INHERITANCE OF NITRATE-N ACCUMULATION IN LETTUCE (LACTUCA SATIVA L.)

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ABSTRACT

INHERITANCE OF NITRATE-N ACCUMULATION IN LETTUCE
(LACTUCA SATIVA L.)

By

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Nitrate accumulation in plants is the result of nitrate uptake in excess of its reduction. A greater capacity for nitrate reduction and subsequent assimilation in a plant is desirable to increase the yield and to breed vegetables for low nitrate content, since nitrate reduction is one of the first steps in protein synthesis. Previous work on lettuce has shown variations in nitrate accumulation in different cultivars.

Experiments reported herein with 66 cultivars showed a differential nitrate accumulation. The plants were grown in sand culture using nutrient solution. The nitrate content was determined on the dry leaf tissues using a nitrate selective electrode. Two low, one medium and three high nitrate accumulators were used to study the inheritance of nitrate accumulation in lettuce.

Accumulation for low levels of nitrate was found to be dominant. Genetic analyses showed that three major genes control the accumulation of nitrate in lettuce.

In the three major gene systems, two genes A and B determined low nitrate accumulation levels in the cultivar Valmaine. The presence of

both A and B dominant genes was essential for low accumulation, i.e., A and B were complimentary to each other.

The low nitrate accumulation levels in the cultivar Wonder Van Voorburg was due to a single dominant gene C_2. Thus low nitrate accumulation can occur due to the presence of both A and B, or C_2 alone or a combination of all three A-B-C_2.

A dominant gene C determined the high nitrate accumulation levels in the cultivar Caravan. The gene C was epistatic to genes A and B, but it was recessive to the dominant allelic gene C_2. Thus the presence of C_2 and C in the same genotype results in low levels of accumulation of nitrate. The genotypes of the cultivars Kordaat and Valore, the high accumulators were similar. The high nitrate accumulating trend of these cultivars was determined by recessive alleles a-b-c. Thus high nitrate accumulation can occur by the presence of C gene alone or the absence of either or both A or B in a genotype.

Accumulation of nitrate for medium levels appeared to be controlled by the allelic gene B_2.

INHERITANCE OF NITRATE-N ACCUMULATION IN LETTUCE (*LACTUCA SATIVA* L.)

By

Ramachandra Subramanya

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INTRODUCTION

The breeding of plants for high yields or quality requires a greater understanding of physiological mechanism. Identifiable physiological traits contributing to yield or quality could provide an alternate selection criteria for a variety improvement program. The determination of these traits needs to be simple and rapid if large populations are to be screened effectively. Nitrate accumulation for example would be suitable for such an investigation. Nitrate reduction is one of the first steps in the synthesis of protein in plants.

In recent years there has been a growing interest in understanding genetic influences on plant nutrition. Low and high-protein cultivars of the same species have been attributed to its nitrogen nutrition (54, 70,86). Considerable work has been directed towards understanding N nutrition. The genetic control of many aspects of N nutrition is, however, missing.

Lettuce (Lactuca sativa L.) and many other crop species are known to accumulate nitrates (6,19,20,23). Nitrates in the diet is undesirable since they may lead to methemoglobinemia in humans (26,42,61,80). Nitrate under certain conditions may be reduced to nitrites in the gastro intestinal tract. These nitrites are known to interact with secondary or tertiary amines to form nitrosamines. Many of the nitrosamines are mutugenic, teratogenic, and carcinogenic to various lab

animals (26); however, carcinogenic concentrations in humans are unknown and, therefore, it is important to have nitrate free food in the human diet. Cultivar difference in their capacity to accumulate NO_3-N have been observed in different vegetable species (6,18,23,68).

The objective of this study was to determine whether nitrate accumulation in lettuce is genetically controlled, and, if so, to learn its heritability and mode of inheritance. The pattern of inheritance would be invaluable for a plant breeder in breeding lettuce for low nitrate content.

LITERATURE REVIEW

Nitrate accumulation in plants has been observed for many years; however, interest in it has intensified due to an increased use of nitrogen fertilizers. The factors influencing nitrate accumulation have been studied extensively.

Nitrate accumulation in plants may affect man and animals directly or indirectly since nitrate in water, food, and feeds can be microbiologically reduced to nitrite which is 10 times as toxic as nitrates (37). Maynard et al. (68), Viets & Hageman (92) and Wolff & Wasserman (95) have recently reviewed the nitrate problem on human health and environment.

One of the effects of nitrite is its toxic interference of the oxygen carrying capacity of blood in both animal and humans, an effect called methemoglobinemia. Nitrates and nitrites can also reduce milk production in animals and cause reproduction problems, or even death if ingested in large quantities (37). Nitrates in forage material under damp conditions can be reduced to nitrites. Consequently, feeding of such material to livestock has been reported to be toxic (95). The release of oxides of nitrogen released from ensiled high-nitrate forage materials can be lethal to both man and animals in silage buildings with no fencing and inadequate ventilation (28,42).

Cyanosis or blue babies caused by nitrates was reported as early as 1945 (25). The low stomach acidity of infants under four months of age permits the growth of microorganisms that can reduce nitrate to nitrite. Poisoning of very young infants fed with spinach high in nitrate content has been reported (80). Deaths of infants fed with water containing high nitrate have also been reported (95).

In recent years, there has been an increased concern about the potential hazard of nitrosamines as toxicants formed in or through eating certain foods or drugs which contain the precursor substances of nitrosamines (46,63).

Nitrite reacts with secondary and tertiary amines, or quaternary ammonium compounds that occur in foods and drugs (63,95) to give nitrosamines which have been shown to be carcinogenic, teratogenic, or mutagenic to various laboratory animals (26,63). Maynard et al. (68) and Wolff and Wasserman (95) recently reviewed the nitrosamine situation. Nitrosamines have not been reported to occur in the commonly consumed vegetables. Although Keybets et al. (57) and Heisler et al. (46) could not detect any nitrosamines in spinach and beets even under conditions favorable for formation of nitrosamines other experimental results cited by Maynard et al. (68) and Wolff and Wasserman (95) suggests a need for extensive research with regard to in vivo synthesis of nitrosamines.

Sprague (89) reported that nitrate reduction is one of the first steps in protein synthesis; therefore, the plants that have the greatest capacity to reduce nitrate will also have a better chance of more protein synthesis. Differences between high and low protein cultivars of

wheat species were attributed to differences in the re-export and translocation of N (54,70,86). Croy and Hageman (29) showed a practical significance of two wheat genotypes, which differed in nitrate reductase activity and their protein yield. The capacity to reduce or assimilate nitrate varies with different crop species (75). In the same study, Olday et al. (75) found the site of nitrate reduction to be concentrated in the leaf blades of cucumber (Cucumis sativus L.), while in pea (Pisum sativum L.) nitrate reduction occurred evenly throughout the plant. The nitrate-N comprised 80% of the N present in the bleeding sap of roots of cucumber plants from which the shoots had been excised; whereas, nitrate-N constituted only 30% of the sap from pea roots, the nitrate reducing capacity of the two species were found to be different in different tissues. In cucumber only 2% of the nitrate reductase activity was in roots; whereas, 92% of the activity was found to be in the leaf blades. In contrast the enzyme activity in pea plants was 18% in the roots and 67% in the leaf blades. They concluded that, because of the greater nitrogen assimilation by pea roots, it uses NO_3-N more efficiently than cucumber resulting in less nitrate and more protein in the pea.

One of the major problems of high nitrates in canned vegetables is the reduced shelf-life caused by the detinning effects of tin lined cans (52,60,87). Farrow et al. (33) reviewed the detinning process and associated factors. High nitrate content in beets can lead to a bitter taste when cooked (79).

Factors influencing nitrate accumulation in plants have been extensively studied. One of the most important factors favoring nitrate accumulation in plants is the nitrate rich environment. The availability of soil nitrate for plant uptake depends upon very complex processes. Soil nitrate can be lost by leaching, volatilization, microbial utilization, or simply be unavailable to the plant for lack of water. Nitrate, an anion, is considered to be contained entirely in the soil solution; therefore, the rate of replenishment of nitrate from other forms (organic and ammoniacal) may be more important than the amount of nitrate present at any given time (12).

Increased demands on agricultural produce for higher yields, rapidly maturing vegetable crops and to maintain a bright green color and succulence especially in leafy vegetable crops like spinach, lettuce, etc., require a liberal or adequate nitrogen fertilizers in the rhizosphere which can result in nitrate accumulation in the plants (68,92,95).

The time and absorption of nutrients by lettuce is crucial. Zink and Yamaguchi (99) reported that most of the nutrient uptake (70 percent) occurs during the period of 21 days preceding harvest, and about one-half of that is absorbed two weeks prior to harvest. Therefore, is it necessary to maintain a high nutrient level in the soil until a few days before harvest.

Barker et al. (6) showed spinach leaves contained more nitrate-N when N was applied broadcast before planting than when applied as a side dress application. This suggests that spinach accumulates nitrates when cultured for a longer period of time in nitrate rich medium.

Greenhouse experiments have shown a direct relationship between time of nitrate fertilization and nitrate concentration in plants (4). Barker et al. (6) also showed the source and timing of N application to affect nitrate content in plants. Ammoniacal fertilizers and materials that mineralize slowly are known to reduce nitrate accumulation in plants (3,6,71) but the possibility of ammonium toxicity exists when all the N is in the ammoniacal form (65). A combination of NH_4-N and NO_3-N with nitrapyrin a nitrification inhibitor was reported to reduce nitrate accumulation in plants without sacrificing the yield (71).

Lettuce (Lactuca sativa L.) has been reported to accumulate nitrate (23). Brown and Smith (13) reported no significant increase in nitrate content of leaf and semihead lettuce plants by increasing the fertilizer rate. Cantliffe and Phatak (23) also reported no significant increase in the plant nitrate content of four lettuce cultivars even at increased nitrogen fertilizer rate. The lack of response to additional N was attributed to the use of muck soil.

Other plant nutrients have been reported to affect nitrate accumulation. Reports on the effect of phosphorus and potassium are varied. A decrease in the nitrate content with an increase in P application (7,39) was noted, but Barker and Maynard (4) observed no effect on nitrate accumulation in spinach when P, K, Ca, and Mg, were moderately deficient. Brown and Smith (14) also observed that insufficient P had no effect on nitrate content of vegetables. Cantliffe (17) working with beets (Beta vulgaris L.) and spinach (Spinacea oleracea L.) plants, grown at different levels of N, P, and K, observed that varying the

level of P had no effect on nitrate accumulation, but plants of both species grown at high K accumulated significant amounts of nitrates.

Molybdenum deficiency lead to nitrate accumulation in higher plants (47,73). Beevers and Hageman (9) in their review indicated that Molybdenum appeared to be involved in the nitrate reductase induction process and not merely activating pre-existing protein. Molybdenum is a constitutive part of nitrate reductase (1,32). Iron (1) and other plant nutrients may be involved in the reduction or accumulation of nitrate but the effect appears to be indirect (68,92).

Light has long been known to influence nitrate content in plants (8,15,19). Low light intensity increased nitrate accumulation (19). Cantliffe (19) observed increased nitrate content in spinach leaves when the light intensity was reduced from 2400 ft-c to 600 ft-c. Shade or inadequate light also resulted in high nitrate accumulation in plants (17). Photoperiod, diurnal variation, and sampling time are known to influence nitrate content in plants (41,44). Cantliffe (20) working with various radish, spinach, and snap bean cultivars harvested at 0, 6, and 12 hours after the initiation of the light period, found radish leaves and snap bean pods contain less nitrate when harvested late in the photoperiod. However, the nitrate content of radish roots and spinach leaves remained the same regardless of the harvest time.

The effect of light on nitrate accumulation is indirect in that light affects nitrate reductase thus affecting the nitrate content. Beevers and Hageman (9) made a comprehensive review on the influence of light on nitrate reductase activity in higher plants.

A direct and absolute requirement of photosynthetic CO_2 fixation for the induction of nitrate reductase in Perilla plant was reported (55). This requirement, however, does not hold true for all tissues since the enzyme can be induced without photosynthetic requirement. Thus, the effect of light appears to provide energy for synthesis of the enzyme or in some way increase and stabilize polyribosome formation (90), or increase the membrane permeability (9,11) so that nitrate transport is enhanced. Effect of light on nitrate reductase induction is also associated with increased protein synthesis (9). Adequate light would also insure photosynthate for reductive energy via carbohydrates and carbohydrate skeletons for amino acid and nucleotide formation (59).

Although Gilbert et al. (39) did not find a temperature effect on nitrate accumulation in various plant species, other workers have observed greater nitrate concentrations in plants grown at high temperature than at lower temperatures (8,21,38). Increased nitrate content with high temperature have also been observed on barley (76), lettuce (36), pasture crops (8), and sorghum-sudangrass (38). Spinach cultivars grown at various temperatures and at various N levels (21) showed an increase in nitrate with an increase in temperature even at zero N treatments. The nitrogen level and higher temperatures appeared to have a synergistic effect on nitrate accumulation. Similar observations have been reported with other crops (7,21,36,38). Cantliffe (21) also indicated a deactivation of nitrate reductase activity at extreme temperatures and nitrate accumulation. Reduction of nitrate reductase activity due to heat stress was also demonstrated in barley seedlings (76).

All of these reports suggest that a general statement about the effect of temperature on nitrate accumulation cannot be made since the processes of absorption, translocation, and assimilation are also affected by temperature. Therefore, it is not uncommon to find contradicting reports of the effect of temperature on nitrate accumulation (68).

Water or moisture is a very important factor in nutrient uptake. Roots come in contact with more nutrient ions when growing in a moist soil than when growing in a dry soil. Ammoniacal N does not readily move but the nitrate-N an anion is considered to be contained entirely in the soil solution, which can readily move in and out of the root zone. When nitrate accumulates under low soil moisture or drought condition, high temperatures are usually associated with it. Both high temperature and moisture stress were shown to reduce or inactivate nitrate reductase activity (97). The enzyme, nitrate reductase was shown to be more sensitive to moisture stress than were certain other enzymes of the photosynthetic system (53). These results suggest that the effect of either high temperature or moisture stress is more adverse on nitrate reduction than it is on the absorption and translocation of nitrate, thus causing nitrate build-up.

Herbicides have been reported to increase nitrate in crops. Cantliffe and Phatak (24) cited many reports indicating an increase in nitrate content of various crops with the use of herbicides. Beevers et al. (10) found that herbicidal levels on 2,4-dichlorophenoxy-acetic acid (2,4-D) had differential effect on cucumber and corn. Nitrate reductase activity was reduced in cucumber whereas an opposite effect

was noted in corn. With both species the nitrate content varied inversely with nitrate reductase activity. Cantliffe and Phatak (24) did not find a dramatic increase of total-N in spinach blades and petioles, by herbicide treatments, they indicated that the increase in nitrate accumulation was the result of a decrease in some phase of nitrate reduction.

Klepper (58) recently reported that the photosynthetic or triazene herbicides inhibited nitrite reductase. He found little influence of these herbicides on nitrate accumulation or nitrate reductase activity.

Sublethal doses of herbicides, especially triazenes have been reported to be beneficial by increasing the protein content and yield (24,49,56). An increase in nitrate reductase activity and nitrate accumulation were also observed (34,81,82,91). Ries et al. (83) treated six different plant species with subherbicidal levels of simazine and found increased nitrate uptake by plants, and in some cases the plants had a high nitrate content. Cantliffe and Phatak (24) working with herbicidal levels of several herbicides found increased nitrate content in spinach petioles with many herbicides; however, with certain herbicides it appeared that the N fertilizer was more efficiently used.

Since nitrate reductase reduces nitrate, interference in the enzyme activity would result in the accumulation of nitrate in the plant. It has been shown that within the same tissue the nitrate content varies inversely with the level of nitrate reductase activity (41).

Other experiments (29,30,74) suggest that nitrogen fertilizer over and above that which is usually considered optimal increases the nitrate reductase level and protein content of the plant or grain.

A review on the characteristics and factors affecting nitrate reductase was made, by Beevers and Hageman (9) and recently by Viets and Hageman (92). Although many endogenous compounds have been reported (35,48,64,84) to inhibit nitrate reductase or repress its synthesis resulting in nitrate accumulation *in vitro* studies, it is not certain that the same endogenous compounds are responsible for nitrate accumulation *in vivo*.

One of the major sources of nitrate intake in foods is from vegetables. Wright and Davison (96) listed Amaranthaceae, Chenopodiaceae, Cruciferae, Compositae, Graminae, and Solanaceae as plant families which have a tendency to accumulate high levels of nitrate. Nitrate-N a natural constituent of plants is not uniformly distributed throughout the plant, or for that matter within the edible portion (e.g., petiole versus leaf blade) (96). The nitrate content within a leaf, root, and fruit, type of vegetables has been known to vary (66). Maynard and Barker (66) stated that nitrate-N generally accumulates in older portions of the plant. Reproductive parts are usually low in nitrate-N, roots slightly higher while leaves even higher. The nitrate content of seeds in general is considered to be negligible. McNamara *et al.* (69) surveyed 113 seed samples representing 37 different species and found among cereals nitrate content was more in corn than either wheat or oats, but the nitrate content of corn was low. Among the major crop

plants tested soybean seeds contained the highest nitrate-N. The weed seeds as a group had the highest average nitrate content. Nitrate content in vegetable seeds varied with the species.

Although high levels of nitrate can accumulate in leaf tissues of plants in the early vegetative growth, the nitrate content decreases in leafy and non leafy tissues as the plant matures. In mature plants, nitrate accumulates primarily in nonchlorophyllus stem and stalk structures which exhibit a low capacity for nitrate assimilation (92). However, a different pattern of nitrate accumulation occurs in leafy vegetables where such as lettuce (99) and spinach (6) the nitrate generally accumulates with age.

Reports of differences in nitrate accumulation among cultivars within the same species which has been recently reviewed by Maynard et al. (68). Cantliffe and Phatak (23) reported cultivar differences in their capacity to accumulate nitrates in their edible parts of lettuce, radish and spinach. Minotti (72) in his initial studies with lettuces found a fourfold difference within crisphead lettuce cultivars. Continued comparisons of two cultivars Minetto and Valrio under varying growth situations showed that nitrate content varied with media, environment, plant part and plant age, but the nitrate content of the cultivar Minetto was consistently higher than that of Valrio.

In recent years there has been an increased interest in genetic influences on plant nutrition. Many aspects of plant nutrition are under genetic control (31). Response to N nutrition being under genetic control was first suggested by Harvey (45), when he showed that corn

and tomato genotypes had differential N requirements and that these differences were inherited.

Savoyed leaf types of spinach were reported to accumulate more nitrate than smooth leaf type spinach (23,67). Barker et al. (5) conducted an experiment with 18 spinach cultivars which were grouped into savoy, semi-savoy, and smooth leaf types, these were grown under various N regimes. Although their results showed that savoy leaf type spinach type accumulated more nitrate than the smooth leaf type when compared as a group, there were marked differences among cultivars in nitrate accumulation within each group. A similar study (18) involving 31 spinach cultivars, including Plant Introduction accessions of various leaf types were grown under two N regimes. In this experiment leaf type was not related to nitrate accumulation and in the presence of high N fertilizer rates individual spinach lines accumulated nitrate. The author (18) suggested that the genetic variations among lines in the N assimilation pathway may account for the differential nitrate accumulation.

Olday et al. (74) explained the physiological basis for differences in nitrate accumulation between the two spinach cultivars America, a high accumulator and Hybrid 424 a low accumulator. The authors observed, with adequate N nutrition, the nitrate content of America was about three times that of Hybrid 424. Nitrate reductase activity in the leaf blades of Hybrid 424 was significantly greater than America. The qualitative and quantitative composition of bleeding sap did not differ for the two cultivars. There was also no indication of reduced

nitrate uptake by Hybrid 424. The lower nitrate content of Hybrid 424 at high N nutrition was attributed to a greater nitrate reductase activity in this cultivar, especially in the leaf blades. Marked variations in the levels of nitrate reductase activity was also reported with several varieties of cauliflower (9). Hageman and Flesher (40), demonstrated an inverse relationship between nitrate accumulation and nitrate reductase activities in two corn (Zea mays L.) lines.

Although the genetics associated with nitrate accumulation have not been reported in the literature, there are a number of reports indicating genetic variations in nitrate reductase (9,92). Corn inbreds and hybrids were reported to differ widely in nitrate reductase activity and the level of activity was under genetic control (98). The heritability of nitrate reductase activity was demonstrated (85) by careful selection of corn inbreds based on enzyme assay, and appropriate inbred combinations, a hybrid could be obtained with a high, medium or low level of nitrate reductase activity. The inheritance of nitrate reductase activity in corn was reported to be controlled by a two gene system (93). The authors (93) concluded that both enzyme synthesis and decay are factors governing the level of nitrate reductase in corn.

Efficiency of N utilization in tomatoes (Lycopersicon esculentum L.) was shown to be a highly heritable trait (77). The efficient plants under severe N stress produced more dry weight than the inefficient strains. The inheritance studies showed that relatively few genes were involved.

Hoener and DeTurk (51) concluded that "high protein" corn strains absorbed and assimilated more nitrate than "low protein" strains. The level of nitrate reductase was reported to be positively correlated, with soluble leaf protein (29,40,43). Croy and Hageman (36) selected wheat cultivars with high nitrate reductase activity and reported a higher grain protein in these selected lines. Others (54,70,86) have reported that the difference between high and low protein cultivars of wheat species were due to their differences in re-export and translocation of N and these differences were under genetic control.

Olday et al. (74) working with two spinach cultivars with differential nitrate reductase activity indicated that the potentially higher growth rate, higher yields, higher protein content and lower nitrate content appear to be interrelated features found in the low nitrate accumulation cultivars. Similar observations were also made by Barker et al. (5) with the low nitrate accumulation spinach cultivars compared with cultivars which accumulated high nitrates.

MATERIALS AND METHODS

Sixty-six lettuce cultivars (Table 1) were screened for NO_3-N accumulation in their leaves after being grown in a modified Hoagland's (50) nutrient solution (Table 2). The plants were grown in the greenhouse in unground silica-sand (Wedron 4030) and watered with nutrient solution to maintain uniformity and a desired level of nutrients in each growing unit. In the preliminary screening study (1973), 236 cc paper cups (cold cups) were used to grow the plants. Holes were made in the bottom of the containers for drainage. A filter paper (Whatman 1) was placed in the bottom to prevent loss of sand through the drainage holes. Each cup contained 315 g of sand. In subsequent plantings (1974 & 1975), 473 cc styrofoam cups were used. Each cup was filled with eight g. of coarse perlite followed by 630 g. of sand.

Benches on which the plants were grown were lined with black polyethylene and arranged so the leachate could be collected at one end of each bench for reuse.

Seeds of each cultivar were sown in a flat containing a mixture of 3 perlite to 1 vermiculite. Seedlings were transplanted at the first true leaf stage. One day prior to transplanting each, silica-filled cup was thoroughly washed with distilled water. Two seedings were planted in each container and watered with a modified Hoagland's solution (Table 2). The following modifications were made in the nutrient solution:

Table 1: Lettuce cultivars screened for NO_3-N accumulation in 1973.

Agilo	
Amplus	Secura
Arlon	Solito
Avoncrisp	Spartan Lakes
Avondefiance	Sucrine
Blondine	Suzan
Bourguignonne	Texas 635- 1967-68
Brioso	Texas 637- 1967-68
Calicel	Texas 641- 1967-68
Calmar	Texas 642- 1967-68
Caravan*	Texas 644- 1967-68
Climax	Tinto
Deciso	Tonika
Delta	Type 57
Empire	Type 69
E 9201A	Valmaine 67074*
Fairton	Valmaine (Weslaco)
Great Lakes	Valore*
Grand Rapids	Valrio
Groso	Valtemp
Hilde	Valverde
Interex	Vanmax
Ithaca*	Variety A (Weslaco)
Klock	Variety B (Weslaco)
Knap	
Kordaat*	Ventura
Korrekt	Wonder Van Voorburg*
Kwiek	Zomerkoningin
Larganda	
Liba	
Magiola	
Marquette	
May Princess	
Montemar	
M.S.U. 71- I46	
Portato	
Proeftuin's Blackpool	
Rapide	
Red Tipped Boston	
Regina	
Resistent	

*Cultivars were used as parents in the genetic analyses.

Table 2: A modified Hoagland nutrient solution used in lettuce sand culture.

Element concentration ppm (mg/l) in nutrient solution		Stock Salt Solution of Major Elements
200	Ca	IM $Ca(NO_3)_2$ $4H_2O$
210	N	IM KNO_3
234	K	IM $MgSO_4$
64	S	IM KH_2PO_4
48	Mg	
31	P	
		Stock Salt Solutions of Trace Elements
0.5	B	H_3BO_3
0.05	Mn*	$MnSO_4H_2O$
0.05	Zn	$ZnSO_47H_2O$
0.02	Cu	$CuSO_45H_2O$
0.01	Mo	MoO_3
2.4	Fe	Na Fe Chelate

*0.5 ppm in 1973 planting.

1. Elimination of chloride by substituting $MnSO_4 \cdot H_2O$ for $MnCl_2$.

2. Substitution of iron chelate for iron tartrate.

The pH of the final solution was 5.7.

In preliminary studies plants watered with this nutrient solution showed manganese toxicity after eight weeks. Hence, in subsequent plantings Mn content was reduced from 0.5 to 0.05 ppm in the solution.

The frequency of watering of the seedlings with nutrient solution was dependent on the stage of the plant growth. At each watering the sand was saturated with nutrient solution. At transplanting seedlings were watered with half the quantity of $CaNO_3$ and KNO_3 in the nutrient solution and continued until the seedlings were established. Watering with a complete nutrient solution followed. Daily watering with the nutrient solution commenced when the plants were large and required it. After daily watering began the sand was saturated weekly with distilled water to leach out salts.

Nutrient solution leachate collected at the end of each bench was reused once or twice before discarding. This was the practice in the first two plantings. In preliminary screening, nutrient solution was used alternating with water.

In addition to natural light plants were supplemented with fluorescent (C.W.V.H.O.) light placed over each bench. Light meter reading at the plant level averaged 750 ft-c. The fluorescent lights were on 16 hrs. a day.

The temperature in the greenhouse was set at $15^{o}C$ and the automatic vents were set at $18^{o}C$. Fans were used to maintain uniform temperature through the greenhouse.

Temperatures varied from 12°C in the night to 27°C during the day from December 2, 1975 to January 2, 1976. The plants were grown in nutriculture for four weeks to obtain a sample for the NO_3-N determination.

Harvesting was done at the beginning of a dark period. Plant tops were cut at the base just above the crown and the fresh weight of leaves were determined. All of the plants were harvested during one period of time.

After the fresh leaves were weighed they were oven dried at 37.5-43.0°C and re-weighed. The dried leaves were ground in a Wiley mill to pass a 20 mesh screen. This plant tissue was analyzed for NO_3-N using an Orion Nitrate Ion Activity Electrode (2,22). The determination of nitrate-N was similar to the method described by Baker and Smith (2), with the following modifications:

1. 0.2 g. of lettuce leaf tissue was extracted in 100 ml of 0.025 M $Al_2(SO_4)_3$ solution.
2. The 0.025M $Al_2(SO_4)_3$ extracting solution also contained 10 µg per ml of NO_3-N and 2 ml per liter of preservative.

The use of 0.2 g. of plant material was necessary since the tissues had high nitrate contents. Preservative was used to prevent biological changes in nitrate concentrations. The extracting solution containing 2 ml per liter of preservative was reported, not to interfere with the nitrate determination (2). The nitrate determination in this study lasted 10-14 days and no changes either in the extracting solution or in the standard solutions could be detected. The nitrate

concentrations were determined on a pH meter with expanded millivolt scale. The extracting solution with the plant material in suspension was read directly without the filtration step. NO_3-N was read as ppm and recorded as percent NO_3-N on a dry weight basis.

To assess the efficiency of nitrate recovery and the sensitivity of the nitrate ion activity electrode, nitrate varying from 10 to 100 ppm was added to the lettuce extract and the amount recovered was ascertained (Table 3). Recovery was nearly complete; these results are in agreement with that of Paul and Carlson (78) and Cantliffe et al. (22).

The preliminary screening was designed to observe: 1) cultivar differences in free NO_3-N in lettuce leaves, 2) effect of age on NO_3-N accumulation, and 3) effect of NO_3-N concentration in the media on the nitrate accumulation in lettuce leaves. Sixteen plants of each cultivar were watered with nutrient solution containing two levels of N (210 and 420 ppm N). There was also an increase in Ca and K in the latter solution, since nitrogen was applied as salts of Ca and K. Design of the experiment was a randomized block with 2 replications, 66 cultivars, 2 harvests, and 2 nitrate, Ca and K levels.

After growing the plants for 4 weeks, one-half of the plants (4 plants) from each nutrient level per replication were harvested. The fresh weight of leaves and roots and the dry weight on the leaves were recorded. The remaining half of the plants were harvested at the end of 8 weeks. On these 8 week old plants a representative sample from each cultivar per treatment was used for NO_3-N determination. The remainder of the plant material was used for the determination of other elements.

Table 3: Nitrate recovery from added nitrate to lettuce tissue extracts as measured by a nitrate selective electrode.

Added NO_3-N (ppm)	Expected	Found	Difference	Percent Recovery
0	63	63	--	--
10	73	72	-1	99
20	83	80	-3	96
40	103	100	-3	97
60	123	124	+1	101
100	163	161	-2	99

The analysis of fresh tissue showed differences among cultivars in nitrate concentration. Eleven cultivars with the largest differences [Low (L), Medium (M), and High (H)] were selected for additional studies. Total N and NO_3-N determinations, based on dry weight, were made for the 11 cultivars on 4 and 8-week old plants grown at both nutrient levels. The total N was determined by Kjeldahl method. Tissue analyses for the other elements were made on 8 week old plants for all cultivars grown with 210 ppm N and for the selected 11 cultivars grown at 420 ppm. On the other elements K was determined by Flame Photometer, all others by Spectroscopy.

Based on the nitrate content of dry tissues, 11 cultivars were selected and all possible crosses and their reciprocals were made. Since lettuce is a cleistogamous plant and the stigma is pollinated prior to flower opening, it was necessary to carry a dominant marker gene in the pollen parent in order to distinguish the crosses from the selfs. The markers were seed coat color, leaf color and the presence of anthocyanin. The four main lettuce types cos or romaine, butterhead, crisphead, and leaf types were included in this study and also served as markers.

Six plants of each parent and 3 to 12 F_1 plants (including reciprocals) were grown in 1974. Six F_1 plants from each cross were not obtained due to the difficulty encountered in making crosses with certain cultivars. A completely randomized design was used. Modified Tukey's test (88) was used for mean comparisons.

For this study the modified Hoaglands's nutrient solution containing the standard nutrient concentration (Table 2) was used. The plants were grown for 35 days prior to leaf sampling. Fresh and dry weights and nitrate-N contents of the dry tissues were determined. The parental and F_1 plants that were sampled for nitrate-N were allowed to flower for hybridization or to obtain F_2 seeds.

In 1975, parental F_1 and F_2 populations were grown. The leachate of the nutrient solution was not reused in this study. The number of plants varied from 10-20 for parents, 9-20 for F_1s, and 50-200 for F_2 populations for each cross. The F_2 populations were randomized on the greenhouse benches. The parental and F_1 populations were interspersed among the F_2 populations to obtain the environmental effects.

All calculations regarding the means, variances, and standard errors were obtained on the individual data rather than from the condensed frequency table. The population means were compared by the use of a "t" test. The ranges of the parental populations and F_1 distribution overlap and the absence of back crosses did not allow for the partitioning procedures as described by Burton (16) and Leonard et al. (62) to obtain a quantitative estimate of the number of gene controlling the trait. Therefore, the procedure of using the mean of recessive parent and the arithmetic mean of the two parents was used to separate the low and high nitrate accumulating phenotypes.

In the use of the recessive parent mean as part of class separation, individuals greater than the mean of recessive parent (high accumulator) in the F_2 population were classified as high accumulators;

therefore, an equal number below this mean were also classified as high accumulators. The total number of plants in the F_2 less the number of high accumulators gave the number of low accumulators or the second phenotypic class.

In a second procedure, the arithmetic mean of the two parents was used as the dividing point between the low and high accumulator classes. This partitioning procedure gave results consistent with the previous procedure in all but one of the crosses (Wonder Van Voorburg x Caravan). In this cross a weighting was used to compensate for the parental overlap. The weighting procedure consisted of 100 minus the percent overlap of recessive class below the arithmetic mean of the two parents/100 $(R_o)(N)$ where R_o is the expected ratio of recessive plants based on the use of mean of recessive parent value, and N is the total number of F_2 plants. The total number of plants in the F_2 less the number of high accumulators gave the number of low accumulators. Calculation of individuals for the low and high accumulator classes were determined from the original data.

Chi-square tests were used to compare the observed and the theoretical ratios.

RESULTS AND DISCUSSION

A. Preliminary Studies

The preliminary observations on the fresh tissue showed cultivar differences in nitrate content in both nutrient regimes (Table 4). Nitrate content on the dry tissues of the selected 11 cultivars also showed cultivar differences (Table 5). The cultivar differences noted in this study are in agreement with earlier reports that lettuce cultivars differ in their capacity to accumulate nitrate (23,68,72). Increased nitrate-N content in leaves of plants grown in higher nitrate regime compared with lower nitrate regime was observed (Tables 4 and 5). This finding supports earlier reports indicating that the available nitrate-N was one of the factors in nitrate accumulation (68,92,95). These results suggested that growing the plants for four weeks using modified (standard nutrient level) Hoaglands's nutrient solution was adequate for the inheritance of nitrate accumulation study.

Total-N

Total-N in the leaf tissue was ascertained to understand the relationship between nitrate-N and total-N. The total-N content of four week old leaf tissues of the two nutrient regimes did not differ, but the total-N content of the eight week old tissues from plants grown at high nutrient regime was greater than those grown in the standard nutrient regime ($P = 0.05$, Table 6).

Table 4: Nitrate-N (percent NO_3-N) content of fresh leaves from 66 lettuce cultivars grown in two nitrogen nutrient regimes for eight weeks.

Cultivar	Nutrient Regime* 1	2
Agilo	0.035	0.054
Amplus	0.050	0.068
Arlon	0.027	0.066
Avoncrisp	0.034	0.053
Avondefiance	0.024	0.074
Blondine	0.029	0.070
Bourguignonne	0.028	0.066
Brioso	0.043	0.070
Calicel	0.026	0.064
Calmar	0.032	0.056
Caravan	0.052	0.084
Climax	0.034	0.050
Deciso	0.029	0.051
Delta	0.034	0.057
Empire	0.042	0.050
E 9201A	0.028	0.044
Fairton	0.026	0.054
Great Lakes	0.031	0.086
Grand Rapids	0.028	0.055
Groso	0.027	0.066
Hilde	0.026	0.068
Interex	0.030	0.072
Ithaca	0.052	0.079
Kloek	0.032	0.066
Knap	0.042	0.079
Kordaat	0.055	0.063
Korrekt	0.039	0.074
Kwiek	0.032	0.066
Larganda	0.034	0.053
Liba	0.048	0.066
Magiola	0.048	0.060
Marquette	0.056	0.067
May Princess	0.038	0.068
Montemar	0.028	0.050
M.S.U. 71-146	0.042	0.066
Portato	0.024	0.040
Proeftuin's Blackpool	0.028	0.047
Rapide	0.030	0.090
Red Tipped Boston	0.029	0.061

continued

Table 4--continued

Cultivar	Nutrient Regime* 1	2
Regina	0.029	0.095
Resistent	0.028	0.075
Secura	0.020	0.058
Solito	0.034	0.083
Spartan Lakes	0.037	0.060
Sucrine	0.029	0.086
Suzan	0.026	0.051
Texas 635- 1967-68	0.034	0.078
Texas 637- 1967-68	0.026	0.054
Texas 641- 1967-68	0.032	0.060
Texas 642- 1967-68	0.025	0.047
Texas 644- 1967-68	0.024	0.048
Tinto	0.029	0.068
Tonika	0.030	0.068
Type 57	0.016	0.054
Type 69	0.030	0.045
Valmaine 67074	0.014	0.058
Valmaine (Weslaco)	0.017	0.051
Valore	0.043	0.066
Valrio	0.036	0.056
Valtemp	0.025	0.060
Valverde	0.039	0.048
Vanmax	0.027	0.056
Variety A (Weslaco)	0.022	0.074
Variety B (Weslaco)	0.022	0.051
Ventura	0.034	0.050
Wonder Van Voorburg	0.016	0.034
Zomerkoningin	0.028	0.068
Mean	0.032	0.062**

*1 Plants grown in modified Hoagland solution containing standard nutrient concentration (Table 2).
2 Plants grown in modified Hoagland solution containing 2 $Ca(NO_3)_2 4 H_2O$ + 2 (KNO_3).

**Means compared by a t-test (P = 0.01).

Table 5: Nitrate-N content (percent NO_3-N on dry weight) of 11 lettuce cultivars grown in two nutrient N regimes and harvested at two growth stages.

Cultivar	Nutrient Regime[z] 1 Age 4 wk[x]	1 Age 8 wk	2 Age 4 wk[x]	2 Age 8 wk	Overall mean per nutrient regime 1	Overall mean per nutrient regime 2
Carvan	1.49g	0.44	1.56e	1.61	0.965	1.585
Kordaat	1.01ef	0.90	1.87f	1.25	0.955	1.560
Korrekt	0.63bc	0.66	1.15cd	1.25	0.640	1.200
Ithaca	1.19f	0.68	1.34de	1.45	0.935	1.395
Marquette	1.09ef	0.70	1.16cd	1.76	0.895	1.460
Spartan Lakes	0.74cd	0.45	1.07c	1.29	0.595	1.180
Type-57	0.97ef	0.49	1.4e	1.8	0.730	1.600
Valmaine-67074	0.35a	0.24	0.66ab	0.88	0.295	0.770
Valmaine-Weslaco	0.48ab	0.14	0.53ab	0.98	0.31	0.755
Valore	0.95de	0.70	1.39e	1.78	0.825	1.585
Valverde	0.96de	0.42	1.18cd	1.2	0.690	1.190
Wonder Van Voorburg	0.46ab	0.26	0.58ab	0.92	0.360	0.750
Mean[y]	0.860**	0.507	1.575[ns]	1.348	0.683**	1.253

[z]Nutrient regime 1--modified Hoagland solution.
Nutrient regime 2--modified Hoagland solution with $2(KNO_3) + 2\ Ca(NO_3)_2 4H_2O$.

[x]Mean separation within column by L.S.D. (P = 0.05).

[y]Treatment means within a nutrient regime.

**Significant (t-test) 1% level.

[ns]Not significant 5% level.

Table 6: Total nitrogen (percent N) of leaf tissues of lettuce cultivars grown in two N regimes and harvested at four and eight week stage.

Cultivar	Growth Stage: 4 week old 1[z]	4 week old 2	8 week old 1	8 week old 2	Nitrate Content
Caravan	4.67	4.26	3.12	4.54	High (H)
Kordaat	4.7	5.38	3.74	4.42	H
Korrekt	-	-	4.25	5.14	Med. M
Ithaca	-	4.65	3.92	4.56	H
Marquette	4.86	4.98	3.67	4.49	H
Spartan Lakes	4.76	4.27	3.94	4.52	M
Type - 57	-	-	3.98	5.78	H
Valmaine - 67-74	4.25	4.29	3.72	4.3	Low L
Valmaine-Weslaco	4.1	4.53	3.32	4.02	L
Valore	-	-	4.46	5.11	H
Valverde	-	-	3.88	4.56	H
Wonder Van Voorberg	4.54	4.88	4.63	5.38	L
Mean	4.55[ns]	4.66	3.89*	4.74	

*Mean values within a growth stage compared by a t-test (P = 0.05).

[z]1. Plants grown in modified Hoagland's nutrient solution (Table 2).
2. Plants grown in modified Hoagland's nutrient solution with $2(KNO_3)$ + 2 $Ca(NO_3)_2 4H_2O$.

-Not enough tissue for analysis.

[ns]Not significant at 5% level.

The total-N of four week old tissues appeared similar at both nutrient regimes, suggesting the plants at this stage had sufficient N in both nutrient regimes, since the uptake of N was similar.

The greater total-N in eight week old leaf tissues from plants grown in the higher nutrient regime appeared to be due to the greater N availability in the media. In the high nutrient regime, nitrate was 26.5% of the total N and at the standard nutrient regime it was 17.6% of the total N. This finding is analogous to an earlier report (99), where lettuce continued to accumulate nitrate with age. This finding also suggests that the available nitrate-N is one of the factors in nitrate-N accumulation.

The total-N in the lettuce leaf tissues of subsequent plantings were not determined since both low and high accumulators of nitrate-N appeared to be similar in their total-N at four week stage. The total-N of eight week old leaf tissues appear to differ among cultivars but did not suggest a relationship between the nitrate accumulation and total-N content.

Other Elements

Other elements were determined on the eight week old leaf tissues for each cultivar grown in the standard nutrient regime to learn if the low and high nitrate accumulation would also differ in other elements (Table 7). Element content of eight-week old leaf tissues on 11 cultivars grown at the two nutrient levels are shown in Table 8. The leaf tissues from the high nutrient regime was greater than those of standard nutrient regime in K, but was lower in P and Mg and B ($P = 0.05$).

Table 7: Element analyses of eight week old leaf tissues of lettuce cultivars grown in the modified Hoagland solution (Table 2) containing standard N, K and Ca concentrations.

No.	Cultivar	K	P	Na	Ca	Mg	Mn	Fe	Cu	B	Zn	Al
		----------%----------					----------ppm----------					
1.	Agilo	5.60	0.45	0.85	2.38	0.82	101.0	283.0	21.0	58.3	44.0	138.0
2.	Amplus	6.14	0.59	1.11	2.29	0.75	104.0	321.0	23.3	48.9	51.0	127.0
3.	Arlon	6.30	0.63	1.11	2.64	0.84	107.0	452.0	22.6	58.3	58.0	111.0
4.	Avoncrisp	5.30	0.48	0.71	2.38	0.82	107.0	274.0	19.5	55.5	44.0	95.0
5.	Avondefiance	5.60	0.45	1.15	2.71	0.97	109.0	239.0	16.4	63.1	44.0	132.0
6.	Blondine	5.74	0.57	0.88	2.74	0.86	89.0	325.0	20.3	55.5	42.0	136.0
7.	Bourguignonne	6.76	0.55	0.93	2.51	0.84	95.0	261.0	19.5	52.7	44.0	111.0
8.	Brioso	5.30	0.52	0.88	2.32	0.80	98.0	302.0	20.3	49.9	49.0	111.0
9.	Calicel	5.02	0.23	0.52	2.26	0.86	89.0	159.0	13.2	34.0	38.0	74.0
10.	Calmar	4.88	0.32	0.75	2.58	0.82	86.0	236.0	21.0	36.8	51.0	202.0
11.	Caravan	4.84	0.38	0.48	1.75	0.67	81.0	196.0	19.5	37.7	35.0	90.0
12.	Climax	6.14	0.39	0.81	2.58	0.89	109.0	252.0	18.7	53.6	44.0	127.0
13.	Deciso	6.14	0.48	0.73	2.23	0.81	107.0	274.0	19.5	60.2	44.0	127.0
14.	Delta	6.26	0.45	0.97	2.45	0.86	104.0	258.0	18.0	54.6	44.0	116.0
15.	Empire	4.88	0.22	0.52	1.98	0.71	86.0	165.0	15.6	34.9	42.0	74.0
16.	E 9201A	5.16	0.42	0.64	1.84	0.73	72.0	171.0	10.8	31.2	26.0	53.0
17.	Fairton	4.40	0.21	0.69	1.84	0.75	72.0	174.0	13.2	31.2	31.0	100.0
18.	Great Lakes	4.64	0.21	0.80	2.51	0.89	81.0	177.0	14.8	33.1	35.0	79.0
19.	Grand Rapids	5.60	0.41	0.81	2.32	0.81	92.0	270.0	18.0	49.9	49.0	116.0
20.	Groso	5.60	0.46	0.72	2.23	0.83	95.0	230.0	17.2	50.8	38.0	116.0
21.	Hilde	4.88	0.41	0.88	2.67	0.99	107.0	196.0	21.0	59.3	51.0	181.0
22.	Interrex	5.74	0.48	0.97	2.81	0.94	107.0	325.0	24.1	63.1	51.0	122.0
23.	Ithaca	5.16	0.25	0.76	2.87	0.91	101.0	261.0	16.4	34.9	44.0	79.0
24.	Kloek	5.16	0.54	0.73	2.84	0.86	104.0	383.0	20.3	59.3	44.0	111.0

continued

Table 7--continued

No.	Cultivar	K	P	Na	Ca	Mg	Mn	Fe	Cu	B	Zn	Al
		----------	----------	%	----------	----------	----------	----------	ppm	----------	----------	----------
25.	Knap	5.84	0.52	0.85	2.67	0.87	124.0	299.0	19.5	61.2	49.0	111.0
26.	Kordaat	6.80	0.48	0.78	2.42	0.82	112.0	312.0	30.7	60.2	58.0	159.0
27.	Korrekt	6.26	0.55	0.82	2.23	0.78	112.0	305.0	22.6	53.6	51.0	106.0
28.	Kwiek	5.74	0.51	0.84	2.58	0.87	118.0	283.0	20.3	62.1	54.0	143.0
29.	Larganda	5.30	0.41	0.92	2.45	0.86	107.0	252.0	27.8	56.5	51.0	122.0
30.	Liba	5.74	0.36	0.97	2.45	0.89	107.0	280.0	18.7	58.3	44.0	116.0
31.	Margiola	5.84	0.48	0.78	2.32	0.82	112.0	318.0	24.1	56.5	51.0	116.0
32.	Marquette	4.88	0.23	0.75	2.32	0.83	83.0	180.0	13.2	32.2	31.0	106.0
33.	May Princess	5.84	0.46	0.69	2.20	0.78	95.0	239.0	15.6	49.9	42.0	100.0
34.	Montemar	4.76	0.19	0.64	2.07	0.87	84.0	159.0	12.4	34.9	28.0	74.0
35.	M.S.U. 71-146	4.52	0.28	0.75	3.21	0.87	92.0	299.0	15.6	36.8	47.0	79.0
36.	Portato	4.76	0.34	0.98	2.14	0.82	86.0	252.0	21.0	39.9	51.0	79.0
37.	Proeftuin's Blackpool	5.60	0.50	0.69	2.23	0.75	98.0	258.0	15.6	52.7	42.0	84.0
38.	Rapida	4.88	0.38	0.65	2.04	0.71	81.0	257.0	21.0	46.1	44.0	116.0
39.	Red Tipped Boston	4.88	0.26	0.63	1.81	0.66	66.0	186.0	12.4	29.4	28.0	63.0
40.	Regina	5.16	0.34	0.81	2.32	0.83	83.0	258.0	15.6	49.9	40.0	106.0
41.	Resistent	5.02	0.36	0.75	2.58	0.89	89.0	227.0	14.8	52.7	44.0	106.0
42.	Secira	4.88	0.37	0.71	1.89	0.71	81.0	202.0	16.4	48.0	42.0	79.0
43.	Solito	5.16	0.42	0.81	2.45	0.84	101.0	318.0	24.1	54.6	54.0	208.0
44.	Spartan Lakes	4.76	0.33	0.59	2.26	0.84	83.0	223.0	13.2	46.1	38.0	68.0
45.	Sucrine	4.76	0.25	0.69	1.84	0.68	109.0	156.0	12.4	46.1	32.0	66.0
46.	Suzan	5.16	0.39	0.62	2.32	0.87	83.0	239.0	18.0	51.8	47.0	95.0
47.	Texas 635 67-68	5.84	0.37	0.84	2.87	0.93	107.0	296.0	15.6	55.5	49.0	90.0
48.	Texas 637 67-68	5.42	0.47	1.19	3.46	1.01	127.0	357.0	24.8	68.7	70.0	111.0
49.	Texas 641 67-68	5.84	0.45	0.57	2.14	0.71	86.0	270.0	14.8	42.4	44.0	68.0
50.	Texas 642 67-68	5.02	0.38	0.59	2.14	0.49	61.0	183.0	8.4	31.2	26.0	53.0

continued

Table 7--continued

No.	Cultivar	K	P	Na	Ca	Mg	Mn	Fe	Cu	B	Zn	Al
		----------%----------					----------ppm----------					
51.	Texas 644 67-68	5.02	0.36	0.64	2.29	0.81	95.0	286.0	21.0	46.1	49.0	122.0
52.	Tinto	5.30	0.47	0.63	2.01	0.72	83.0	334.0	18.0	47.1	51.0	68.0
53.	Tonika	5.30	0.37	0.95	2.32	0.89	95.0	302.0	21.0	56.5	51.0	106.0
54.	Type 57	5.30	0.32	0.97	2.14	0.80	107.0	270.0	30.0	63.1	61.0	154.0
55.	Type 69	6.00	0.42	0.82	2.71	0.86	112.0	321.0	21.8	53.6	58.0	100.0
56.	Valmaine 67074	5.02	0.27	0.50	1.69	0.67	72.0	217.0	13.2	34.9	31.0	68.0
57.	Valmaine (Weslaco)	4.64	0.23	0.58	1.88	0.71	95.0	147.0	18.0	36.8	49.0	106.0
58.	Valore	6.60	0.55	1.04	2.81	0.88	127.0	325.0	28.5	57.4	70.0	132.0
59.	Valrio	5.30	0.30	0.59	2.20	0.86	101.0	211.0	24.1	48.9	49.0	267.0
60.	Valtemp	6.26	0.35	0.73	2.42	0.71	104.0	255.0	22.6	45.2	54.0	106.0
61.	Valverde	4.88	0.31	0.67	2.71	0.89	92.0	227.0	18.7	41.5	44.0	289.0
62.	Vanmax	4.88	0.19	0.97	1.95	0.73	75.0	156.0	13.2	34.0	26.0	79.0
63.	Variety A (Weslaco)	5.30	0.30	0.51	2.07	0.69	78.0	214.0	26.3	44.3	47.0	181.0
64.	Variety B (Weslaco)	5.6	0.37	0.76	2.26	0.87	101.0	277.0	30.7	50.8	49.0	289.0
65.	Ventura	6.4	0.61	0.75	2.94	0.75	130.0	354.0	24.8	50.8	68.0	132.0
66.	Wonder Van Voorburg	5.3	0.46	0.75	2.45	0.87	101.9	227.0	18.7	50.8	58.0	111.0
67.	Zomerkoningin	5.84	0.41	0.59	2.81	0.83	101.0	242.0	18.0	56.5	51.0	95.0

Table 8: Element contents of eleven selected cultivars grown in two nutrient regimes.

1. Plants grown in modified Hoagland solution containing standard N, K and Ca concentrations.

Cultivar	K	P	Na	Ca	Mg	Mn	Fe	Cu	B	Zn	Al	Nitrate content
	----------		%----------			----------		ppm----------				
Caravan	4.84	0.38	0.48	1.75	0.67	81.0	196.0	19.5	37.7	35.0	90.0	H
Ithaca	5.16	0.25	0.76	2.87	0.91	101.0	261.0	16.4	34.9	44.0	79.0	H
Kordaat	6.80	0.48	0.78	2.42	0.82	112.0	312.0	30.7	60.2	58.0	159.0	H
Korrekt	6.26	0.55	0.82	2.23	0.78	112.0	305.0	22.6	53.6	51.0	106.0	M
Marquette	4.88	0.23	0.75	2.32	0.83	83.0	180.0	13.2	32.2	31.0	106.0	H
Spartan Lakes	4.76	0.33	0.59	2.26	0.84	83.0	223.0	13.2	46.1	38.0	68.0	M
Type 57	5.30	0.32	0.97	2.14	0.80	107.0	270.0	30.0	63.1	61.0	154.0	H
Valmaine 67074	5.02	0.27	0.50	1.69	0.67	72.0	217.0	13.2	34.9	31.0	68.0	L
Valmaine-Weslaco	4.64	0.23	0.59	1.86	0.71	95.0	147.0	18.0	36.8	49.0	106.0	L
Valore	6.60	0.55	1.04	2.81	0.88	127.0	325.0	28.5	57.4	70.0	132.0	H
Valverde	4.88	0.31	0.67	2.71	0.89	92.0	227.0	18.7	41.5	44.0	289.0	H
Wonder Van Voorberg	5.30	0.46	0.75	2.45	0.87	101.0	227.0	18.7	50.8	58.0	111.0	L
$\bar{X}$	5.37*	0.37*	0.73	2.29^{NS}	0.81*	97.0^{NS}	241.0^{NS}	20.23^{NS}	45.77*	47.5^{NS}	122.0	

continued

Table 8—continued

2. Plants grown in modified Hoagland solution containing $2Ca(NO_3)_2 4H_2O$ and $2KNO_3$.

Cultivar	K	P	Na	Ca	Mg	Mn	Fe	Cu	B	Zn	Al	Nitrate content
	----------%----------					----------ppm----------						
Caravan	7.40	0.19	0.75	2.29	0.67	83.0	186.0	19.5	32.2	42.0	116.0	H
Ithaca	6.76	0.23	0.59	2.35	0.65	86.0	223.0	25.6	30.3	47.0	213.0	H
Kordaat	7.08	0.18	0.72	2.26	0.53	69.0	208.0	18.7	32.2	47.0	74.0	H
Korrekt	6.50	0.23	0.84	2.38	0.64	83.0	196.0	18.0	38.7	44.0	84.0	M
Marquette	6.76	0.19	0.57	1.89	0.58	72.0	162.0	19.5	27.5	35.0	100.0	H
Spartan Lakes	6.58	0.18	0.85	2.32	0.60	78.0	174.0	18.0	30.3	44.0	68.0	M
Type 57	7.20	0.23	0.78	2.32	0.56	83.0	239.0	21.0	34.9	49.0	95.0	H
Valmaine 67074	5.42	0.15	0.46	1.55	0.43	49.0	90.0	11.6	21.1	22.0	58.0	L
Valmaine-Weslaco	6.00	0.13	0.38	1.69	0.46	69.0	105.0	18.0	24.7	42.0	58.0	L
Valore	8.32	0.26	0.63	1.92	0.51	63.0	199.0	16.4	26.6	38.0	74.0	H
Valverde	6.76	0.18	0.42	1.89	0.54	63.0	159.0	17.2	26.6	35.0	90.0	H
Wonder Van Voorberg	6.50	0.20	0.59	2.01	0.54	66.0	174.0	18.7	26.6	42.0	63.0	L
$\bar{X}$	6.76	0.19	0.63	2.07	0.56	72.0	176.0	17.68	29.31	41.0	91.0	

*Mean values within a column compared by a t-test (P = 0.05).

NSNot significant at 5% level.

Ca and other micro nutrients of leaf tissues were similar for both nutrient regimes.

The higher K in leaf tissues of plants grown at the high nutrient regime was probably due to the greater availability of K in the media. Calcium contents were similar in leaf tissues at both nutrient regimes although the Ca content was twice as high in the high nutrient regime. A decrease in P content with increased N was also noted with beet leaf blades and spinach leaves (17). A decrease in Mg content of tomato shoot tissue was also reported by Wilcox et al. (94) when the plants were grown with nitrate-N in the media for seven days as compared with three day treatment.

Micro elements content, except B, were similar in leaf tissues from both nutrient regimes; this was probably due to similar concentrations in both nutrient solutions.

The other elements were not determined in the subsequent plantings, since the preliminary observations did not show a marked difference in the elements between the low and high nitrate accumulating cultivars as a group.

Fresh and Dry Weights

Fresh and dry weights of leaves and fresh weight of roots were determined on the four week old plants of various cultivars grown at both nutrient regimes (Table 9).

Analysis of variance of the fresh weight of leaves, suggested cultivar differences in each nutrient regime. The fresh weight of roots

Table 9: Mean fresh weight of leaf, root and dry weight of leaf of four week old lettuce plants grown in two nutrient regimes for the cultivars used in NO_3-N study.

	Plant Part					
	Fresh				Dry	
	Leaf[y]		Root		Leaf	
	Nutrient Regime[z]					
Cultivar	1	2	1	2	1	2
Agilo	20.95	22.79	7.775	8.675	0.9988	1.1425
Amplus	29.00	24.62	9.100	6.865	1.3275	1.2525
Arlon	24.96	15.86	6.925	5.065	1.3650	0.9825
Avoncrisp	25.95	16.37	8.690	5.025	1.0988	0.9100
Avondefiance	19.71	11.12	6.305	3.205	0.8225	0.4575
Blondine	24.59	21.13	7.190	5.780	1.1725	1.1063
Bourguignonne	21.29	20.83	6.450	6.930	1.1050	1.0210
Brioso	20.34	20.93	6.100	5.580	0.9188	0.9688
Calicel	22.73	16.08	4.705	4.950	1.2304	1.2350
Calmar	25.55	12.73	3.990	3.300	1.0775	0.7867
Caravan	27.42	26.17	6.615	6.400	1.2013	1.1875
Climax	22.70	24.73	5.265	5.415	1.0800	1.2938
Deciso	28.38	18.92	6.355	5.030	1.2475	1.0435
Delta	23.08	17.78	8.250	6.050	1.2555	1.1025
Empire	23.38	13.90	4.515	3.300	1.2325	0.8300
E 9201 A	11.64	9.48	4.725	3.365	0.6863	0.5142
Fairton	21.57	20.59	4.425	5.315	1.0388	1.0925
Great Lakes	21.79	24.10	3.155	4.380	1.0963	1.4113
Grand Rapids	30.12	23.20	7.290	5.850	1.3900	1.2113
Groso	22.40	17.03	5.515	6.275	1.1713	0.9988
Hilde	26.73	24.88	7.975	8.005	1.5363	1.6125
Interex	23.55	23.40	6.725	7.000	1.3913	1.3475
Ithaca	22.36	27.93	3.815	4.900	1.0450	1.3913
Kloek	26.37	22.70	5.775	6.140	1.4113	1.2938
Knap	23.82	32.08	4.915	8.230	1.2513	1.7838
Kordaat	22.67	25.79	5.325	6.405	1.2325	1.3113
Korrekt	22.22	29.43	6.100	8.840	1.3555	1.5625
Kwiek	26.22	23.63	6.655	7.465	1.4713	1.4588
Larganda	26.48	24.79	6.230	7.525	1.7635	1.7325
Liba	21.12	18.68	5.590	4.675	0.9550	1.0975
Magiola	24.48	21.55	4.230	6.825	1.3525	1.2813
Marquette	26.47	21.48	4.100	4.740	1.2913	1.2050
May Princess	21.80	24.52	6.865	8.050	1.0290	1.2888
Montemar	13.99	11.72	2.950	4.415	0.7163	0.6400
M.S.U. 71-146	20.14	20.79	3.780	4.325	1.0575	1.0825
Portato	21.07	16.76	6.465	6.390	1.1588	1.2713
Proeftuin's Blackpool	21.28	20.36	7.515	7.250	1.2621	1.1788

continued

Table 9--continued

Cultivar	Plant Part: Fresh Leaf[y] Nutrient Regime[z] 1	Fresh Leaf 2	Fresh Root 1	Fresh Root 2	Dry Leaf 1	Dry Leaf 2
Rapide	20.75	17.62	4.205	3.765	1.0863	1.0463
Red Tipped Boston	19.04	15.07	5.315	3.690	1.0713	0.9613
Regina	20.58	18.03	6.100	6.150	1.2488	1.2238
Resistent	21.19	17.99	5.415	5.555	1.2350	1.1000
Secura	21.15	17.62	5.640	5.600	1.1225	1.0450
Solito	21.12	22.88	5.750	6.625	1.1663	1.4813
Spartan Lakes	17.72	22.54	4.005	5.355	1.9725	1.3625
Sucrine	17.37	21.30	5.015	7.680	1.1000	1.4500
Suzan	19.50	20.53	5.540	6.940	1.1875	1.4700
Texas 635-1967-68	22.67	21.70	6.525	6.015	1.3325	1.2675
Texas 637-1967-68	20.13	20.90	6.015	7.415	1.1113	1.4763
Texas 641-1967-68	15.27	22.84	4.380	6.950	0.8525	1.4125
Texas 642-1967-68	23.25	14.58	7.300	5.430	1.4325	1.1400
Texas 644-1967-68	22.57	18.54	6.490	5.840	1.2625	1.3363
Tinto	20.59	20.78	5.505	5.950	1.0338	1.2025
Tonika	25.09	22.73	7.460	4.240	1.3629	1.2263
Type 57	24.23	21.88	6.350	5.775	1.2488	1.1913
Type 69	21.72	21.25	6.375	4.890	1.2325	1.1163
Valmaine 67074	21.03	22.62	6.780	7.500	1.3700	1.6025
Valmaine (Weslaco)	25.53	19.27	6.630	6.985	1.6038	1.6013
Valore	22.38	25.94	5.125	6.675	0.9800	1.3838
Valrio	18.17	15.80	5.530	2.625	0.9650	0.5950
Valtemp	25.12	14.27	8.440	5.025	1.4050	1.0225
Valverde	23.88	21.34	4.830	5.980	1.2489	1.0388
Vanmax	19.96	12.14	3.017	4.430	1.0350	0.6975
Variety A (Weslaco)	18.73	18.13	5.340	6.565	1.1388	1.1563
Variety B (Weslaco)	20.54	17.50	6.500	4.940	1.1088	0.8325
Ventura	17.40	19.37	5.790	5.275	0.9200	1.0850
Wonder Van Voorburg	19.44	17.41	5.725	5.680	1.1238	1.2125
Zomerkonigin	22.78	21.45	7.055	7.115	1.1825	1.3775
Mean	22.20	20.17	5.858	5.829	1.1923	1.1823

[y]Mean separation LSD 0.05 = 3.79.

[z]1. Plants were grown using modified Hoagland solution (Table 2).
2. Plants were grown using modified Hoagland solution containing 2 Ca $(NO_3)_2 4H_2O$ + 2 KNO_3.

and dry weight of leaves for the two nutrient regimes was similar. The fresh leaf weight from the standard nutrient regime was greater ($P = 0.05$) than those grown from a higher nutrient regime (Table 9).

Preliminary observations on the nitrate-N content and yield (fresh weight) for the various cultivars failed to show a relationship between these two variables at four weeks of age.

B. Genetic Experiments--1974

Based on the results of preliminary screening, ten lettuce cultivars were selected for further studies based on their low (L), medium (M), and high (H) nitrate content. The mean nitrate-N contents of these cultivars and their reciprocal crosses are summarized in Table 10.

Although the results from the preliminary studies suggested that cultivars may be classified as low, intermediate and high nitrate-N accumulators (Tables 4 and 5) in 1974, the medium and high accumulators could not be separated from each other. The nitrate-N content of Valmaine (L) and Wonder Van Voorburg (L) were lower than Caravan (H), Kordaat (H), Ithaca (H), Marquette (H), Korrekt (H), Valore (H), Valverde (H) and Type-57 (H) (Table 10) in 1974 as in the previous year.

The nitrate-N contents of F_1 plants from low x high nitrate crosses were not significantly different from the low accumulator parent which suggests a dominance for low nitrate accumulation. Nitrate-N content of F_1 plants from low x low crosses was low and did not differ significantly from the parents. Results from crosses involving only high nitrate accumulators were variable.

Table 10: Mean nitrate-N (percent NO_3-N on dry weight) of ten cultivars and their reciprocal crosses.

Generation		No. of Plants	Mean Nitrate-N[y]	s^2	Nitrate content
Valmaine 67074	P_1	4	1.0494	0.0631	L
Valmaine Weslaco	P_2	6	0.7733	0.0233	L
Wonder Van Voorburg	P_3	6	1.1567	0.0396	L
Caravan	P_4	6	1.6867	0.1742	H
Kordaat	P_5	4	1.9181	0.0978	H
Ithaca	P_6	4	1.9663	0.2181	H
Marquette	P_7	3	1.7442	0.1890	H
Korrekt	P_8	4	1.6765	0.0075	H
Valore	P_9	6	1.7133	0.0886	H
Valverde	P_{10}	4	1.8250	0.0081	H
Type-57	P_{11}	4	1.8844	0.0762	H
					Cross
F_1	P_1xP_3	7	0.6370	0.0296	LxL
	P_3xP_1	8	0.6635	0.0052	LxL
	P_4xP_1	4	1.0906	0.0135	HxL
	P_1xP_5	4	0.9506	0.3130	LxH
	P_5xP_1	8	0.9763	0.033	HxL
	P_1xP_6	4	0.9370	0.0022	LxH
	P_6xP_1	3	0.9225	0.0515	HxL
	P_1xP_7	4	1.0850	0.0578	LxH
	P_1xP_8	4	0.9700	0.0076	LxH
	P_8xP_1	4	1.0500	0.0430	HxL
	P_1xP_{11}	4	0.9900	0.0587	LxH
	$P_{11}xP_1$	4	0.8500	0.0296	HxL

continued

Table 10--continued

Generation	Cross	No. of Plants	NO_3-N	s^2	Cross
F_1	P_2xP_3	6	0.6100	0.0084	LxL
	P_3xP_2	6	0.5967	0.0109	LxL
	P_4xP_2	3	1.1333	0.0702	HxL
	P_2xP_5	4	0.9050	0.0111	LxH
	P_5xP_2	4	0.8009	0.0003	HxL
	P_2xP_6	4	0.9788	0.0958	LxH
	P_6xP_2	4	0.8748	0.0785	HxL
	P_2xP_7	4	1.1019	0.0282	LxH
	P_2xP_8	4	0.8965	0.0100	LxH
	P_8xP_2	4	0.9841	0.1044	HxL
	P_2xP_9	6	0.7567	0.0036	LxH
	P_9xP_2	5	0.7520	0.0091	HxL
	P_4xP_3	6	0.9100	0.0150	HxL
	P_3xP_4	2	0.8913	0.0330	LxH
	P_3xP_6	4	0.8044	0.0016	LxH
	P_6xP_3	4	0.8880	0.0242	HxL
	P_3xP_9	4	1.300	0.0355	LxH
	P_9xP_3	4	1.2850	0.0404	HxL
	P_4xP_5	4	1.1583	0.0790	HxH
	P_4xP_6	4	2.2678	0.1045	HxH
	P_5xP_7	4	1.2330	0.0039	HxH
	P_7xP_5	4	1.235	0.0490	HxH
	P_5xP_8	4	2.238	0.1997	HxH
	P_8xP_5	4	2.1675	0.0970	HxH
	P_6xP_7	4	1.7131	0.1331	HxH
	P_7xP_6	4	1.6718	0.1175	HxH

continued

Table 10--continued

Generation	Cross	No. of Plants	NO_3-N	s^2	Cross
F_1	P_9xP_5	4	1.6718	0.1829	HxH
	P_9xP_8	4	1.9650	0.0596	HxH
	$P_{10}xP_9$	4	1.1781	0.0200	HxH

[y]Mean comparison, modified Tukey's method.

To compare two means differing in their sample size use lower 'n' value, for example P_1 = 1.0494, n = 4 and P_2 = 0.7733, n = 6. The minimum significant value (n =4) = 0.4831.

The two P_1 and P_2 means do not differ by this value, therefore, these means are not significantly different at the 5% level.

n minimum significant difference

2 = 0.4831

3 = 0.3945

4 = 0.34164

5 = 0.20596

6 = 0.27896

7 = 0.2583

The F_1 generation of three of eight such crosses [Caravan (P_4) x Kordaat (P_5), Kordaat (P_5) x Marquette (P_7), and Valverde (P_{10}) x Valore (P_9) Table 5] showed a lower nitrate-N content than either parent. Low accumulation trait of the F_1 generation resulting from two high accumulator parents suggests that the F_1 plant contained two or more dominant alleles, each locus probably controlled an enzymatic step essential for low nitrate accumulation.

The nitrate-N contents of five other high x high F_1 plants remained high and were similar to their parents (Table 10). Nitrate-N contents of reciprocal crosses were not significantly different.

Variations in Nitrate-N Content of Lettuce Cultivars at Different Plantings

The nitrate content of parental lines which appeared to be medium or high accumulators was variable from year to year. For example, cultivar Korrekt, Type-57, Valore and Valverde appeared to be medium or medium-high accumulators in 1973, but all were found to be high accumulators in 1974 (Table 11). Cultivar Ithaca which appeared to be a high accumulator in the 1974 and 1974 planting, was found to have medium levels of nitrate in 1975. Based on this information it was assumed that the medium and high accumulators were probably the same and the inconsistency observed was due to the sensitive nature of the trait. The difference between the cultivars designated low and high was consistent for the three years of testing.

The mean nitrate content was lowest in 1973 and highest in 1975, this was due to the increased frequency of watering the plants with nutrient solution.

Table 11: Leaf nitrate-N (percent) content of various cultivars grown in sand culture using modified Hoagland solution on four week old dry tissues for three years.

Cultivar	1973	1974	1975
Caravan	1.49	1.6867	2.0233
Ithaca	1.19	1.9663	1.6075
Kordaat	1.01	1.9181	2.2700
Korrekt	0.63	1.6765	-
Marquette	1.09	1.7442	-
Type-57	0.97	1.8844	-
Valmaine-Weslaco	0.48	0.7733	1.2525
Valmaine-67074	0.35	1.0494	-
Valore	0.95	1.7133	2.0525
Valverde	0.96	1.8250	-
Wonder Van Voorburg	0.46	1.1567	1.4038
Mean	0.871	1.581	1.768

Individual Crosses--1975

Nitrate in plant tissue is always in a dynamic state since it represents the difference between absorption and nitrate reduction (assimilation). Nitrate absorbed can also move into or out of a particular plant part. Hence, one factor may alter tissue nitrate concentrations by affecting any one or all the processes of absorption, assimilation, and translocation (68). For example, factors such as nitrate availability (68,92,95), light (9,68), shade (17), temperature, and other factors (9,68,92,95) have been known to influence the tissue nitrate-N content. To avoid the diurnal fluctuations in tissue nitrate levels, all the lettuce plants were harvested at one time under dark conditions. Thus the nitrate measured was the excess unreduced portion at that time. Although every effort was taken to obtain a uniform environment, the parental lines showed a wide range in their nitrate content.

The mean nitrate-N contents of parents and F_1 plants are shown in Table 12. The low nitrate accumulator lines used were Valmaine and Wonder Van Voorburg. The high accumulator parents were Caravan, Kordaat and Valore (Table 12). The cultivar Ithaca which was used in one cross was a medium accumulator in 1975 but was high in 1974 and 1973 (Table 11).

The nitrate-N content of F_1 populations of crosses low x low and low x high were low accumulators. The reciprocal crosses did not differ significantly and therefore were pooled.

Table 12: Mean nitrate-N (percent NO_3-N) content (dry weight) of parents and F_1 plants of crosses used in the genetic analyses of nitrate-N study.

Parent Lines	Percent NO_3-N [z]
P_1 Valmaine	1.2525 a
P_2 Wonder Van Voorburg	1.4038 ab
P_3 Caravan	2.0233 c
P_4 Kordaat	2.2700 c
P_5 Ithaca	1.6075 b
P_6 Valore	2.0525 c
$\underline{F_1 \text{ Population}}$	
$P_1 x P_2$ [y]	1.0355
$P_1 x P_3$	1.5475
$P_1 x P_4$	1.2292
$P_1 x P_6$	1.1825
$P_2 x P_3$	1.4275
$P_2 x P_6$	1.4675

[z] Mean separation within the column (t-test 5% level).

[y] Reciprocal crosses included.

Cross: Valmaine (P_1) x Caravan (P_3) (Low x High)

In order to obtain the best estimate of the gene number involved in the crosses, two partitionint procedures were considered to separate the phenotypes. The first procedure consisted of using the recessive parental mean. Plants in classes greater than the recessive parental mean in the F_2 population were classified as high accumulators, and an equal number of plants lower than this mean was also classified as high accumulators. The total number of plants in the F_2 less the number of high accumulators gave the number of low accumulators or the second phenotype.

In the second method the arithmetic mean of the two parents were used as the dividing point between the low and high accumulator classes.

The population means were compared by a t-test at P = 0.05. The P value of Chi-square tests indicate that the calculated value is significant at the first P value but not at the second. For example, in the cross Valmaine (L) x Caravan (H), the division of phenotypes suggested a segregation ratio of 9 Low:55 High. The calculated X^2 value for goodness of fit to the proposed 9:55 ratio was 0.6644 with a P value of 0.5-0.25. The Chi-square 0.6644 was significant at the 50% level but not at the 25% level. Therefore, the proposed ratio of 9:55 was accepted on the basis of lack of significance at 25% level.

Frequency distributions of nitrate-N content for Valmaine (P_1) Caravan (P_3), and their F_1 and F_2 populations are summarized in Table 13. The nitrate content of Caravan was greater than that of Valmaine and the F_1 generation (P = 0.05). The mean of the F_1 population (1.5475)

Table 13.

Table 13: Frequency distribution of Nitrate-N (percent NO_3-N)/plant (dry weight) for the parental, F_1 and F_2 generations of cross of Valmaine x Caravan.

	No. of Plants	Upper Limit of Class 0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	Mean[z]	s^2
Valmaine	20	1	4	3	6	3	2	1						1.2525[a]	0.0919
Caravan	15					3	1	4	1	4	-	2		2.0233[b]	0.0813
F_1	10		1	-	1	4	4							1.5475[a]	0.0710
F_2	200		2	11	10	18	30	41	49	20	6	8	5	1.9364[b]	0.1800

[z]Mean separation within column by a t-test (P = 0.05)

approximated the arithmetic mean of the two parents (1.6379). These two values were not significantly different from each other. The F_1 mean was not significantly different from Valmaine, the low accumulator, suggesting dominance toward low nitrate accumulation.

The F_2 population mean was greater (P = 0.05) than the low accumulator parent Valmaine and the F_1 but not significantly different from the high accumulator parent Caravan. The parental types were recovered in the F_2 population and a majority of the segregating population fell in the high accumulator range (Table 13).

Using the recessive parental mean to separate the two classes (low and high) the number of plants which fell beyond (greater) the mean (2.0233) in the F_2 population was calculated and multiplied by 2 to obtain the total number of high accumulators (49+20+6+8+5)2 = 176 (Table 13). The number of low accumulators is calculated by subtracting the high accumulators from the total F_2 population (200-176 = 24), thus giving a ratio of 24 low:176 high accumulators.

Since the arithmetic mean of the two parents (1.6379) approximates the F_1 mean (1.5475) and the arithmetic mean of the parents was greater (P = 0.05) than the low accumulator parent. The dividing point between the low and high accumulators appears to be between the 1.4 and 1.6 class (Table 13). The observed 23 low:177 high is similar to the previous partitioning (24:176) procedure. This observed phenotypic ratio suggests a 9:55 model. The X^2 value gave an acceptable fit to the model (P = 0.5-0.25, Table 14). The observed F_2 mean, 1.9364, approximates the expected F_2 mean of 1.9149 for a 9:55 model (P = 0.990-0.975, Table 15).

Table 14: Chi-square test for goodness of fit to the postulated model for nitrate-N data in the F_2 generation.

Cross	Nitrate-N Accumulation	Low:High[1]	Low:High[2]	Expected Low:High	Model	X^2 1	X^2 2	P^y 1	P^y 2
Valmaine x Caravan	Low x High	24:176	23:177	28:172	9:55	0.6644	1.0380	0.5-0.25	0.5-0.25
Valmaine x Kordaat	Low x High	116:84	111:89	112.5:87.5	9:7	0.2490	0.0453	0.75-0.05	0.9-0.75
Valmaine x Valore	Low x High	112:88	111:89	112.5:87.5	9:7	0.005	0.0453	0.95-0.9	0.9-0.75
Wonder Van Voorburg x Caravan	Low x High	152:48	160:40[x]	150:50	3:1	0.0900	2.625	0.9-0.75	.25-0.1
Wonder Van Voorburg x Valore	Low x High	158:42	154:46	150:50	3:1	1.707	0.455	0.25-0.1	0.5-0.25
[a]Valmaine x Wonder Van Voorburg	Low x Low	180:20	--	178:22	57:7	0.222	--	0.75-0.5	--
[a]Caravan x Ithaca	High x Medium	--	23M:27H	18.75:31.25	6:10	1.541	--	0.25-0.1	--

[z]1. Partitioning F_2 population based on the recessive parental mean value.
2. Partitioning F_2 population based on the arithmetic mean of two parents.

[x]Weighted value.

[y]The calculated X^2 values significant at the high P level but not the low P.

[a]Partitioning of F_2 population based on F_2 distribution.

Table 15: Chi-square test for goodness of fit of the F_2 mean to the postulated model.

Cross	Model	OBS	EXP	X^2	P
Valmaine x Caravan	9:55	1.9364	1.9149	0.0002	0.990-0.975
Valmaine x Kordaat	9:7	1.7868	1.6977	0.0047	0.975-0.900
Valmaine x Valore	9:7	1.6352	1.6025	0.0007	0.990-0.975
Wonder Van Voorburg x Caravan	3:1	1.5822	1.620	0.0009	0.990-0.975
Wonder Van Voorburg x Valore	3:1	1.5836	1.566	0.0002	0.990-0.975
Caravan x Ithaca	6:10	1.8445	1.7364	0.0119	0.950-0.900

Based on the F_2 progeny of the cross Valmaine x Caravan, a 3-major-gene system is suggested for the inheritance of low and high nitrate-N accumulation in this cross. The F_1 mean of low x high was low, suggesting a dominance toward low accumulation. Apparently, both parents may have contributed dominant gene(s) towards the observed F_1 phenotype. The F_2 distribution shows a unimodal distribution and is skewed towards high accumulation. The F_2 and P_3 population means were not significantly different from each other, and a major portion of the F_2 population fell in the high accumulator range suggesting that a dominant gene was contributed by the high accumulator parent. Based on the ratios observed in this cross (Table 14) the proposed genotype of Valmaine is AA BB cc and that of Caravan is aa bb CC (Table 16) where the presence of A and B genes are necessary to bring about low nitrate accumulation, and C is the dominant gene controlling high nitrate accumulation.

The F_1 genotype is Aa Bb Cc and was classified as a low accumulator, but the observed ratio of 9 low; 55 high in the F_2 population suggests that the genotypes having A-B-C, A-b-C, a-B-C, a-b-C, A-b-c, a-B-c and a-b-c are high accumulators; while, the genotype having A-B-c were classified as low accumulators. Although the F_1 generation was classified as a low accumulator, the F_1 distribution shows a large portion (80%) of its population in the parental overlap (Table 13). The F_1 mean and the arithmetic mean of the low parents were not significantly different from each other. The F_1 mean 1.5475 was greater than the P_1 (L) mean (1.2525) at 10% level but not at 5% level. The F_1 plants also showed heterosis in fresh and dry weights (see Table 24, page 76).

Table 16: Proposed genotypes of the lettuce parent lines used in nitrate-N study.

Cultivar	Nitrate-N Accumulation	Genotype
Valmaine P_1	Low	AA BB cc
Wonder Van Voorburg P_2	Low	aa bb C_2C_2
Caravan P_3	High	aa bb CC
Kordaat P_4	High	aa bb cc
Ithaca P_5	Medium	aa B_2B_2 cc
Valore P_6	High	aa bb cc

All of the observations suggest that the observed low accumulator phenotype of the F_1 generation probably was due to hybrid vigor and/or due to a dilution effect. The observed 9L:55H in the F_2 population suggests that the presence of C gene controlling high accumulation, is epistatic to the A and B alleles. The genes A and B are complimentary to each other which results in low accumulation. The contribution of the recessive allele c was not detected because of the F_2 population size.

Cross: Valmaine (P_1) x Kordaat (P_4) (Low x High)

Frequency distributions for nitrate-N content of Valmaine (P_1), Kordaat (P_4), and their F_1 and F_2 populations are summarized in Table 17. The nitrate content of Kordaat was greater than that of Valmaine and the F_1 generation ($P = 0.05$). The means for the F_1 and Valmaine were not significantly different from each other suggesting dominance for low accumulation.

The F_2 population mean was greater than Valmaine (1) and the F_1 mean, but less than Kordaat (H) in its nitrate content ($P = 0.05$). Using the recessive parental mean to separate phenotypic classes of high and low, the number of plants which fell beyond the mean of Kordaat (2.27) in the segregating population was multiplied by 2 to obtain the number of high accumulators 42 x 2 = 84. The number of low accumulators was 200 - 84 = 116. This observed ratio of 116 L:84 H suggested a 9:7 model and gave a good fit to the model ($P = 0.75$-0.5, Table 14). Thus, a two major gene system controlling nitrate accumulation was suggested.

Table 17: Frequency distribution of Nitrate-N (percent NO_3-N)/plant (dry weight) for the parental, F_1 and F_2 generations of cross of Valmaine x Kordaat.

		Upper Limit of Class													
	No. of Plants	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	Mean[z]	s^2
Valmaine	20	1	4	3	6	3	2	1						1.2525[a]	0.0919
Kordaat	15						1	4	1	2	4	3		2.2700[b]	0.1394
F_1	9		1	3	2	3								1.2292[a]	0.0387
F_2	200	1	8	24	19	33	26	25	22	19	8	8	7	1.7868[c]	0.2873

[z]Mean separation within column by a t-test (P = 0.05).

The ratio also suggested that low nitrate accumulation was the result of an epistatic effect.

Similarly, ratios were obtained by using arithmetic mean of the two parents (1.7613) as the dividing point between the lows and highs. The number of plants in the F_2 population below the arithmetic mean of the two parents (low accumulators) was 26+33+19+24+8+1 = 111 and the number of high accumulators was 25+22+19+8+7 = 89 (Table 17). This observed 111 low:89 high suggested a segregation ratio of 9:7 and gave a good fit to the model (P = 0.90-0.75, Table 14). The observed F_2 mean of 1.7868 approximated the expected F_2 mean 1.6977 for a 9:7 model (P = 0.975-0.90, Table 15).

A two major gene system is suggested based on the following observations: the F_1 plant was completely dominant for low accumulation, which was contributed by Valmaine, whose genotype was designated as AA BB cc; based on the observed F_2 ratio the proposed genotype of Kordaat is aa bb cc (Table 16); the genotype of the F_1 was Aa Bb cc, and the observed F_2 ratio suggested that the presence of both the A and B alleles are necessary to bring about low nitrate accumulation; the genotypes having A-b or a-B and a-b were high nitrate accumulators while the A-B genotypes were low accumulators; the genes A and B are complimentary to each other; both Valmaine and Kordaat were homozygous for the recessive c gene; therefore, the presence of a third gene was not evident.

Cross: Valmaine (P_1) x Valore (P_6) (Low x High)

Frequency distribution of nitrate-N content of Valmaine (P_1), Valore (P_6) F_1 and F_2 populations are summarized in Table 18. The mean nitrate content of Valore was greater than that of Valmaine and the F_1 population (P = 0.05). The F_1 and P_1 population means were not significantly different from each other (Table 18), suggesting dominance for low accumulation of nitrate-N. The nitrate content of the F_2 population mean was greater than that of Valmaine (L) and the F_1 (P = 0.05), but less than Valore (H) (P = 0.05).

The partitioning procedure using the recessive parent (Valore) mean (2.0525) to calculate the number of high accumulator was 44 x 2 = 88 and the number of low accumulators was 200 - 88 = 112. Thus an observed ratio of 112 low:88 high suggested a segregation ratio of 9:7. These data gave a good fit to the model (P = 0.95-0.9, Table 14), based on a 2 major gene system with epistasis.

Similar results were obtained by using the arithmetic mean of the two parents (1.6525) as the dividing point between lows and highs. The number of low accumulators was 34+36+22+8+1 = 111 and the high accumulators was 41+14+29+6+7+2 = 89 (Table 18). Thus, the observed 111 low:89 high also gave a good fit to the 9:7 model (P = 0.9-0.75, Table 14). The results of this cross were similar to the previous cross, Valmaine x Kordaat. The genotype of Valore (high accumulator) is designated as aa bb cc and for Valmaine AA BB cc (Table 16). The observed F_2 mean, 1.6352 approximates the expected F_2 mean 1.6025 for a 9:7 model (P = 0.990-0.975, Table 15).

Table 18: Frequency distribution of Nitrate-N (percent NO_3-N)/plant (dry weight) for the parental, F_1 and F_2 generations of cross of Valmaine x Valore.

	No. of Plants	Upper Limit of Class 0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	Mean[z]	s^2
Valmaine	20	1	4	3	6	3	2	1						1.2525[a]	0.0919
Valore	15					1	3	2	3	4	1	1		2.0525[b]	0.1308
F_1[y]	13		4	5	1	3								1.1825[a]	0.0904
F_2	200	1	8	22	36	34	41	14	29	6	7	-	2	1.6352[c]	0.1776

[y]Reciprocal crosses were pooled.

[z]Mean separation within column by a t-test (P = 0.05).

Cross: Wonder Van Voorburg (P_2) x Caravan (P_3) (Low x High)

Frequency distribution of nitrate-N content of Wonder Van Voorburg (P_2), Caravan (P_3), and their F_1 and F_2 populations are summarized in Table 19. The mean nitrate-N content of Caravan (H) was greater than that of Wonder Van Voorburg (L) and F_1 ($P = 0.05$). The F_1 and P_1 population means were not significantly different from each other (Table 19) suggesting complete dominance for low accumulation of nitrate-N.

The population means of the P_2, F_1, and F_2 generations were not significantly different from one another, but their nitrate content was lower than the P_3 parent mean ($P = 0.05$). Using the recessive parental mean to separate the lows and highs, the number of plants in the F_2 distribution beyond P_3 mean (2.0233) were calculated from the original or raw data and multiplied by 2 (24 x 2 = 48). The number of low accumulators was 200 - 48 = 152. Thus, the observed ratio of 152 low: 48 high suggested a segregation ratio of 3:1 and gave a good fit to the model ($P = 0.9$-0.75, Table 14) which suggested a single major gene differentiated between these two parents for accumulation of nitrate.

Partitioning the F_2 population into low and high classes, by using the arithmetic mean of the two parents, 1.71, a slightly different ratio was observed. This was perhaps due to a 20% overlap of the parental distributions. Therefore, the classes were given weighted values. The weighting procedure consisted of 100 minus the percent overlap of the recessive class below the arithmetic mean of the two parents, divided by 100 (R_o) (N); where R_o is the expected ratio of recessive plants based on the mean of recessive parent value and N is

Table 19: Frequency distribution of Nitrate-N (percent NO_3-N)/plant (dry weight) for the parental, F_1 and F_2 generations of cross of Wonder Van Voorburg x Caravan.

		Upper Limit of Class												
	No. of Plants	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	Mean[z]	s^2
Wonder Van Voorburg	10		2	1	2	3	-	1	-	1			1.4038[a]	0.1907
Caravan	15					3	1	4	1	4	-	2	2.0233[b]	0.0813
F_1	10			1	4	3	2						1.4275[a]	0.0367
F_2	200	2	9	21	35	42	48	21	17	4	1		1.5822[a]	0.1321

[z]Mean separation within column by a t-test (P = 0.05).

the total number of F_2 plants. The number of low accumulators was 200 - 40 = 160. Thus the observed ratio 160 low:40 high suggested a segregation ratio of 3:1 and gave an acceptable fit to the model (P = 0.25-0.1, Table 14) which again suggests a single major gene.

Based on the segregation in the F_2 population of this cross and the information obtained from the cross Valmaine (L) x Caravan (H), the proposed genotype for Wonder Von Voorburg is aa bb C_2C_2 and for Caravan (P_3) aa bb CC (Table 16). The allele C_2 is dominant over C where the presence of C_2 results in low nitrate accumulation. The F_1 genotype aa bb C_2c was dominant for low accumulation of nitrate-N. The expected F_2 mean (1.620) approximates the observed F_2 mean (1.5822) for a 3:1 model (P = 0.990-0.975, Table 15).

Cross: Wonder Van Voorburg (P_2) x Valore (P_6) (Low x High)

Frequency distribution of nitrate-N content of parents, F_1, and F_2 populations are summarized in Table 20. The nitrate content of Valore (P_6) was higher than the means of Wonder Van Voorburg and F_1 population (P = 0.05). The F_1 and F_2 means were not significantly different from each other suggesting a complete dominance for low accumulation of nitrate-N.

The P_2, F_1 and F_2 population means did not significantly differ from one another and were lower than the P_6 mean (P = 0.05, Table 20). Using the recessive parent mean to separate the classes the number of plants in the F_2 population beyond the recessive parent mean (2.0525) was calculated 21 x 2 = 42. The number of low accumulators was 200 - 42 = 158. The observed 158 low:42 high suggested a segregation

Table 20: Frequency distribution of Nitrate-N (percent NO_3-N)/plant (dry weight) for the parental, F_1 and F_2 generations of cross of Wonder Van Voorburg x Valore.

	No. of Plants	Upper Limit of Class 0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	Mean[z]	s^2
Wonder Van Voorburg	10		2	1	2	3	-	1	-	1			1.4038[a]	0.1907
Valore	15					1	3	2	3	4	1	1	2.0525[b]	0.1308
F_1	10			1	4	3	1	1					1.4675[a]	0.0679
F_2	200		1	14	43	63	40	18	13	6	1	1	1.5836[a]	0.0808

[z]Mean separation within column by a t-test (P = 0.05).

ratio of 3:1 and gave an acceptable fit to the model (P = 0.25-0.1, Table 14).

Partitioning the F_2 population into low and high phenotypes using the arithmetic mean of two parents (1.73), the observed ratio of 154 low:46 high determined from the raw data suggested a segregation ratio of 3:1 and gave an acceptable fit to the model (P = 0.5=0.25, Table 14). Nitrate accumulation in this cross appears to be controlled by one major gene with dominance for low nitrate accumulation. The observed F_2 mean (1.5836) approximated the expected F_2 mean (1.566) for a 3:1 model (P = 0.990-0.975, Table 15). The P_2 genotype, as assigned in the previous cross Wonder Van Voorburg X Caravan, was aa bb C_2C_2. The genotype proposed for Valore in the cross Valmaine (P_1) x Valore (P_6) was aa bb cc (Table 16). The F_1 genotype in this cross is aa bb C_2c. The presence of C_2, which is a dominant gene, was responsible for the low accumulation of nitrate-N and the absence of it resulted in high nitrate-N accumulation.

Cross: Valmaine (P_1) x Wonder Van Voorburg (P_2) (Low x Low)

Frequency distribution of nitrate-N content of Valmaine (P_1), Wonder Van Voorburg (P_2) and their F_1 and F_2 populations are summarized in Table 21. The mean of the parental and F_1 were not significantly different from one another. The mean of parental and F_2 populations were not significantly different; however, the F_2 population mean was greater than the mean of the F_1 generation (P = 0.05, Table 21).

Table 21: Frequency distribution of Nitrate-N (percent NO_3-N)/plant (dry weight) for the parental, F_1 and F_2 generations of cross of Valmaine x Wonder Van Voorburg.

	No. of Plants	Upper Limit of Class 0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	Mean[z]	s^2
Valmaine	20	1	4	3	6	3	2	1				1.2525[ab]	0.0919
Wonder Van Voorburg	10		2	1	2	3	-	1	-	1		1.4038[ab]	0.1907
F_1[y]	19	4	5	4	5	1						1.0355[b]	0.1219
F_2	200	7	24	33	40	32	36	17	6	4	1	1.4078[a]	0.1426

[y]Reciprocal crosses were pooled.

[z]Mean separation within column by a t-test (P = 0.05).

Observation of the parental F_1 and F_2 distributions suggests that the parents may be of the same genotype. Based on the results of crosses of Valmaine (L) and Wonder Van Voorburg (L) with the high accumulator parents (Caravan and Valore) however, it was demonstrated that Valmaine and Wonder Van Voorburg are genetically different.

Partitioning was based on the F_1 and F_2 distributions. The F_2 distribution showed that a major portion of the F_2 population centered around the class 1.4 (Table 21). The original data showed that the maximum number of plants fell between the classes 1.25 and 1.4. The mean of these two classes is 1.33 which is also the arithmetic mean of the two parents (1.3269). The number of individuals in the F_2 population dropped in the 2.0 to 2.6 classes. The 2.0 class approximates the mean of the three high accumulators (Caravan + Kordaat + Valore/$_3$ = 2.1153). In order to partition the low and high accumulators, the number of plants below the arithmetic mean of the two parents (1.3269) were calculated to obtain the low accumulators (89 x 2 = 178). Twenty-two (200 - 178) individuals were classified as high accumulators. The observed 178 low:22 high gave an acdeptable fit to the model of 57L:7H (P = 0.75-0.5, Table 14). This ratio suggests that three major genes make up the parents Valmaine (P_1) and Wonder Van Voorburg (P_2). The proposed genotype of P_1 is AA BB cc and that of P_2 is aa bb C_2C_2, Table 16.

The F_1 genotype is Aa Bb C_2c and is a low nitrate-accumulator. In the F_2 population the following genotype A-B-C_2, A-B-c, A-b-C_2, a-B-C_2, and a-b-C_2 would be low accumulators while the genotypes A-b-c,

a-B-c, and a-b-c would be high accumulators. The recombination of the genes yielded a few high accumulators from this low x low cross. This would only be possible based on the proposed genotypes of Valmaine (AA BB cc) and Wonder Van Voorburg (aa bb C_2C_2).

Cross: Caravan (P_3) x Ithaca (P_5) (High x Medium)

Frequency distributions for the nitrate-N content of Caravan (H), Ithaca (M) and their F_2 populations are summarized in Table 22. The mean nitrate content of Caravan was greater than that of Ithaca ($P = 0.05$, Table 22).

This cross was made to obtain information as to whether high accumulators would recombine and produce segregants that were higher nitrate accumulators than the parents. Initially this cross was planned as a high x high; however, the parent Ithaca in the 1975 planting was classified as a medium accumulator. The F_2 distribution suggests that the segregation for nitrate-N fell within the parental distributions. The majority of the segregants were scattered about the mean of Caravan (H). It appears that the genotype of Ithaca (M) was different from that of Caravan.

The mean of the F_2 population (1.8445) approximates the arithmetic mean of the two parents (1.8154), which was not significantly different from either parent. The absence of the F_1 generation in this planting made it difficult to identify the dominance direction. Using the parental arithmetic mean, the division of the medium and high phenotype were identified. The observed 23 medium:27 high suggests a two-major

Table 22: Frequency distribution of Nitrate-N (percent NO_3-N)/plant (dry weight) for the parental, and F_2 generations of cross of Caravan x Ithaca.

	No. of Plants	Upper Limit of Class 0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	Mean[z]	s^2
Caravan	15					3	1	4	1	4	-	2	2.0233[a]	0.0813
Ithaca	10				3	2	3	2					1.6075[b]	0.0653
F_2	50				4	8	11	7	12	7	1		1.8445[ab]	0.1463

[z]Mean separation within column by a t-test.

gene difference between the parents. This observed classes also suggested a ratio of 6M:10H and gave an acceptable fit to this model (P = 0.25-0.10, Table 14).

The proposed segregation ratio is based on the genotype of Caravan as aa bb CC (Table 16), and that of Ithaca to be aa B_2B_2 cc. The F_1 genotype would be aa B_2b Cc and a high accumulator phenotype. The ratio of 6M:10H is based on the genotypes of medium to be aa B_2B_2 cc, aa B_2B_2 CC, aa B_2B_2 Cc and aa B_2b cc, while the genotype having aa B_2b CC, aa B_2b Cc, and aa bb cc would be high accumulators. The observed F_2 mean (1.8445) approximates the expected F_2 mean (1.7634) for a 6:10 model (P = 0.95-0.90, Table 15).

SUMMARY AND CONCLUSIONS

Data from the segregation of F_2 population from crosses, involving Valmaine (L), Wonder Van Voorburg (L), Caravan (H), Kordaat (H), and Valore (H), suggest that three major genes determine the inheritance of nitrate accumulation in lettuce.

In the three major gene systems the two genes, A and B, appear to determine the inheritance of nitrate accumulation in the crosses of Valmaine (L) x Kordaat (H) and Valmaine (L) x Valore (H) (Table 23). In these crosses, the mean nitrate content of the F_1 population approximate the mean for Valmaine (L). The F_2 population showed a ratio of 9L:7H suggesting two major genes with epistasis. The presence of both A and B alleles was necessary to give Valmaine (L) phenotypes, i.e., A and B were complementary to each other.

The presence of a third major gene, C, was demonstrated from the cross Valmaine (L) x Caravan (H) (Table 23). In this cross, the F_1 appeared partially dominant towards low accumulation with the F_2 generation demonstrating a segregation ratio of 9L:55H. This suggested a third major gene may be involved in controlling nitrate accumulation. Based on crosses involving Valmaine (L), the genotype of Valmaine (L) is designated as AA BB cc and the genotype of Caravan (H) as aa bb CC based on the cross Valmaine x Caravan. Gene C from Caravan (H) controls high nitrate accumulation. The C allele is dominant over c and the

Table 23: Genotypes and phenotypes of the F_2 populations from various crosses of nitrate-N accumulation study.

Cross	Model	Genotypes		
		Low	Medium	High
Volmaine x Caravan (High)	9L:55H	A-B-c	--	- -C A-b-c a-B-c a-b-c
Valmaine (L) x Kordaat (H)	9L:7H	A-B-c	--	A-b-c a-B-c a-b-c
Valmaine (L) x Valore (H)	9L;7H	A-B-c	--	A-b-c a-B-c a-b-c
Wonder Van Voorburg (L) x Caravan (H)	3L:1H	a-b-C_2 a-b-C_2C	--	a-b-C
Wonder Van Voorburg (L) x Valore (H)	3L:1H	a-b-C_2	--	a-b-c
Valmaine (L) x Wonder Van Voorburg (L)	57L:7H	a-b-C_2 A-B-c	--	A-b-c a-B-c a-b-c
Caravan (H) x Ithaca (M)	6H:10M	--	aa-B_2B_2-cc aa-B_2B_2-Cc aa-B_2B_2-CC	aa-B_2b-CC aa-B_2b-Cc aa-bb-cc

presence of C is epistatic to A and B genes. The low nitrate accumulation genotypes in this cross were A-B-c and the high accumulator types were - - C, A-b-c, a-B-c, and a-b-c.

The presence of an allelic gene, C_2, controlling nitrate accumulation for low levels was demonstrated in the cross Wonder Van Voorburg (L) x Caravan (H) (Table 23). In this cross, the F_1 appeared dominant toward low accumulation and the F_2 population segregated 3L:1H. The genotype of Caravan (L) was designated as aa bb CC in the cross Valmine (L) x Caravan (H). The designated genotype for Wonder Van Voorburg (L) is aa bb C_2C_2. The allelic gene C_2 is dominant over C. Thus two dominant allelic genes are identified at the 'c' locus with the direction of dominance $C_2 > C > c$, where C_2 controls low nitrate accumulation and is dominant over the C and c alleles. The allelic gene C controls high nitrate accumulation and is dominant over c whereas the recessive allele, c, did not appear to have an influence on either low or high accumulations of nitrate-N.

The presence of gene C_2 was also noted in the cross Wonder Van Voorburg (L) x Valore (H). The presence of A, B, and C_2 genes were also demonstrated in the cross Valmaine (L) x Wonder Van Voorburg (L). The segregation of high accumulators in this Low x Low cross would only be possible from the proposed genotype of Valmaine (AA BB cc) and Wonder Van Voorburg (aa bb C_2C_2). The recessive genes a, b, and c appear to contribute little to the nitrate accumulation. The high accumulation with a-b-c genotype may be due to the absence of both A-B or C_2 genes, rather than the presence of the recessive genes.

The low nitrate accumulation was dominant over high nitrate accumulation as noted in the F_1 generation of all L x H crosses (Table 12). Low nitrate accumulation may occur in three ways, first low nitrate accumulation may be the result of both genes A and B which are complimentary to each other as noted in crosses involving Valmaine (L). Alternatively, low nitrate accumulation may be controlled by a single dominant gene C_2 as noted in crosses involving Wonder Van Voorburg (L). Moreover a combination of A-B and C_2 as noted in the cross of Valmaine (L) x Wonder Van Voorburg (L) may also yield low nitrate accumulators (Table 23).

High nitrate accumulation may be due to the absence of genes A and B in a genotype, as demonstrated from crosses involving Valmaine (L). Otherwise, it may be due to the presence of a dominant gene C as noted in the cross between Valmaine (L) x Caravan (H). The presence of gene C (high accumulator) from Caravan resulted in high nitrate accumulation even in the presence of genes A and B suggesting that gene C is epistatic to genes A and B (Table 23). The gene B_2 determined a medium level of nitrate accumulation in the parent Ithaca as noted in the cross Ithaca (M) x Caravan (H) (Table 23).

Preliminary studies on total-N of the parental lines at the four week old stage suggested that the difference in nitrate accumulation between low and high accumulators was due to the greater nitrate reduction capabilities of low accumulators rather than a greater nitrogen (nitrate) uptake by the high accumulators.

Although the preliminary observations suggested cultivar difference in the other elements, a comparison of each of these elements within and between the low and high nitrate accumulator did not show a marked variation in their content. Probably the two variables are not related.

Preliminary studies on the fresh and dry weight of parent lines did not suggest a relationship between the nitrate content and yield (fresh or dry weight) at the four week stage. However, the F_1 generation between a low and a high accumulator were higher in yield than one or both parents (Table 24), probably due to hybrid vigor. The fixing of low nitrate content along with higher yields in the segregating populations is worthy of further investigation.

In order to better understand the mechanism of nitrate accumulation in lettuce, a knowledge of nitrate reductase activity and total nitrate uptake by the low and high nitrate accumulators of lettuce is essential.

Table 24: Mean fresh and dry weights of parents and their F_1 population for two plantings.

	December 1975		January 1976	
	Fresh[z] wt	Dry wt	Fresh wt	Dry wt
P_1	35.160[a]	1.7665[a]	35.162[a]	2.6859[a]
P_2	32.315[a]	1.6100[a]	33.797[a]	2.2578[b]
F_1	49.193[b]	2.7901[b]	40.089[b]	2.9574[a]
P_1	35.16[a]	1.7665[a]	35.162[a]	2.6859[a]
P_3	38.28[a]	1.8240[a]	40.570[b]	2.8622[a]
F_1	42.51[b]	2.1760[b]	41.609[b]	3.1673[b]
P_1	35.16[a]	1.7665[a]	35.162[a]	2.6859[a]
P_4	40.113[a]	1.4853[a]	36.129[a]	1.9266[b]
F_1	52.789[b]	2.6078[b]	40.117[a]	2.9878[a]
P_1	35.16[a]	1.7665[a]	35.162[a]	2.6859[a]
P_6	43.36[a]	1.8780[a]	38.197[a]	2.2014[b]
F_1	56.115[b]	2.8723[b]	43.618[b]	3.0468[a]
P_2	32.315[a]	1.6100[a]	33.797[a]	2.2578[a]
P_3	38.28[a]	1.824[a]	40.570[b]	2.8622[b]
F_1	55.34[b]	2.615[b]	46.389[b]	3.4286[c]
P_2	32.315[a]	1.6100[a]	33.797[a]	2.2578[a]
P_6	43.36[b]	1.8780[ab]	39.197[b]	2.2014[a]
F_1	48.905[b]	2.2990[b]	42.938[b]	2.5453[a]

P_1 Valmaine
P_2 Wonder Van Voorberg
P_3 Caravan
P_4 Kordaat
P_5 Valore

[z]Mean separation using a "t" test (P = 0.05) within a column and within a cross.

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