high NUE potential can be revealed through repeated experiment

4.4 Effect of soil moisture content on nitrate uptake

Correlation analysis of percentage soil moisture content and amount of N taken up from each of the four soil layer showed highly significant ($p<0.001$) positive association with correlation coefficient of 0.4677. From Figure 10 it can be seen that in the first (top) layer both N uptake and moisture percentage was higher. Both N uptake and moisture availability decreased considerably as the soil depth increased.

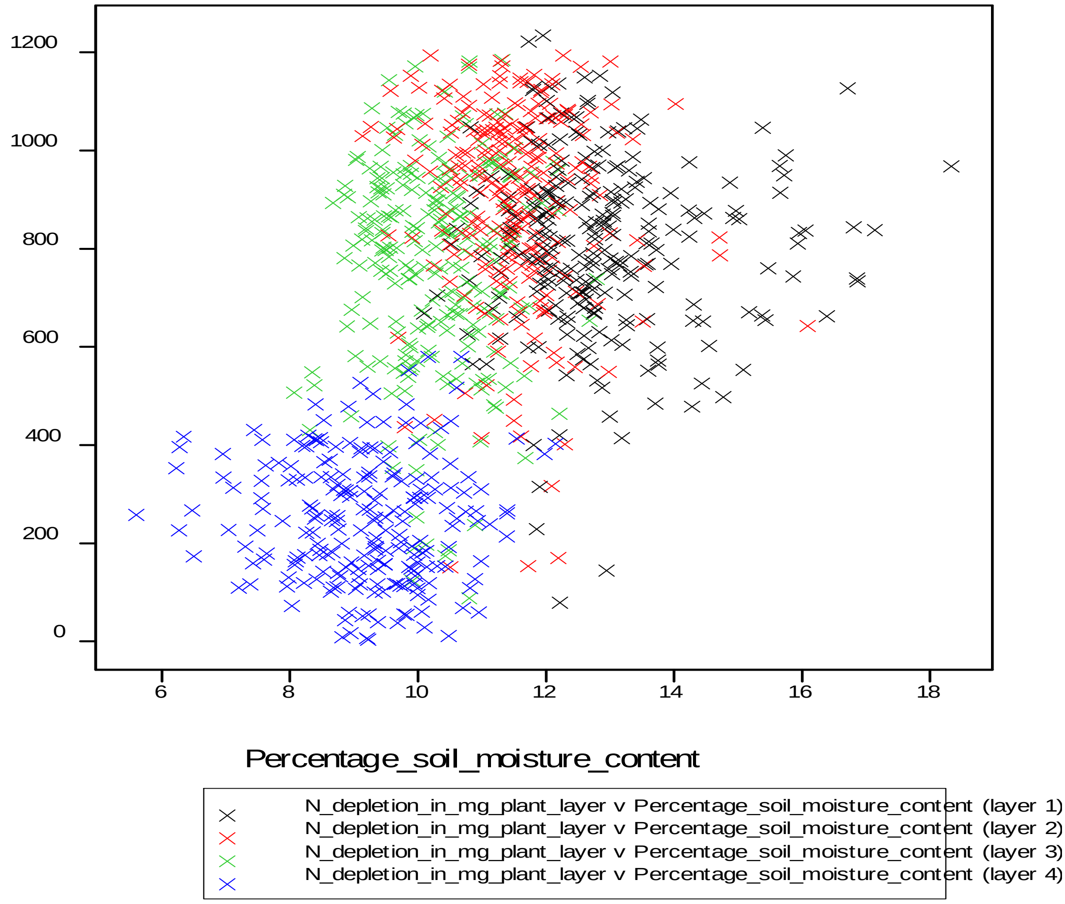

Figure 10: Relationship of soil moisture percentage and N uptake (mg) per soil layer

Analysis of variance showed that percentage soil moisture content per genotypes over four layers varied among genotypes ($p<0.001$). The result may be an indication of variation in water uptake of genotypes. This can be associated with difference in evapotranspiration water demand because of differences in leaf area. It can also associate with their variation N demand.(Cárdenas-Navarro et al., 1999) reported positive correlation between nitrate and water contents of lettuce genotypes; they underline the importance of water for homoeostasis of nitrate concentration.

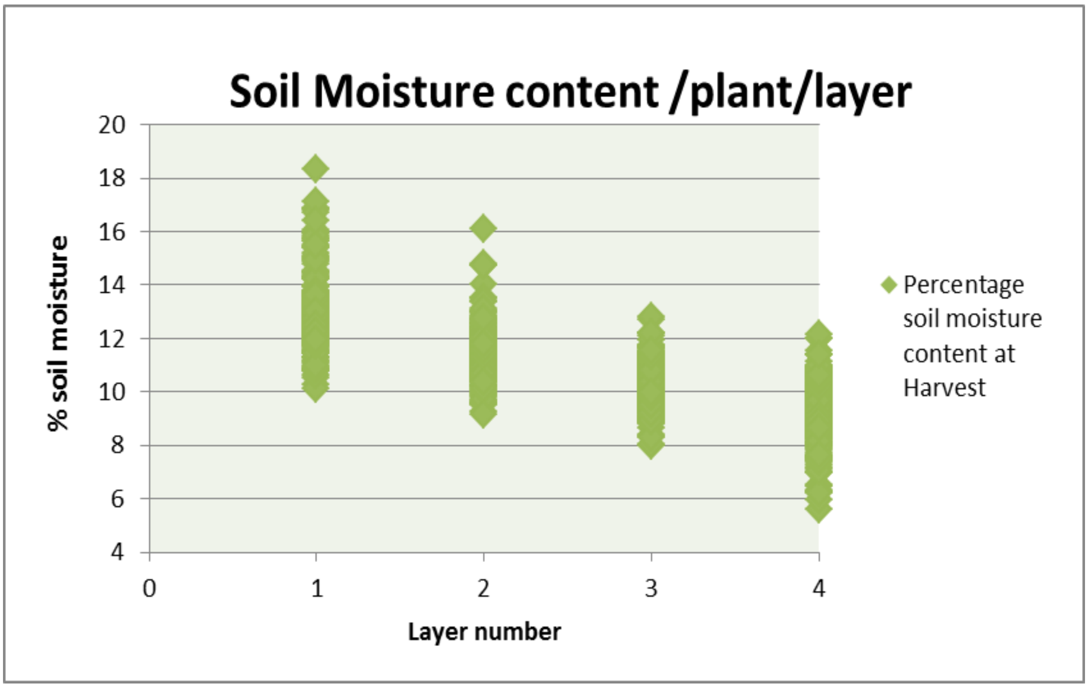

Figure 11: Soil moisture percentage of four soil layers under different genotypes

Since water is the carrier of nutrients to the root surfaces several authors reported the tight relationship of soil moisture availability with nutrient uptake (Garwood and Williams, 1967), nutrient utilization (McMichael and Quisenberry, 1993) root development and shoot to root ratio (Gonzalez Dugo et al., 2010). Limited availability of water is reported to have reduction effect on N demand of shoot because of reduced growth rate associated with reduction of stomatal conductance that inhibits carbon assimilation and reduced leaf area expansion (Gonzalez dugo et al., 2010).

Soil moisture availability and crop water requirement largely depends on weather conditions. There is also genetic difference in requirement and uptake capacity of water and nutrient. As it can be seen from Figure 11, genotypes can differ in their soil moisture uptake. Thus, there is genotype by environment interaction in determination of rooting pattern, root to shoot ratio, crop water and nutrient requirement. During the current experiment the growing condition can be assumed as optimal based on the weather data and the crop performance. Mean daily temperature was above 15 ^{o}C throughout the growing period (

Appendix 2.2 Daily temperature fluctuation during the growing period) and the soil moisture percentage on top layer was 13 % on average (Figure 12). The field performance evaluation showed that some of genotypes showed mild stress symptoms (a score of 3) where the scoring scale was ranging from 1= no stress up to 9= severe stress. The score for individual genotypes can be seen from Appendix 1: Genotypes yield and field performance score.

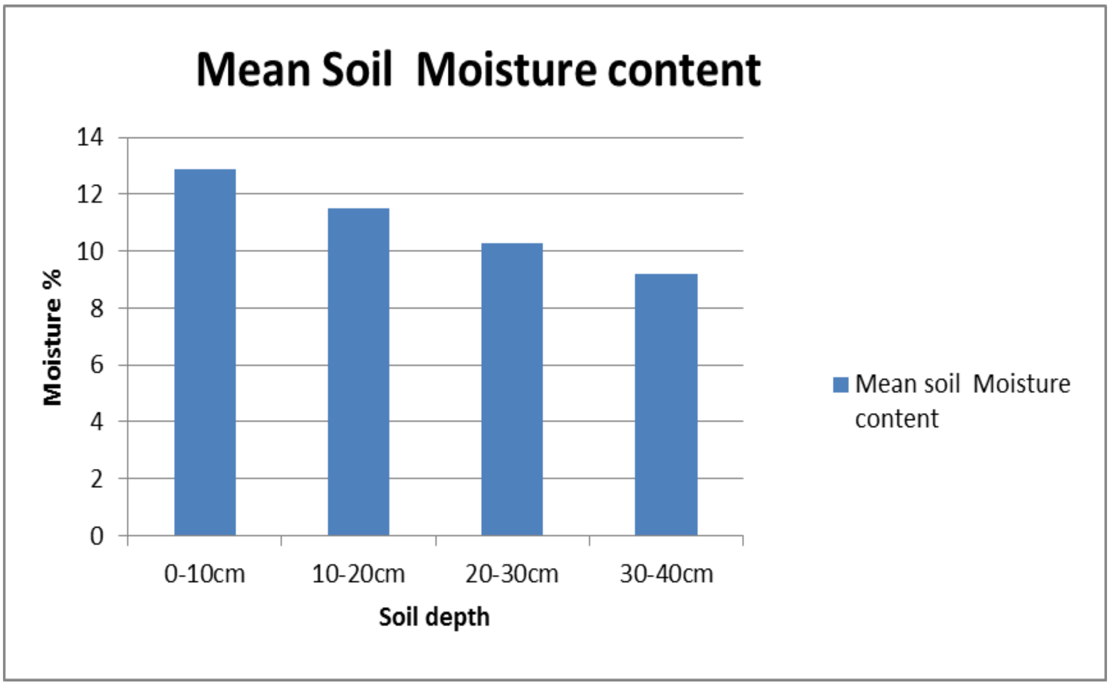

Figure 12: Mean percentage soil moisture content of four soil layers over all genotypes

4.5 Effect of genotype on nitrate uptake per soil horizon

Analysis of variance for total N uptake per genotypes showed slightly significant ($P=0.04$) difference in nitrogen uptake of lettuce genotypes. This shows variation in N uptake capacity of genotypes.

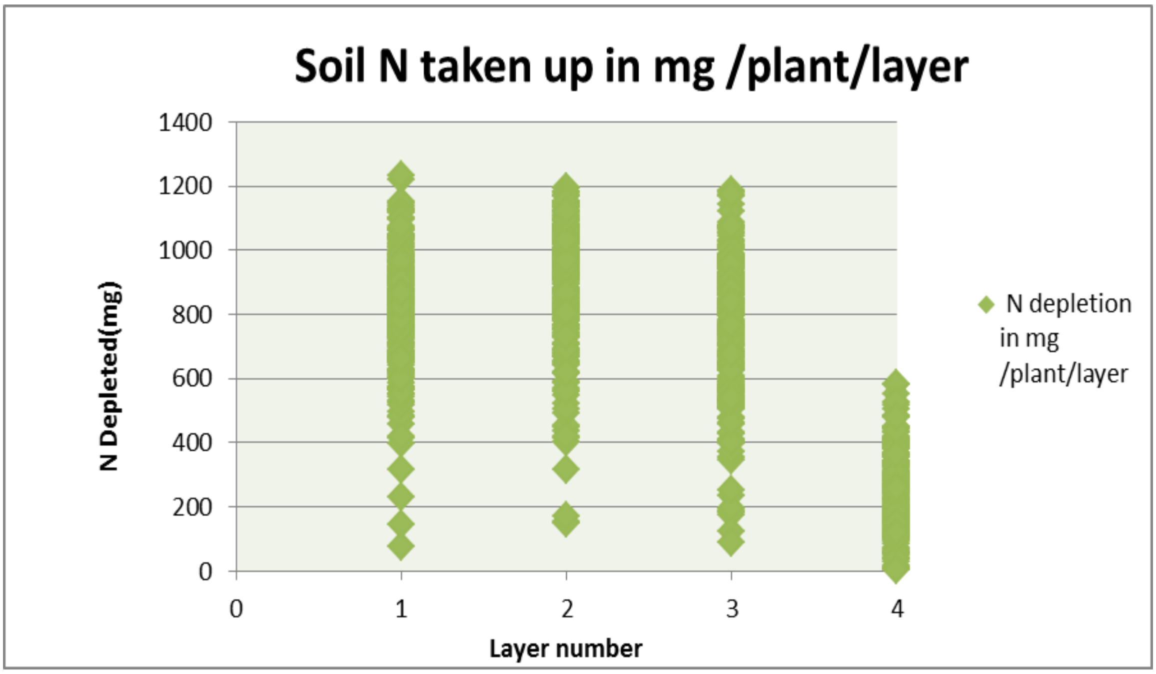

Figure 13: Soil N taken up from four soil layers under different genotypes

Nitrogen uptake from four soil depth irrespective of genotype (Figure 14) indicates that lettuce genotypes took up the higher amount of nitrogen from the three top most layers. Uptake from deeper soil layer (layer 4) was very low as lettuce in general is a shallow-rooting crop. Genotypes that took up relatively higher amount of nitrogen from the deeper soil layers can be interesting for further experiment and breeding effort.

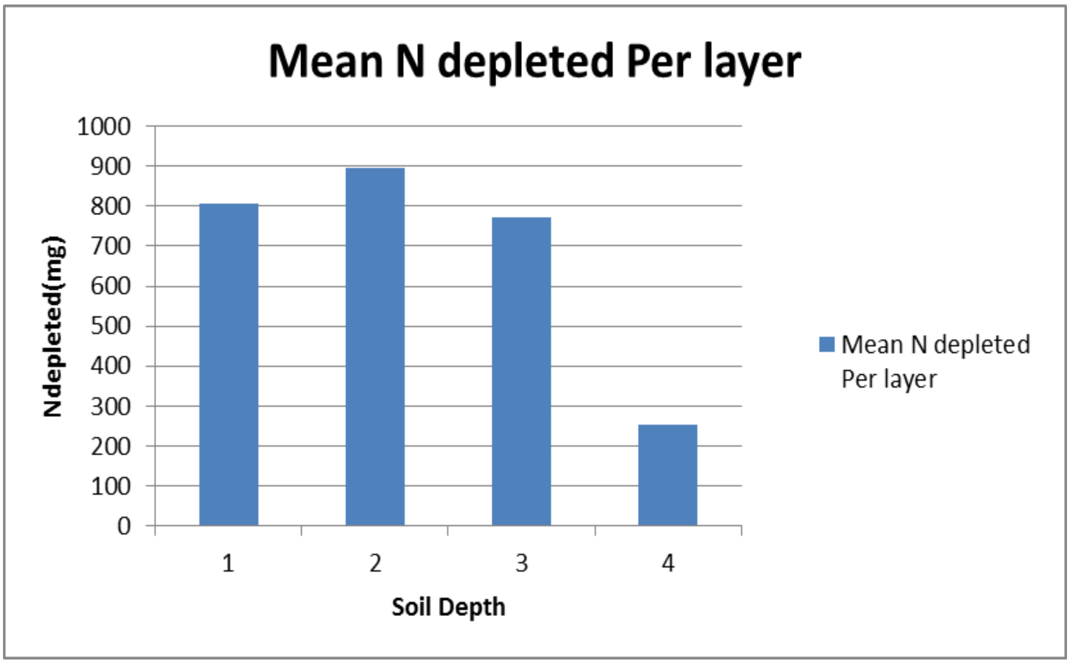

Figure 14: Mean N (mg) taken from four soil layers over all genotypes

There was significant genotype by soil layer interaction (p=0.039). The result indicates variation of genotypes in rooting pattern.

Figure 15 shows the difference in N uptake pattern of genotypes selected from the high yielding group (Mantilia and Sprinter), the medium yielding group (Du Bon Jardinier and Verte De Perpignan) and from the low yielding (Furchtenichts and White Boston).

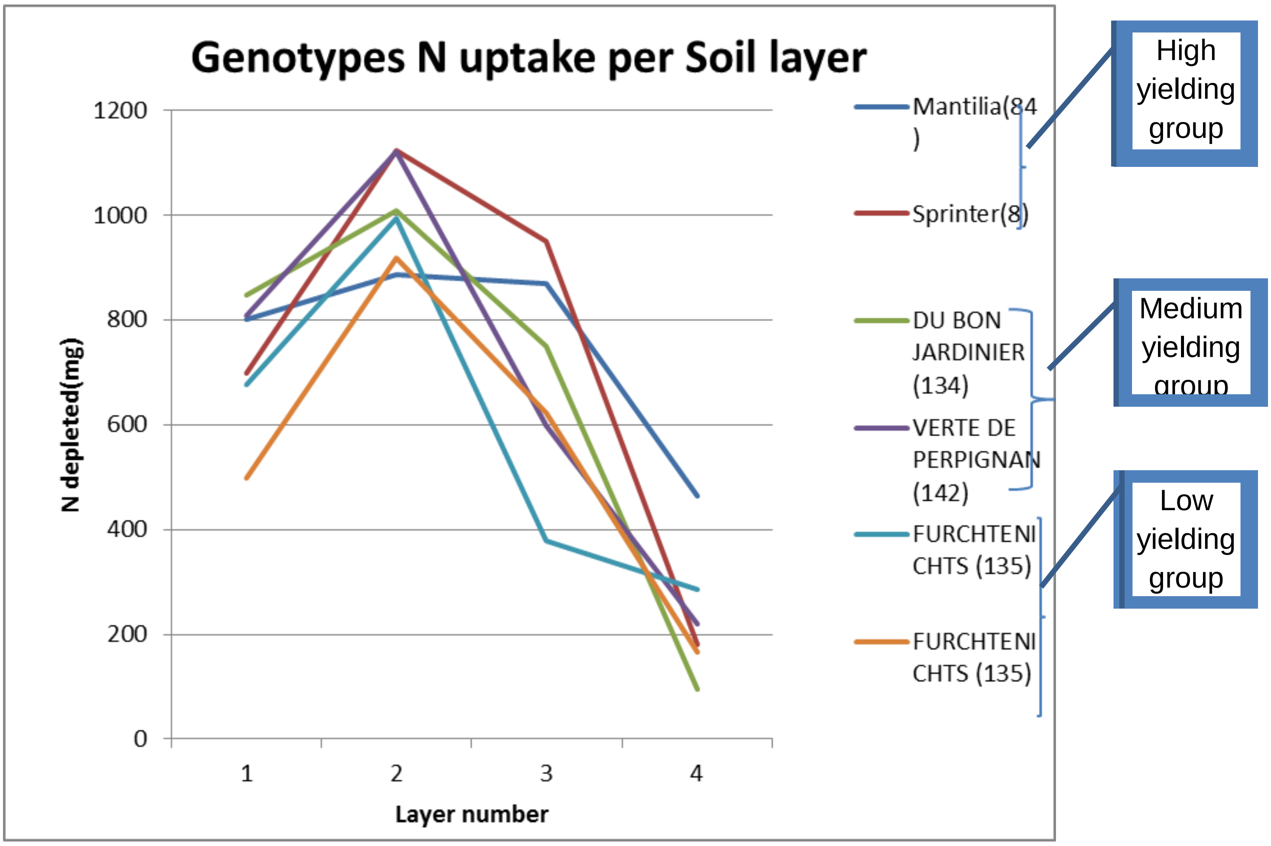

Figure 15: N uptake (mg) trend of some selected genotypes from four soil depths.

Mantilia took up more or less an equal amount of N from the first three layers and lower amount of N from the fourth layer. Sprinter took up higher amount of N from the first three layers but less amount of N from the fourth layer. Du Bon Jardinier showed a very low N uptake from the fourth layer compared to the rest of the genotypes. Verte De Perpignan took up higher amount of N from layer one and two but very low from third and fourth layer, whereas Furchtenichts took up higher amount from the first and second layers and lower amount from the third and fourth layer. White Boston showed an uptake pattern similar to that of Verte De Perpignan as it took up higher amount of N from layer one and two and very low from third and fourth layer. Both the amount of

N uptake and the variation in uptake pattern among genotypes were minimum at the fourth layer.

Genotype Mantilia took up higher amount of N from the fourth layer than the rest of the genotypes. Mantilia also had a high dry biomass yield in addition to its good uptake pattern from deeper soil layer. This result indicated its good rooting character.(Lairon et al., 1984), based on their experiment to examine the effect of organic and inorganic fertilizer, also indicated the good ability of genotype Mantilla in utilization of nitrogen fertilizers.

Only few references were available related to N uptake measurement under field condition. (Gonzalez dugo et al., 2010) reported that uncertainty about the size of the actual soil N pool due to N transformations under field conditions makes it difficult to make a quantitative assessment of N uptake by the plant. In contrast, Thorup-Kristensen and Sørensen (1999) studied the difference in N uptake efficiency of carrot, leek and white cabbage under field condition. They found significant variation among the three crops in amount of N (inorganic) left in the upper and sub soil layer. They reported that the result was in agreement with available knowledge on rooting pattern of the crops and they highlighted the significance of existing differences in rooting depth for the difference in ability among the three crop species in utilizing available N reserves from deeper soil layers. In another experiment conducted to study the significance of rooting depth of lettuce carrot, early cabbage and onion for the utilization of green manure nitrogen (N). In which roots were observed by minivideo camera in order to record visible roots on the minirhizotron surface, the result clearly reflected the role of differences in rooting depth in the ability of the four crops to deplete N from the soil layers of 0.25 to 1.50 m depth (Thorup-Kristensen, 2006).

As information about rooting pattern of these genotypes was hardly available in order to cross check the current result on uptake pattern of genotypes repeated experiment is important. However, the significant difference in yield and quality among lettuce genotypes indicates the difference in suitability of genotypes for low input cultivation. Commercial cultivars that are known for their suitability for organic farming have given high dry biomass yield (Table 2) in the current experiment also. However, the relatively low dry matter yield obtained from some of these genotypes indicates the importance of fresh biomass yield, size and quality in determination the choice of genotypes for commercial purpose.

Table 2: Characteristics of some commercial cultivars that were used in the experiment

Variety no	Variety name	DM yield(g)	Growing season	Resistance	Characteristics	Source
63	Analena	53.38	spring, autumn	Berimia and Lettuce Mosaic Virus resistant	Very voluminous with a fresh green colour. Good head closing	Vitalis Seed Company
64	Barilla	43.85	spring, autumn	Very strong on internal tip burn	Slow closing variety, Nice bottom and easy to cut	Vitalis Seed Company
76	Lucan	57.82	summer	slow bolting and features good tip burn resistance , Berimia resistant	Features a very large framed that has excellent weight	http://rogersadvantage.com/products/lettuce.asp
100	Maditta	49.49	spring, autumn	Very strong on internal tip burn tolerance	Voluminous variety with a well filled heart, not too dense, so easy to peel.	Vitalis Seed Company
70	Margarita	41.91	spring, autumn	Slow bolting and good tip burn tolerance	Dark green variety, Good field standing ability	Enza Zaden seed Company
101	Optima	49.61	spring, autumn	Downy mildew, bottom rot and tip-burn	extremely hardy, Big framed and thick leaved	http://gardenseeds.gardeninng.com/organic-butterhead-optima-lettuce-seeds/
147	Pronto	43.98	spring, summer, autumn	Berimia and LM V, resistant, slow bolting	Voluminous, shiny mild green variety, loose heading.	Vitalis Seed Company

Probability values from correlation analysis presented below (Table 3) showed that soil nitrogen depletion per layer was non-significantly associated with field performance parameters. Plants may take up high amount of nitrogen mainly from the top 20 cm soil depth because of high nutrient availability in the top most soil layers and also higher proportion of their root can be concentrated in top most layers (Fageria et al., 2011). In addition, significant association between nitrogen uptake per soil layer and field performance parameter was observed recently in other replicate of this current experiment (Kerbiriou et al. 2011, unpublished data). Thus, relatively strong association of field performance parameters with uptake from the top most soil layers was expected. However, in the current experiment there was a non-significant association between uptake per soil layer and field performance parameters. The results showed that the genotypes' responses can largely vary across growing seasons, years and environmental conditions. In addition, these results also indicated that head performance was largely determined by the total amount of nitrogen translocation to the shoot than uptake from individual layer.

Table 3: Probability (p=0.05) values of correlation analysis results of N uptake per layer versus field Performance parameters

N uptake mg/ plant	Dry matter yield	Head N content	Overall performance	Size	Uniformity
Layer 1	0.4236	0.3394	0.5450	0.8165	0.8831
Layer 2	0.4534	0.0910	0.3559	0.1622	0.3592
Layer 3	0.7171	0.0513	0.5164	0.7891	0.3938
Layer 4	0.3966	0.6185	0.4906	0.7171	0.4891

5. Conclusion and recommendation

In the current experiment significant interaction of nitrogen uptake per soil layer with genotype indicates genetic difference in rooting patterns. The observed difference in total nitrogen uptake per genotype indicates variation in uptake potential among lettuce genotypes. The impact of rooting pattern difference is expressed on DM yield and NUE, as the genotypes showed significant difference in their NUE and DM yield. The correlation of total nitrogen uptake with head nitrogen content and dry matter yield was positive, as expected. However, slightly weak relationships observed can be due to the fact that soil nitrogen content cannot be 100% homogenous as N mineralization and organic N content may vary from spot to spot under field condition. Thus, based on the investigation of the other repeated experiments across seasons and years it may be useful to estimate the contribution of this source of variability in order to get better estimate of the amount of soil N taken up. From the current experiment it can be concluded that variation in rooting pattern of genotypes to some extent can be explained based on the amount soil N depleted from different soil depths. However, it is important to check the response of genotypes under different environmental conditions to validate the results.

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Appendix

Appendix 1: Genotypes yield and field performance score

Variety number	Variety Name	Dry weight g/plant	Head N content in mg/ plant	size	Uniformity	Overall performance	Early stress	Late stress
2	Salad Bibb	48.72	929	-	-	-	-	-
3	Bobby	45	1017	7	6	7	4	3
4	Edwina	50.74	1334	8	6	6	7	3
5	Floret	50.77	1310	7	5	3	8	3
6	Jiska	45.92	1179	7	5	3	6	4
8	Sprinter	62.02	1088	7	6	5	6	3
9	Arno	49.41	1193	8	7	6	4	3
10	Enrica	49.76	1120	7	6	5	5	3
12	Alanis	55.52	1382	7	7	7	3	3
13	Alber '2'	47.45	971	7	5	4	7	3
14	Amos	51.41	1245	6	7	7	3	3
15	Autan	38.7	1074	7	5	4	5	3
16	Berdine	43.9	1217	7	7	6	3	3
17	Camilla	44.25	887	6	5	1	9	3
18	Caterina	44.41	1079	8	7	6	5	3
19	Charlotta	41.36	677	6	5	4	7	3
20	Divina	41.71	914	6	4	4	8	3
21	Domino	45.73	1209	8	5	6	7	3
22	Dorinta	51.63	1125	6	6	6	6	3
23	Dynamite	55.95	938	7	6	5	5	3
24	Edito	44.63	1129	6	5	5	7	3
25	Edox	42.57	997	8	7	8	3	3

Appendix 1: Genotypes yield and field performance score (continued)

Variety number	Variety Name	Dry weight g/plant	Head N content in mg/ plant	size	Uniformity	Overall performance	Early stress	Late stress
26	Escada	44.16	1018	7	6	5	5	3
27	Estelle	43.97	1147	8	7	8	3	3
28	Frauke	39.45	1078	7	6	5	5	3
29	Giotto	58.09	1277	6	5	4	7	3
30	Idara	42.53	989	7	6	6	5	3
31	Josina	51.86	1030	3	6	6	3	3
32	Kermit	50.31	763	6	7	8	3	3
33	Kerouan	44.64	1018	7	7	6	4	3
34	Libusa	48.49	764	6	6	5	6	3
35	Lores	48.55	1303	7	5	9	7	3
37	Maxina	50.33	1006	7	7	7	5	3
38	Mehari	48.4	1291	5	5	3	8	3
39	Mercury	51.23	1129	7	5	5	6	3
40	Mirian	44.15	1091	7	6	5	6	3
41	Nadine	49.24	956	7	7	7	5	3
42	Naima	47.15	1109	7	5	4	6	3
43	Plenty	38.56	814	6	5	4	7	3
44	Ponchito	49.17	1218	7	6	5	5	3
45	Pontiac	47.37	940	6	5	3	7	3
46	Princess	53.5	1012	8	5	3	7	3
47	Sagess	43.47	675	7	5	3	7	3
48	Sandrine	43.76	1463	7	5	4	7	3
49	Softan	50.29	1143	8	6	6	5	3
50	Soraya	49.73	1134	6	6	8	3	3

Appendix 1: Genotypes yield and field performance score(continued)

Variety number	Variety Name	Dry weight g/plant	Head N content in mg/ plant	size	Uniformity	Overall performance	Early stress	Late stress
51	Sorenza	59.59	895	7	6	6	5	3
52	Sumian	54.64	863	6	6	5	5	3
53	Sylvesta	41.74	1015	7	5	4	7	3
54	Tequila	42.72	921	6	6	7	4	6
55	Torpedo	58.93	1228	6	5	3	8	3
56	Tremino	52.15	1041	7	6	5	7	3
57	Vinka	50.9	978	5	4	3	8	3
58	Votan	37.72	822	8	8	7	2	3
59	Walter	47.66	871	6	5	4	7	3
60	Daguan	50.46	1075	6	4	3	7	3
61	Garuda	48.81	911	7	6	5	7	3
62	Melodion	59.79	1293	7	6	5	6	3
63	Analena	53.38	1171	8	6	5	6	3
64	Barilla	43.85	1005	8	6	4	6	3
65	Jumbis	44.71	1202	7	7	6	5	3
66	Casanova	48.49	969	7	6	5	5	3
67	Palomino	48.34	1046	8	6	5	5	3
68	Rheinia	48.84	1099	6	5	4	7	3
69	E13.4410	51.69	1124	7	6	5	6	3
70	Matilda	41.91	718	7	6	5	5	3
71	Gisella	42.89	1271	7	5	5	6	3
72	Cilento	52.98	1109	5	5	5	3	5
73	Santoro	41.16	731	7	5	3	8	3
74	Marenia	40.26	1016	7	5	5	6	3

Appendix 1: Genotypes yield and field performance score(continued)

Variety number	Variety Name	Dry weight g/plant	Head N content in mg/ plant	size	Uniformity	Overall performance	Early stress	Late stress
75	Fabietto	51.94	915	7	7	7	3	3
76	Lucan	57.82	1301	6	5	5	6	3
77	Nobellan	50.8	868	7	5	3	7	3
78	Mafalda	43.07	1100	6	7	3	8	3
79	Trofis	41.3	768	8	7	6	3	3
80	Allex	47	1003	6	5	4	7	3
81	Audran	47.74	1310	7	3	4	8	3
82	Augusta	42.48	1457	4	6	7	3	3
83	Eline	44.58	1150	7	5	4	7	3
84	Mantilia	57.59	1559	5	6	8	3	3
85	E13,0974	46.26	878	6	4	3	7	3
86	Crufia	43.88	1011	8	6	5	6	3
87	Justine	47.55	1043	5	7	7	3	3
88	Aljeva	63.6	1276	6	6	5	6	3
89	Touareg	50.27	1129	7	5	3	8	3
90	Monique	43.36	1228	7	6	6	4	2
91	Italina	53.11	1555	6	7	7	4	3
92	Tizian	44.56	879	6	5	4	7	3
93	Caliente	44.05	1244	7	5	4	7	3
94	Kagraner Sommer	51.71	1333	6	5	5	6	3
95	Marianna	53.18	1374	6	6	5	5	3
96	Oriana	46.03	1222	7	7	7	3	3
97	Tolima	52.09	893	7	6	6	5	3
98	Habana	49.57	913	7	6	5	6	3

Appendix 1: Genotypes yield and field performance score (continued)

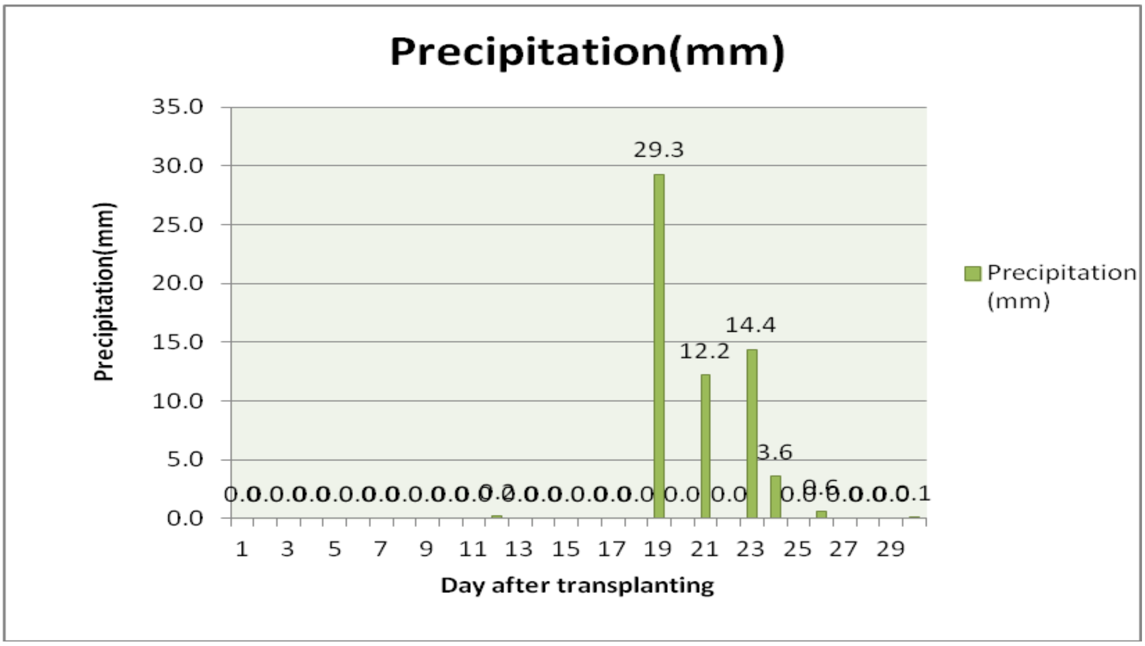
Variety number	Variety Name	Dry weight g/plant	Head N content in mg/ plant	size	Uniformity	Overall performance	Early stress	Late stress
99	Nerea	45.43	934	7	6	6	5	3
100	Margarita	49.49	1191	6	5	8	4	3
101	Optima	49.61	1225	8	6	6	4	3
102	Salad Pak	43.65	822	7	6	6	5	3
108	BAUTZENER DAUER	52.31	1306	8	6	6	4	3
109	BLONDE DÉTE	52.25	1224	5	5	3	8	3
111	Dakota	49.41	859	6	5	5	7	3
112	Donar	52.8	998	7	7	7	3	3
113	DU BON JARDINIER 2	40.19	840	8	6	6	3	4
114	Fastian	47.5	1036	6	6	6	4	3
116	HILDE 2	48.49	1009	4	1	1	9	3
117	Hilro	50.33	812	7	5	5	6	3
118	HOCHSOMMER	67.71		5	5	5	6	3
120	LA CHAUME	57.29	1020	6	7	7	3	3
121	Lido	50.93	1366	8	5	4	7	3
122	Lutine Rivoire	49.8	1180	7	5	3	8	3
123	Magda	49.36	1127	7	5	3	7	3
124	Nancy	47.07	846	7	5	5	7	3
125	Noran	51.78	1257	3			3	3
126	Peson	50.02	1253	6	5	3	7	5
128	Printania	48.49	1351	8	5	3	7	3
129	Steenkrop	48.09	803	-	-	-	-	-
131	Vista	46.57	1298	7	5	4	6	3
132	Zorro	48.28	1111	6	5	3	7	3

Appendix 1: Genotypes yield and field performance score (continued)

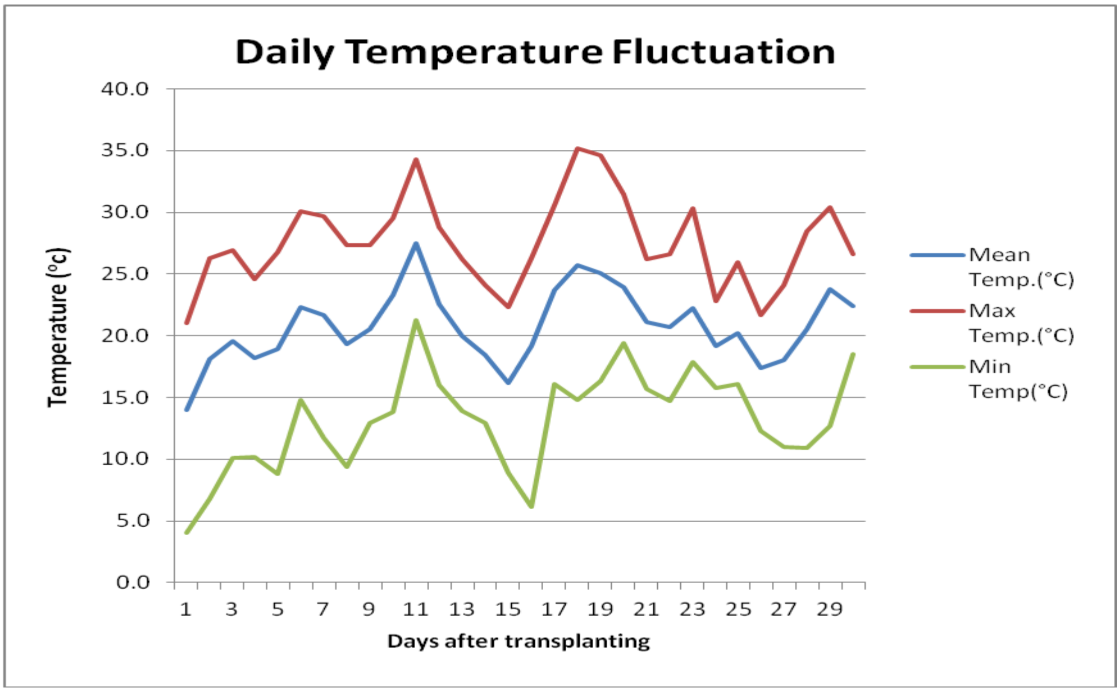
Variety number	Variety Name	Dry weight g/plant	Head N content in mg/ plant	Size	Uniformity	Overall performance	Early stress	Late stress
133	Cobham Green	46.56	1022	5	5	7	4	4
134	Du bon Jardinier 1	53.93	1109	8	6	7	8	4
135	Furchtenichts,	38.47	1115	7	6	7	4	3
139	Resistente Estate Di Kagraner Sommer	50.54	1254	7	7	8	3	3
140	Spat Aufsch. Grosse	52.11		7	5	5	6	3
141	Suzan	51.78	967	6	5	3	9	3
142	Verte De Perpignan	51.89	872	4	5	5	4	3
145	White Boston	46.49	1112	4	4	6	4	3
147	Pronto	43.98	1157	-	-	-	-	-
148	Matilda	46.48	1071	-	-	-	-	-

Appendix 2: Weather data

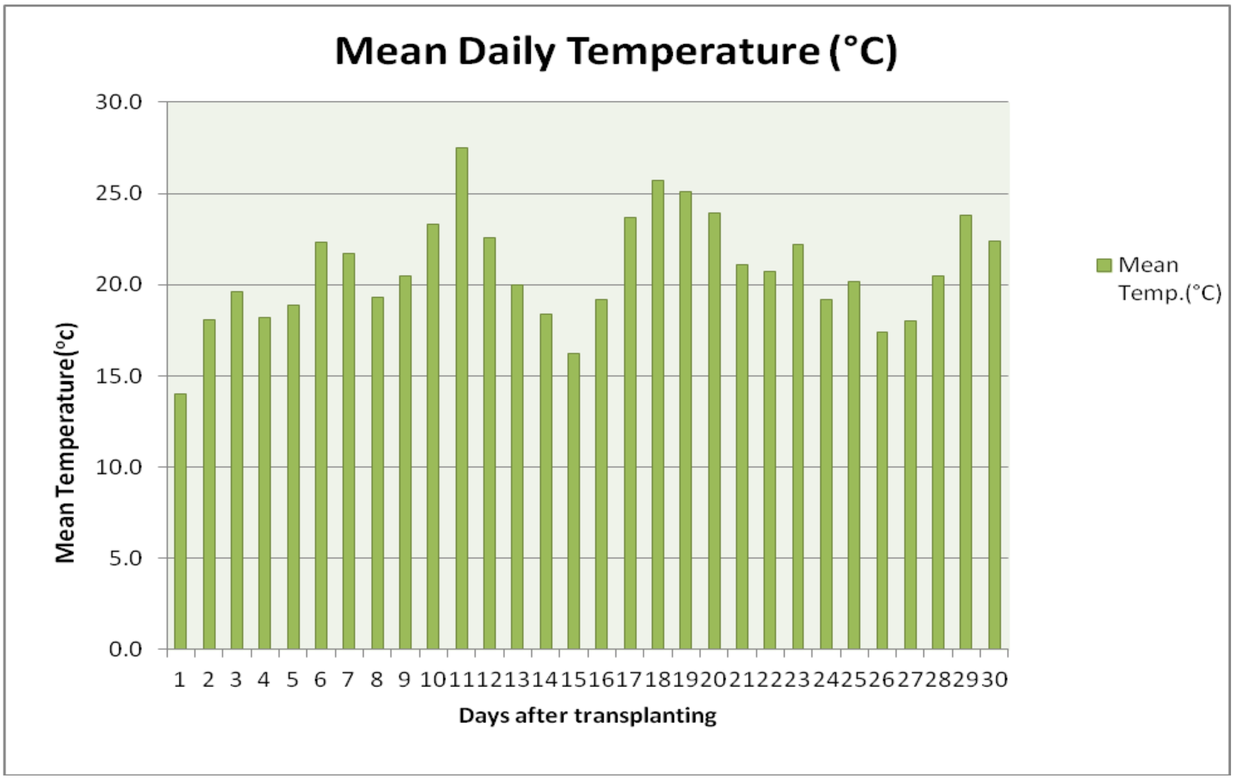
Appendix 2.1 Daily precipitation data during the growing period



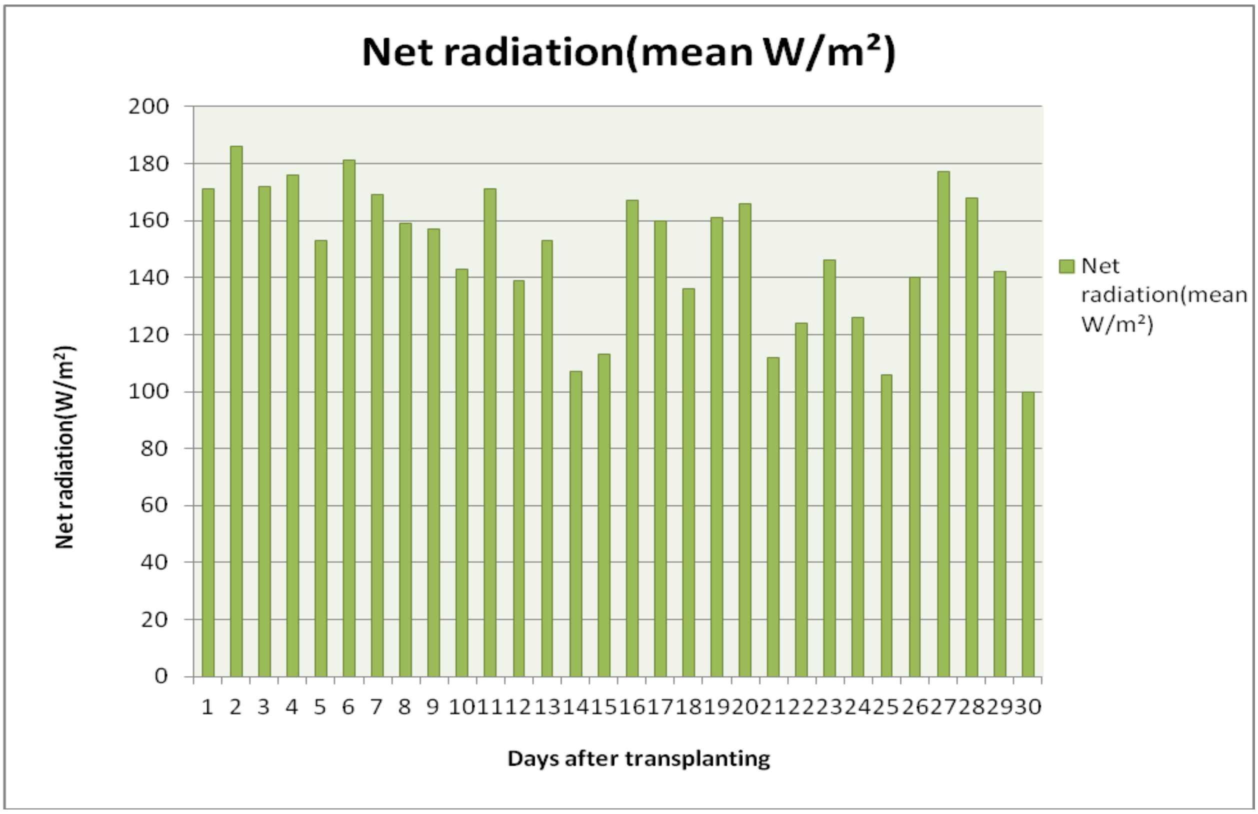
Appendix 2.2 Daily temperature fluctuation during the growing period



Appendix 2.3 Daily mean temperature during the growing period



Appendix 2.4 Daily net radiation during the growing period



Appendix 3: Summary statistics of field performance parameters

Parameter	Mean	Minimum	Maximum	Range
Dry weight/ genotype	48.49	32.15	69.51	37.36
Head N content	1077	675	1559	884
Total N taken up from soil	2553	951.1	3456	2504
N uptake layer 1	807.5	403.5	1067	663.5
N uptake layer 2	807.5	403.5	1130	725.8
N uptake layer 3	773.6	290.9	1139	847.9
N uptake per layer 4	252.3	54.4	507.3	452.9
Soil moisture % layer 1	12.88	10.10	18.33	8.239
Soil moisture % layer 2	11.49	9.133	16.09	6.956
Soil moisture % layer 3	10.25	8.004	12.79	4.788
Soil moisture % layer 4	9.206	5.601	12.15	6.550